**BEHAVIOURAL AND ANTICONVULSANT EFFECTS OF METHANOL EXTRACT OF *FICUS VALLIS* CHOUDAE (MORACEAE) STEM BARK**

**BY**

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**ZARIA, NIGERIA**

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**STEM BARK**

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**DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS FACULTY OF PHARMACEUTICAL SCIENCES**

**AHMADU BELLO UNIVERSITY**

**ZARIA, NIGERIA**

**FEBRUARY, 2009**

## DECLARATION

I declare that the work in the thesis entitled „Behavioural and anticonvulsant effects of methanol extract of *Ficus vallis* choudae (moraceae) stem bark‟ has been performed by me in the Department of Pharmacology and Therapeutics under the supervision of Prof. (Mrs) H.O. Kwanashie and Prof. I.M. Hussaini.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

Name of student Signature Date

# CERTIFICATION

This thesis entitled “BEHAVIOURAL AND ANTICONVULSANT EFFECTS OF METHANOL EXTRACT OF *FICUS VALLIS* CHOUDAE (MORACEAE) STEM

BARK” by Malami Sani meets the regulations governing the award of the degree of Master of Science (Pharmacology) of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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## ABSTRACT

*Ficus vallis* choudae is a plant widely spread in tropical Africa including Nigeria. It is said to be of value in ethnomedicine especially in the treatment of jaundice, gastrointestinal problems, giddiness and epilepsy. The anticonvulsant activity of methanol stem bark extract of *Ficus vallis* was studied in mice and two-day old cockrels. This study was conducted using three models; pentylenetetrazole-induced seizure test, maximal electroshock seizure test and 4-aminopyridine-induced seizure test. Also, the sedative as well as motor coordination deficit effects were studied in mice; using diazepam-induced sleep, hole-board test, and walking beam assay models respectively. The extract afforded 25.0%, 50.0% and 33.3% protections at doses of 80mg/kg, 40mg/kg and 20mg/kg respectively against pentylenetetrazole seizure model. It also significantly increased the onset of seizures at doses of 40 mg/kg and 80 mg/kg (p<0.005 and p<0.05 respectively). The extract did not exhibit significant activity in maximal electroshock seizure model. In the 4-amino pyridine-induced seizure test, at doses of 20 mg/kg, 40 mg/kg and 80 mg/kg, there was only 16.7%, 16.7% and 0% protection against seizure. The corresponding mortality rates were also high: being 83.3%, 83.3% and 100% respectively. However, onset of seizures was found to be significantly different from saline control at p<0.001, p<0.05 and p<0.05 for 20, 40, and 80 mg/kg respectively.. The extract resulted in significant decreases in the onset of sleep at doses of 20 mg/kg, 40 mg/kg and 80 mg/kg (p<0.05, p<0.005 and p<0.005 respectively); and significant increases in the duration of sleep (same graded doses of 20, 40 and 80 mg/kg) at p<0.005, p<0.05 and p<0.005 respectively. The number of head dips were significantly decreased at p<0.001 for all the tested doses of the extract. From the beam walking test for motor deficits, the result showed a significant increase in the

number of foot slips at doses of 20 mg/kg, 40 mg/kg and 80 mg/kg (p<0.001, 0.005 and p<0.005 respectively); with no significant difference in the time taken to cross the two ends of the beam (time taken to complete the task). The median lethal dose (LD50) values of *F. vallis* extract were found to be 471.2 mg/kg (i.p.) and >5,000 mg/kg (p.o.) in mice whereas that found in chicks were 774.6 mg/kg (i.p.) and >5,000 mg/kg (p.o.). The preliminary phytochemical screening reveals the presence of saponins, alkaloids, flavonoids, tannins and glycosides. These results suggest that, *F. vallis* extract possess biologically active compounds that have anticonvulsant properties, and are sedative in nature.

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## LIST OF ABREVIATIONS

< Less than

5HT 5-hydroxytryptophan

ABU Ahmadu Bello University

AC Adenyl cyclase

AEDs Antiepileptic drugs

AMPA Alpha amino- 3- hydroxyl- 5- methyl isoxazole- 4- pripionic acid ATP Adenosine triphosphate

BBB Blood-brain barrier

BDZ Benzodiazepines

Ca2+ Calcium ions

Cl- Chloride ions

CNS Central nervous system

Co Company

CRH Corticotropin- releasing hormone

DZ Diazepam

EEG Electroencephalograph

Etc Ecetra

FV Ficus vallis

GABA Gamma amino butyric acid

GAD Glutamic acid decarbixylase

HLTE Hind limb tonic extension

i.p. Intraperitoneal

K+ Potassium

KA Kainite

|  |  |
| --- | --- |
| Kg | Killogram |
| LD | Lethal dose |
| Ltd | Limited |
| mA | Milli ampere |
| MEST | Maximal electroshock test |
| mg | Milligram |
| MGluR | Metabotropic receptors |
| ml | Milli litre |
| ms | Millisecond |
| MTLE | Mesial temporal lobe epilepsy |
| MTZ | Metrazole |
| n | Number of animal |
| Na+ | Sodium ions |
| NAPRI | National Agricultural Production and Reseach Institute |
| NMDA | N- methyl- D- aspartate |
| NREM | Non rapid eye movement |
| p.o. | Per oral |
| PH | Phenytoin |
| PHB | Phenobarbital |
| PI | Phosphoinositide |
| PTZ | Pentylenetetrazole |
| REM | Rapid eye movement |
| s.c. | Subcutaneous |
| SEM | Standard error of mean |
| SSRI | Selective serotonin reuptake inhibitors |

|  |  |
| --- | --- |
| TCA | Tricyclic antidepressants |
| USA | United States of America |
| VGCCs | Voltage-gated calcium channels |
| VGKCs | Voltage-gated potassium channels |
| VGSCs | Voltage-gated sodium channels |
| WHO | World Health Organisation |

## \CHAPTER ONE

## INTRODUCTION

### Statement of Research Problems

Several types of insults such as status epilepticus, hypoxia and trauma are known to alter the normal function of the central nervous system (CNS). Modalities that protect the brain against such insults have been very difficult and challenging. It is important to know that, epilepsy, as one of such CNS disorders, alter the normal function of brain; and its treatment is all about neuroprotection, either to reduce the duration of seizures or to suppress the occurrence of seizures (Arzimanoglou *et al*., 2002). According to Avanzini and Franceschetti (2003), complete seizures suppression can only be obtained in about 60-70% of patients using conventional antiepileptic agents. The remaining (30 - 40%) are referred to as being affected by refractory, intractable or drug resistant epilepsies.

Epilepsy refers to a disorder of brain function characterized by the periodic and unpredictable occurrence of seizures. These seizures are transient alteration of behavior due to the disordered, synchronous and rhythmic firing of populations of brain neurons (McNamara, 2001). This group of disorders are diverse, and they all appear to have in common, the feature of aberrant synchronized discharge of neurons leading to alteration in electroencephalograph (EEG) activity and behavior (Nicholas *et al*., 2002).

The causes of seizures are many and include the full range of neurological diseases, from infection to neoplasm and head injury. In some sub-groups of epilepsy, hereditory is known to be a major contributing factor. These may explain why monotherapy in epilepsy is difficult (Roger, *et a*l., 2004).

Epilepsy is the second most common neurological disorder after stroke; and approximately 1% of the world‟s population has epilepsy (Roger, *et al*., 2004). About 2 million people in the United States have epilepsy and 3% of persons in the general population will have epilepsy at some point in their lives (Bernard, *et al*., 2003). The incidence is highest in the first year of life, and decreases with age throughout child hood and adolescence (Mizrahi and kellaway, 1998). Chronic seizures disorder, is known to predisposes individual to anxiety disorder (Loberg, 1996); a mental illness affecting up to 10% of general population at some time in their lives (Kessler and Moller,1994).

### Justification of the Study

Most of the synthetic centrally acting drugs used in the management of epilepsy and /or as anxiolytics, sedatives, as well as muscle relaxants(e.g. Benzodiazepines), are known to cause untoward effects ranging from teratogenicity, hepatotoxicity, sedation, anterograde amnesia, addiction, and impaired cognitive and mental behavior. Moreover, they are mostly used in combination, and affordability and compliance remain a great problem. These necessitate the research for alternative therapeutic agents especially from natural source with higher therapeutic efficacy, fewer side effects, cost effectiveness, and availability.

### Theoretical Frame Work

Drugs involved in the central nervous system (CNS), can be broadly classified according to whether they have a general stimulatory or depressant action, with further sub-division regarding specific actions such as anticonvulsant and psychopharmacological activities (Evans, 1996). However, it should be noted that, an

overlap of pharmacological actions do exist among some centrally acting drugs. For instance, benzodiazepines are known to exhibit antiepileptic activity, anxiolytic effect, muscle relaxing properties, as well as sedative and hypnotic actions to a greater or lesser extent (Mycek, *et al*., 2000). It has also been reported that, pentylenetetrazole (metrazole)-induced convulsion model, used primarily to evaluate antiepileptic drugs; can as well be used as a tool to predict anxiolytic activity of compounds. Thus, most of the anxiolytic agents are also able to prevent or antagonize metrazole-induced convulsions (Vogel and Vogel, 1997). Nevertheless, the metrazole (MTZ)-antagonism has been proposed to study centrally acting skeletal muscle relaxants (Domino, 1964). Hence, evaluation of biologically active compounds with benzodiazepines like pharmacological activity is of immense contribution in the development of health care systems.

### Phytochemical screening

Phytochemical determination has been employed as one of the strategies used in screening and evaluation of traditional medicine resources (Diaz *et al*., 1989). The therapeutic benefits of traditional remedies are often attributed to a combination of active constituents (Amos*, et al.,* 2001). For instance, flavonoids were found to inhibit phosphodiesterases which are involved in cell activation, whose effect depend upon the biosynthesis of protein cytokines that mediate adhesion of circulating leucocytes to the sites of injuries, thus, possess anti-inflammatory activity. According to Wagner, *et al.,* (1983), saponins have been shown to have sedative property in experimental animals as well as antagonistic activity against amphetamine.

### Acute toxicity study

Median lethal dose (LD50) is a parameter used in assessing acute toxic effect of a compound using the smallest number of animals possible. It is the dose that kills approximately 50% of the animals. The determination of LD50 is of significance importance, in the sense that, it has been a valuable tool employed to compare toxicities of compounds relative to their therapeutic doses (Berkowitz, 2004). In studying unknown compounds (e.g. a plant extract), appropriate doses of the extract should be determined by preliminary studies of acute toxicity. Such studies are also essential to prevent over dose of drug which may interfere with results of experiment, and provide a clue for the toxicity profiles of the plant extract (Ozbek *et al*., 2004).

### Pentylenetetrazole-induced seizure test

Pentylenetetrazole is a known chemical convulsive agent. The anticonvulsant activity in subcutaneous PTZ test identifies compounds that can raise the seizure threshold in brain (White *et al*., 1998). The model is used to evaluate antiepileptic drugs which are effective in the therapy of generalized absence or myoclonic seizures (petitmal epilepsy). Agents like phenobarbitone, valproic acid, ethosuxamide and benzodiazepines are known to suppress seizure patterns induced by PTZ (Loscher *et al.,* 1991).

### Maximal electroshock test (MEST)

Maximal electroshock test is an assay used primarily to establish compounds which are effective in the treatment of generalized tonic-clonic seizures (grandmal epilepsy) and partial seizures. It is characterized by hind limb tonic extension (HLTE), and there are

no false-negatives in the model. Agents such as phenytoin, carbamazepine, oxcarbazepine and lamotrigine are known to suppress HLTE (Rho and Sankar, 1999). Inhibition of HLTE is the common feature of maximal electroshock in rodents, cats, monkeys and humans, and the response of rodent brain to the anticonvulsant is similar to that of humans (Swinyard, 1972). Protection against HLTE also indicates the ability of testing compound to inhibit or prevent seizure discharge within the brain stem seizure substrate (Browning, 1992).

### 4- aminopyridine-induced seizure test

4-aminopyridine is a powerful convulsive agent, it is known to block potassium ion channel. It causes the inducement of seizure by enhancing spontaneous and evoked neurotransmitter release. The agent is highly lethal, induces clonic-tonic convulsions. Both excitatory and inhibitory synaptic transmission is facilitated by 4-aminopyridine, the epileptiform activity induced by the drug is predominantly mediated by non-NMDA type excitatory amino acid receptors. It is noted that, this model is different from other chemoconvulsant models and more similar to those that prevent THLE in maximal electroshock seizure test. Hence, the mode of action of anticonvulsant drugs can be predicted using the 4-aminopyridine-induced seizure model (Yamaguchi and Rogawski, 1992).

### Diazepam-sleeping time

This model is used to evaluate CNS activity of compounds. Prolongation of sleeping time is believed to be a measure of a sedative and/or hypnotic property (Fujimori, 1965), and hence suggests central depressant activity (Perez, *et al*., 1998). The time interval from drug administration to loss of righting reflex is measured as a criterion for

the onset of sleep, and the time from the loss of righting reflex to recovery is considered as the duration of sleep (Amos*, et al*., 2004).

### Hole-board test

Hole-board test is a measure of exploratory behavior or curiosity in mice (File and Wardill, 1975). The ability of a compound to decrease this parameter is considered to have a sedative property (File and Pellow, 1985). However, antianxiety drugs have been shown to increase the exploratory behavior i.e. increase in the number of head dips by mice (Takeda *et al.,* 1998).

### Walking-beam assay

This assay is aimed at detecting motor deficits caused by damage to the motor cortex in both rats and mice. The most commonly used model to assess BZD-induced ataxia is the rotarod assay. However, in rotarod assay, about 70% receptor occupancy with a full BZD agonist is required to provide a significant impairment on the rotarod. In contrast, walking-beam assay is more sensitive in the sense that, a significant deficit can be observed at about 25-30% GABAA receptor occupancy. The increased sensitivity may be afforded by the fact that, the beam-walking assay allows individual performance to be monitored more closely. Also, it allows multiple measures to be taken; i.e. number of foot slips (most sensitive measure) and falls, as well as time spent on the beam (Stanley, *et al*., 2005).

### Objectives of the Study

This research was aimed at screening the stem bark methanol extract of *Ficus vallis* for anticonvulsant activity as claimed by traditional herbalists. The screening was conducted through the use of chemoconvulsant agents; pentylenetetrazole and 4-

aminopyridine; and an electrically induced seizure model (maximal electroshock seizures). Also, the extract was subjected for other behavioral tests to ascertain some of its other central effects.

The Specific aims were as follows:

* To conduct preliminary phytochemical screening on the crude extract
* To conduct acute toxicity studies in mice and chicks, via intraperitoneal and oral routes
* To screen the extract for anticonvulsant activity in mice and chicks
* To ascertain its sedative property in mice
* To predict its effect on motor coordination deficits in mice.

### Research Hypothesis

The stem bark extract of *Ficus vallis* contains biologically active compounds that have anticonvulsant activity, cause sedation and other CNS effects.

## CHAPTER TWO

## LITERATURE REVIEW

### Traditional Medicine

Recently, there has been increasing interest in the use of plants to treat diseases. The plant kingdom has become a target for the search of new drugs and biologically lead compounds by multinational drug companies and research institutes (Evans, 1996). They are believed to be important source of new chemical compounds with potential therapeutic effects (Eisner, 1990). As such, they have been used in development of new drug and continue to play an important role in drugs discovery process (Fansworth, 1994).

A number of medicinal plants have been found and put into use in ethnomedicine by traditional healers in the management of many ailments for many years (Sofowora, 1993). The World Health Organization (WHO) has recommended, especially in developing countries, the initiation of programmes designed to use medicinal plants more effectively in traditional health care systems (WHO, 1978). The resolution of the 31st WHO assembly requested a complete inventory, evaluation of the efficacy and safety as well as standardization of medicinal plants (Fansworth, 1980). Hence, these traditional medicines have been exploited and serve in the discovery and development of new drugs.

To a large extent, drug development has become a global activity; aimed at providing information on the optimal use of a new drug in the treatment or prevention of diseases. Efficacy and safety are the main criteria of interest in drug development both from natural and synthetic origin (Peter, 2001). Today, a number of plants are used as

medicines, and the plant kingdom and the forest are often referred to as the sleeping giant of drug development and God‟s own pharmacy respectively (Chindo, 1999).

### Plant Description

### Family

*Ficus vallis* belongs to the moraceae family having about 53 genera and 1400 species. They are mainly tropical and sub-tropical shrubs or trees containing latex. The fruit is often multiple, as in *ficus*, the fig (Evans, 1996).

### Genus

The large genus ficus (about 800 species) includes trees and shrubs of very varied habit. The latex is often antihelmintic owing to proteolytic enzyme *ficin* (Evans 1996). According to Dalziel (1955), the West African species seem to be of little economic importance, the latex of several species is used as bird lime; some bear figs, which are in diverse degrees regarded as edible by the people; as shade trees, they are the most familiar forms in all towns and villages, giving dense shade usually wholly or in part evergreen, and easily raised from stakes.

Medicinally, the bark of several species is chewed with kolanut either to relieve thirst or as a remedy for sore throat. It is also known that, in folk medicine superstitious practices are common among *ficus* species. Many of them begin life as epiphytes, the seed being carried by birds. The aeriel roots eventually embrace the stem of the host and subsequently replace it by their growth, uniting and forming the compound trunk of an eventually independent tree. *Ficus* may grow to maturity and bear fruit, even before their aerial roots have established themselves in the ground. According to Danthu *et al*.,

(2002), *ficus* species are multipurpose trees well known by rural populations in Sahelian and Sudanian zones of Africa. These species are commonly used as food by local people (e.g *ficus sycomorus*); others are used for fire wood (e.g *ficus ovata*), as well as traditional medicines. For example, leaves of *ficus thonningi* are used to treat malaria and yellow fever. Infusions of the dried fruits of *ficus sycomorus* is taken orally for the management of tuberculosis (Arnold and Guluman 1984).

About 70 species of *ficus* are found naturally in West Africa, and they are sometimes over-exploited leading to their rarity or even disappearance locally (Danthu, *et al*., 2002)*.* In South Africa, there are about 24 indigenous species of *ficus*, and the fruit of most of them is edible although not so palatable as *ficus carica*. The monkey and many species of birds delight eating them. The fruit is often infested with insects to a degree which makes it disagreeable to the human palate. They are known for their use as an antidote to arrow poison (Watt, 1962).

* + 1. ***Ficus vallis* choudae**
			1. *Common traditional names in Africa*

### Country Local name

Niger Kamasagi

Ghana Doma

Senegal Ga-nak

Ivory Coast Aloma

Togo Buboku

Nigeria Dullu, bargomi (Hausa); Oguro (Yoruba)

* + - 1. *Geographical distributions*

*Ficus vallis* is widely spread in tropical Africa, ranging from damp sites, stream banks and into dry savanna; in Senegal to Southern Nigeria (Burkill, 1997). It is also found in northern Nigeria.

* + - 1. *Morphological description*

*Ficus vallis*-choudae, is a tree of about 60 ft high, with short bole and spreading crown; branchlets and leaves nearly globorus (Hutchinson and Dalziel, 1958). According to Burkill (1997), it is sometimes shrubby, 3-5 m high, with a spreading crown of conspicuously large tooth ovate leaves. The wood is pale yellowish brown to golden yellow, with straight grain and good working characteristics. The bark is fibrous and contains abundance latex. The fruit is the largest of the West African figs, commonly 3- 5 cm across, sometimes grows up to 5-6 cm. it is succulent and generally eaten with relish, and the fertilizing wasp is often to be found within.

* + - 1. *Non medicinal uses*

*Ficus vallis* has been used in different locations for several purporse. According to Burkill (1997), the wood is used for cheap furniture and fittings in East Africa. It is usually converted into charcoal in South-East Senegal. In central African Republic, the wood is termed as woman‟s fire wood because it last long in hearth and cooking place, burning with a hot flame and making minimal smoke. The bark can be stripped off for making bark-cloth. The leaves are cooked and eaten in Sierra Leone as palaver sauce, also Tenda people of South-Eastern part of Senegal eat the young leafy shoots.

* + - 1. *Ethnomedical uses*

The leaves and young leafy stems are decocted and taken for jaundice, nausea, bronchial and gastro-intestinal problems. Such preparations are emollient and astringent. In Ghana and Zaire, the combined part of the plant is used as an antidote for poisons. Also, leaf-sap is taken by mouth and rubbed over the head for giddiness (Burkill, 1997). In Northern Nigeria, the bark is used in the treatment of haemarrhoid and epilepsy (oral communication).

* + - 1. *Previous work done on the plant*

An intensive search of the internet and other resources show that much has not been done on the plant especially in relation to pharmacological activity. However, the ethanolic bark extract has been investigated for antifungal activity, and was found to be effective against *Trichoderma viride* (Adekunle *et al*., 2006).

### Epilepsy

### Major types of epilepsy

According to Benbadis, (2001), epilepsy syndrome falls into two broad categories: generalized and partial (or localization-related) syndromes. In generalized epilepsies, the predominant type of seizures begins simultaneously in both cerebral hemispheres. Many forms of generalized epilepsy have a strong genetic component; in most neurologic function is normal. In partial epilepsies, seizures originate from one or more localized foci, although they can spread to involve the entire brain. Most partial epilepsies are due to one or more central nervous system insult, but in many cases the nature of the insult is never identified.

Epilepsy syndromes can also be classified according to the type of seizures, the presence or absence of neurologic or developmental abnormalities, and electroencephalographic (EEG) findings (Idem, 1987).

* + - 1. *Absence epilepsy*

Absence epilepsy is a generalized epilepsy syndrome that begins between the ages of four and eight years with absence seizures and, more rarely, generalized tonic-clonic seizures (Panayiotopoulos, 1997). During absence seizures the patients stare and cease normal activity for a few seconds, then return immediately to normal and have no memory of the event. The mechanism that generates absence seizures is now believed to involve an alteration in the circuitry between the thalamus and the cerebral cortex (Kostopoulos, 2001). Three neuronal populations are involved in this circuitry; thalamic relay neurons, thalamic reticular neurons, and cortical pyramidal neurons. The thalamic relay neurons can activate the cortical pyramidal neurons either in a tonic mode, which occur during wakefulness and rapid-eye-movement (REM) sleep, or in a burst mode, which occurs during non-REM sleep. The burst mode is made possible by T-type calcium channels, which allow for low-threshold depolarization on which burst of action potentials (mediated by voltage-gated sodium channels) are superimposed (Nowyeky, *et al*., 1985).

In absence epilepsy, the abnormal circuit causes rhythmic activation of the cortex (typical of normal non-REM sleep) during wakefulness, which results in the characteristic EEG discharges and clinical manifestations of absence seizures (Kostopoulos, 2001). Some data suggest that the T-type calcium channels may be the

primary culprits (Kim, *et al*., 2001). Other work has emphasized the importance of altered (GABA) receptor function (Caddick and Hosford, 1996).

The above concept helps to explain the unique pharmacologic treatment of absence epilepsy. Ethosuximide, a drug that suppress absence seizures, appear to work by causing voltage-dependent blockade of T-type calcium currents (Glauser, 2001). This mechanism, as might be expected, is believed to inhibit the burst mode of thalamic- relay-neuron firing. Valproic acid, an antiepileptic drug used for absence seizures and other types of seizures, also act on the T-type calcium channels, as well as other substrates (Kwan *et al*., 2001). Benzodiazepines that activate an inhibitory GABAA receptor subtype on thalamic reticular neurons can also be effective in suppressing absence seizures (Panayiotopoulos, 1999).

* + - 1. *Partial epilepsy*

Partial epilepsy is the most common seizure disorder in adults, often stemming from focal lesions such as head trauma, strokes, and tumors (Annegers, 2001). The most common of these syndromes features complex partial seizures arising from the mesial temporal lobe (Benbadis, 2001 and Engel, 2001). Recordings from intracranial depth electrodes have clearly demonstrated an ictal onset in mesial temporal structures such as the hippocampus, amygdala, and adjacent parahippocampus cortex; surgical resection of these areas in suitable patients usually abolishes the seizures (Kim and Spencer, 2001). These seizures can begin with olfactory or gustatory hallucinations, an epigastric sensation, or psychic symptoms such as depersonalization. Once the seizures progress to a loss of awareness, the patients may stare blankly, speak unintelligently, or exhibit lip smackling, picking at clothing, or other automatism (French, *et al.,* 1993).

The most common lesion in surgically resected tissue from patients with mesial temporal-lobe epilepsy is hippocampal sclerosis (Mathern, *et al*., 1997). In hippocampal sclerosis, there is selective loss of neurons in the dentate hilus and the hippocampal pyramidal-cell layer, with relative preservation of dentate granule cells and a small zone of pyramidal cells (in the cornu ammonis, field 2, of the hippocampus). The dense gliosis that accompanies the loss of neurons causes shrinkages and hardening of tissue (Falconer and Taylor, 2000). Hippocampal sclerosis has been seen in a wide variety of epileptic conditions, including cryptogenic temporal-lobe epilepsy (Sutula, *et al*., 1985) and epilepsy that follows febrile seizures or other brain insults early in life (Mathern, *et al*., 1995).

### Epileptogenesis

It has been suggested that the selective vulnerability of certain neurons may be a mechanism of epileptogenesis in hippocampal sclerosis. The excitatory interneurons located within the dentate gyrus, which normally activate inhibitory interneurons, appear to be selectively lost (Idem, 1987). Loss of these excitatory feed-back and feed- forward mechanisms that act on dentate granule cells, result in hyperexcitability (Idem, 1991).

Glial cells, although considered to be merely supporting cells in the central nervous system, may also have an important role in epileptogenesis (Delgado-Escueta, 1999). Glial perform key buffering functions that help to maintain the uptake of potassium and glutamate and other aspects of the extracellular milieu of neurons. Disruption of these glial functions could cause neuronal hyperexcitability, since increased levels of

extracellular potassium decreased the threshold for neuronal firing and increased levels of glutamate could increase neuronal activation. It has been shown that astrocytes (a subtype of glia) near the dysplastic region have a profound reduction in inward potassium currents. Thus, a change in the neuronal microenvironment may be another mechanism of epileptogenesis (Bordey, *et al.,* 2001).

It is important to note that, epileptogenesis could as well result due to the side effects of drugs, e.g. β-lactam antibiotics. According to Sonek, *et al*., (2008), a wide range of possible neurotoxic complications such as confusion, disorientation, agitation, myoclonus, convulsions, non-convulsive status epilepticus and coma have been observed after cefepime (4th generation cephalosporin) administration. Convulsion and non-convulsive status epilepticus are the most frequently observed neurological effects. The epileptogenic effect of β-lactam antibiotics is believed to be as result of competitive antagonism with main inhibitory neurotransmitter GABA especially in renal insufficiency. This is because the concentration of cefepime in the spinal fluid is said to rise due to competitive inhibition of active transport from cerebrospinal to blood by accumulation of toxic organic acids, higher BBB permeability and low-serum protein binding.

### Common causes of seizures

The occurrence of seizures may be due to several number of factors, which include: cerebrovascular disease; brain tumor; Alzheimers disease and degenerative diseases; alcohol withdrawal, metabolic disorders such as uremia, hepatic failure, electrolyte abnormalities, hypoglycemia, illicit drug use, trauma, infection, perinatal hypoxia and ischemia; as well as febrile seizures (Lowenstein, 2004).

### Refractory epilepsies

According to Kwan and Brodie (2000), refractory epilepsy is that which optimal doses of all of the antiepileptic drugs indicated for the specific type of epilepsy fail to control seizures. Drug refractoriness may be due to development of antiglutamic acid decarboxylase (GAD) autoimmunity, which may reduce sensitivity to drugs that act on the GABA-ergic system (Peltola, *et al*., 2000). Also, it may be due to enhanced activity of the multi-drug transporters (e.g. p-glycoprotein) that carry antiepileptic drugs from nerve tissue to the vascular compartment (Rizzi*, et al*., 2002).

* + - 1. *P-glycoprotein*

P-glycoprotein is a brain microvascular endothelial cell protein, which possesses several essential pharmacological functions of drug portage and expulsion, and hence, termed as an active drug efflux transporter protein. While P-glycoprotein is involved in protecting the brain from exposure to a variety of pharmacologically active hydrophobic agents, it is an impediment to the treatment of various CNS diseases such as primary brain tumors and epilepsy (Dwibhashyam and Nagappa, 2008).

* + - 1. *Blood-brain barrier*

The BBB is one of the most challenging barriers in body known to protect brain from exogenous substances. The shielding effect of the BBB is further strengthened by the presence of P-glycoprotein in the luminal membrane of the cerebral capillary endothelium. BBB is created by the way the blood vessels in the brain are organized. Brain capillaries are different from capillaries of other parts of the body in that normal brain endothelia have fewer pinocytic vesicles, more mitochondria, and adjacent cells are maintained in close opposition by tight junctions. This protective barrier as well,

restrict the entry to the brain from the periphery of compounds that might be of therapeutic value in the treatment of fatal CNS diseases such as epilepsy, cerebrovascular disease and neurodegerative disorders, and of other pathologies. Thus, delivery of drugs to the brain is a challenging task because of the efficient protective mechanisms (Dwibhashyam and Nagappa, 2008).

### Mechanisms of action of antiepileptic drugs

The primary mechanism of action of the majority of antiepileptic drugs involves blockade of voltage dependent Na+ currents; including their persistent action, which contributes to sustained membrane depolarisations and repetitive firing e.g. phenytoin. Other mechanisms include potentiation of GABA-mediated inhibition and Ca2+ channel blockade e.g. zonisamide. Felbamate and topiramate reduce glutamate-mediated transmission at the postsynaptic level. Antiepileptic drugs that act on Na+ channels also decrease glutamate release at presynaptic terminals (Avanzini and Franceschetti, 2003). Others like retigabine, act via sub-threshold voltage-gated K+ channel (KCN Q2/Q3), thereby causing shifts in the voltage-dependence of channel activation (Rundfelt and Netzer, 2000).

### Neurotransmitters

According to McNamara (2001), synapses are known to mediate interneuronal communication via synaptic neurotransmitters and as such reduction of inhibitory synaptic activity might be expected to trigger occurrence of seizures. Amino acids including glycine as well as gamma amino butyric acid (GABA) are the inhibitory neurotransmitters. On the other hand, glutamate is the principal excitatory neurotransmitter acting through N-methyl-D-aspartate (NMDA), alpha amino-3-

hydroxy-5-methyl isoxazole-4-propionic acid (AMPA) and kainite (KA) receptor subtypes.

### Gamma amino butyric acid (GABA)

GABA is the predominant inhibitory neurotransmitter in the brain, and the expression and function of GABA receptors also are developmentally regulated (Russell and Frances, 2001).

* + - 1. *GABAA receptors*

GABAA receptors mediate post synaptic responses to GABA in central neurons, and are expressed at embryonic stages (Laurie, *et al*., 1992). However, in the first postnatal week, activation of GABAA receptors causes membrane depolarization rather than hyperpolarisation typical of mature GABA-ergic synapses (Swann, *et al*., 1999). This difference is not due to receptor composition, but rather results from maturational changes in the transmembrane chloride ion gradient, as this largely governs the equilibrium potential for GABAA channels (Stanley, *et al*., 1995). Inhibitory (hyperpolarisation) GABAA receptor-mediated potentials gradually appear over the first 3 postnatal weeks (Ben-Ari, *et al*., 1997) and are correlated with the induction of expression of the neuronal K+/Cl- cotransporter, which extrudes Cl- from cells. Thus, although functional GABA receptors are present very early in development, the delayed onset of GABA-ergic inhibition may contribute to the enhanced excitability of immature brain (Rivera, *et al*., 1999).

* + - 1. *GABAB receptors*

The G-protein-coupled GABAB receptors are activated both pre- and postsynaptically, with opposite effects on transmission (Gaiarsa, *et al*., 1995). Postsynaptic GABAB receptors mediate relatively slowly activating and long-lasting membrane hyperpolarisation through the activation of a K+ conductance, whereas the activation of presynaptic GABAB receptors decreases neurotransmitter release through the inhibition of Ca2+ channels (Gaiarsa, *et al*., 1995).

### Glutamate receptors

* + - 1. *Ionotropic glutamate receptors*

These include the NMDA, AMPA, and KA subtypes (Dingledine, *et al*., 1999). AMPA and KA receptors mediate fast excitatory signaling as they exhibit rapid activation and desensitization. NMDA receptors play a more modulatory role, as their activation requires concurrent glutamate binding and membrane depolarization and results in slower and longer-lasting excitation. NMDA-receptor channels are highly permeable to Ca2+; in addition to Na+ and K+ (Aamodi and Constantine-paton, 1999).

* + - 1. *Metabotropic glutamate receptors*

There are at least eight cloned metabotropic glutamate receptors: mGluR1, mGluR2, mGluR3, mGluR4, mGluR5, mGluR6, mGluR7 and mGluR8 (Conn and Pin, 1997). These have been classified into three groups based on sequence homology, coupling to second-messenger systems and pharmacological sensitivities. Group 1 receptors are coupled to phosphoinositide (PI) hydrolysis that leads to Ca2+ mobilization from intracellular stores, whereas Groups 2 and 3 receptors are negatively coupled to adenylyl cyclase (AC) activity. Although the consequences of mGluR activation vary

depending on receptor type, neuronal type, or brain region, some general principles regarding the effects of mGluR activation in relation to seizures have emerged (Wong, *et al*., 1999). For instance, postsynaptic group 1 mGluR activation causes an increase in the intrinsic excitability of principal neurons (particularly in hippocampal CA1 and CA3 subfields), mainly by down modulation of voltage-gated potassium channels (Gerber and Gahwiler, 1994), and therefore, activation of PI-coupled mGluRs is likely to promote seizure activity. Conversely, presynaptic Groups 2 and 3 receptor activation tends to depress excitatory synaptic transmission by inhibiting glutamate release (Glaum and Miller, 1994), and therefore, activation of AC-coupled mGluRs is likely to inhibit seizure activity.

### Serotonin

Serotonin is a chemical derived from the amino acid tryptophan. It plays a key part in a number of reactions in the brain and other tissues. In the brain, serotonin is a neurotransmitter, affecting the activity of nerve cells. Brain concentrations are affected by diet in quite a complex way depending on the ability of tryptophan to cross the barrier between blood and the brain. Generally, carbohydrate-rich diets increase tryptophan levels, accelerating serotonin production. Some protein-rich diets (those containing the amino acids tyrosine, phenylalanine, leucine, isoleucine, and valine) compete with tryptophan to get across the blood-brain barrier, depress tryptophan uptake into the brain, and reduce serotonin levels. Changes in serotonin levels can alter mood: increases have a calming effect, relieving depression, insomnia, and irritability; decreases are associated with wakefulness and greater sensitivity to pain (Anonymous, 2003).

### Non-receptor Regulatory Mechanisms in Epilepsy

* + 1. **Neuromodulators (neuropeptide Y and corticotropin-releasing hormone)**
			1. *Neuropeptide Y* (*NPY*)

This is a neuromodulatory substance that appears to be of central importance in the regulation of neuronal excitability, particularly tuning interneuron discharge propensity (Baraban and Tallent, 2004). NPY is able to decrease synaptic transmission by reducing presynaptic calcium influx and can suppress epileptiform activity via Y2 receptor activation (Avoli-massimo, 2005). According to Tu, *et al*., (2005), tonically released, endogenous NPY may decrease excitability in recurrent mossy fibre projections in a limbic epilepsy model.

* + - 1. *Neuropeptide corticotrophin-releasing hormone (CRH)*

The excitatory neuropeptide CRH, is the most potent epileptogenic peptide, and may play a critical role in the triggering of seizures (Wasterlin and Mazarati, 1997).

### GABA transporters

GABA transporters may be altered in epilepsy as seen in human epileptogenic tissue from MTLE, the research reveals the presence of reduced level of GABA transporters. The K+ stimulated release of GABA is increased and glutamate induced Ca2+ independent release of GABA is decreased in the epileptogenic hippocampus (Avoli, *et al*., 2005).

### Gene expression patterns

Widespread changes of gene expression patterns do occur in human epileptic hippocampus, some of which overlap with changes found in pilocarpine-induced

chronic MTLE condition. These include protein involved in, cell-matrix interactions, cell growth and differenciation, transcriptional regulation and cellular signaling (Becker, *et al*., 2003).

### Gap junction

Gap junctions play an important role in synchronizing neuronal networks under physiological and pathological conditions such as epileptic seizures (Nakase and Naus, 2004). They allow flow of electrical signals and small molecules including dyes, between cells, thus promoting neuronal synchrony. Procedures capable of enhancing or blocking the function of gap junction increase or decrease epileptiform synchronization, respectively (Carlen, *et al*., 2000). It is important to note that, the constituent of gap junctions are proteins called *connexins* (Hormuzdi, *et al*., 2004).

### Voltage-Gated Ionic Channels

Multiple numbers of ion channel mutations are linked to paroxysmal network synchronization and hence, play a role in episodic neurological disorders, especially those where excessive membrane excitability has been implicated e.g. epilepsy (Jeffrey, 2003). These include Na+, K+, and Ca2+ channels.

### Voltage-gated sodium channels (VGSCs)

Under normal physiological conditions, VGSCs are required for excitatory synaptic transmission, therefore over stimulation can result in profound neuronal damage. Antagonists of these channels may confer beneficial neuronal protection as well as antiepileptic properties. The anticonvulsant agent phenytoin and ameltolide served as templates for the design and synthesis of additional derivatives with potential as

antiepileptic agents with sodium channel activity (Nicholas, *et al*., 2002). Other examples are carbamazepine, lamotrigine, topiramate.

### Voltage-gated calcium channels (VGCCs)

VGCCs are key regulators of Ca+ entry into neurons, and are known to control a variety of cellular processes that regulate neuronal excitability. Voltage-gated calcium channels can be divided into two groups; high-voltage activated and low-voltage activated.

High voltage activated controls the release of neurotransmitters such as the excitatory neurotransmitter glutamate, while low voltage activated (T-type) controls membrane potential that lead to low threshold stimulation in thalamic neurons, which may underlie the synchronizing discharges characteristic of epilepsy. These channels have been shown to be blocked by known antiepileptic drugs such as ethosuximide, gabapentin and levetiracetam (Nicholas, *et al*., 2002).

### Voltage-gated potassium channels (VGKCs)

According to Nicholas *et al*., (2002), it is known that, voltage-gated potassium channels indirectly modify presynaptic Ca2+ entry, neurotransmitter release and action potential. Hence, more subtle mechanism of seizure control than voltage-gated calcium channel. Presynaptic voltage-gated potassium regulates the release of both excitatory and inhibitory neurotransmitters throughout the brain. Generally, if VGKCs open; the membrane potential will move toward the potassium equilibrium potential, whereas if close, other ionic conductances active in neurons will drive membrane potential changes, often, as in case of VGSCs and VGCCs, in a depolarizing direction thus, VGKCs openers can be considered as good antiepileptic because they speed the

repolarization of the presynaptic terminals, which will shorten the duration of action potential toward potassium equilibrium potential (EK). Examples of VGKCs opener are Flupirtine and Retigabine.

According to Wickenden (2002), potassium channels play a major role in the control of all aspect of neuronal excitability that need to be exploited for the development of new AEDs, this may serve as an alternate or adjunct therapy for the treatment of drug- resistant or refractory epilepsy. For instance, inward rectifiers, is a class of potassium channels primarily functions to control neuronal excitability. These channels pass current over a hyperpolarized voltage range; this will lead to the maintenance of resting membrane potential, responsiveness to synaptic inputs and neurotransmitter release. Gating of some inward rectifiers is tightly regulated by intracellular ATP levels, providing a link between cellular metabolism and neuronal excitability. Others are activated by G-protein coupled receptors; both are referred to as ATP-sensitive K+ channels and G-protein activated K+ channels respectively.

Another class of VGKCs is KCNQ2 and KCNQ3. They are predominantly expressed in the CNS, and are found both pre- and post-synaptically in brain regions that are known to be important for the control of neuronal network oscillations and synchronization (Cooper, *et al*., 2001).

### Analeptics

According to Burgen and Mitchell (1985) and Anonymous (2001), analeptics are drugs that produce CNS stimulation as their primary action. The common ones are: strychnine, picrotoxin, bicuculin, leptazole and 4- aminopyridine.

### Strychnine

Strychnine is an alkaloid (derived from the nux vomica plant) which acts in the spinal cord by competitively antagonizing the post-synaptic inhibitory action of glycine at motor neurons. Glycine is released from Renshaw cell nerve terminals and from other inhibitory inputs to motor neurons, so this action of strychnine results in the exaggeration of spinal reflex activity and causes tonic spasms of muscles. Neither strychnine nor glycine has any presynaptic actions, and their post-synaptic actions are confined to the spinal cord and brain stem.

### Picrotoxin

Picrotoxin has its convulsant action mainly in the brain, although its effects do occur at cellular level in the spinal cord. It works by selectively blocking the presynaptic inhibitory action of GABA. This inhibitory action of GABA prevents the release of excitatory transmitter from afferent nerve terminals. Hence, results in the release of more excitatory transmitters leading to the observed convulsions.

### Bicuculin

Bicuculin is as potent convulsant as strychnine, it is known to be a fairly specific and reversible antagonist of the inhibitory post-synaptic actions of GABA in the brain and spinal cord, and this effect results in generalized convulsions.

### Leptazole

Leptazole stimulates the brain and, to a less extent, the spinal cord. It does not seem to act by blocking pre- or post- synaptic inhibition nor does it have direct excitatory actions on central neurons. It is known to decrease neuronal recovery time by

shortening refractory time. Also, it appears to interact with binding sites for picrotoxin and to block the enhanced binding of GABA. Perhaps the most important use of leptazole is in producing models for petitmal epilepsy in animals for screening of potential new antiepileptic drugs.

### 4-aminopyridine

This chemical agent is an antagonist of d- tubocurarine, ketamine-diazepam anaesthesia and neurolepts. It crosses the blood-brain-barrier and stimulates the CNS as well as stimulate the respiratory centre and produce an increased respiratory gas exchange. According to Yamaguchi and Rogawski (1992), 4-aminopyridine causes the inducement of seizure by enhancing spontaneous and evoked neurotransmitter release. The agent is highly lethal and induces clonic-tonic convulsions. Both excitatory and inhibitory synaptic transmission is facilitated by 4-aminopyridine, the epileptiform activity induced by the drug is predominantly mediated by non-NMDA type excitatory amino acid receptors.

### Hypnotics and Sedatives

Sedative hypnotic drugs (such as benzodiazepines barbiturates, paraldehyde, chloral hydrate) are known to depress the CNS in a relatively non-selective and dose-dependent manner; producing progressively calming or drowsiness (sedation), sleep (hypnosis), unconsciouness, coma, surgical anaesthesia and fatal depression of respiration and cardiovascular regulations (Theodore, 1990).

Hypnotic drug is known to produce drowsiness and facilitate the onset and maintenance of sleep state that resembles natural sleep in the EEG characteristic and from which the

recipient can be aroused easily**.** On the other hand, a sedative drug decreases activity, moderates excitement and calms the recipient. The pharmacological properties of sedative-hypnotic drugs are quite similar to those of general anaesthetics. For instance, when large doses are used as a suicide attempt or when a barbiturate such as thiopental is used to induce general anaesthesia, the difference between a sedative-hypnotic and general anaesthetic disappears. These drugs act via different mechanisms to induce depressant action on the CNS. However, behavioural and electrophysiological studies supports the predominant view that most, if not all, the actions of sedative-hypnotic drugs occur as a result of neuronal inhibition that is mediated by GABA (Chindo, 1999).

According to Tobler, *et al*., (2001), ligands acting at the benzodiazepines (BDZ) site of GABAA receptors currently are the most widely used hypnotics. Also, fast synaptic inhibition in the mammalian CNS is largely mediated by activation of GABAA receptors. GABAA receptor function can be enhanced by allosteric modulators, e.g., benzodiazepines, barbiturates and neurosteroids. This enhancement of neuronal inhibition by GABA is one of the most powerful therapeutic strategies for treatment of CNS diseases such as sleep disturbances, anxiety disorders, muscle spasms, and seizure disorders. Classical BDZ like diazepam bind to GABAA receptors that contain the α subunits called α1, α2, α3 or α5 GABAA receptors. GABAA receptors containing the α4 or α6 subunits are insensitive to diazepam. The α1 GABAA receptors represent about 60% of all diazepam-sensitive GABAA receptors in the brain and are found mainly in the cerebral and cerebellar cortex, thalamus and pallidum. It is important to note that, α1 GABAA mediate the sedative (reduction of motor activity) and amnestic actions of

diazepam, whereas the anxiolytic, muscle relaxant, motor impairing, and ethanol potentiating effects are mediated by GABAA receptors other than α1.

BDZ hypnotics have distinct effects both on sleep and the sleep electroencephalogram (EEG). They induce dose-dependent increases of non-rapid eye movement (NREM) sleep, a reduction of REM sleep in humans. These effects are common for agonists acting at the BDZ site, irrespective of whether they are BDZ or non-BDZ compounds such as zolpidem and zopiclone (Tobler, *et al*., 2001).

The fact that BDZ attracted much therapeutic optimism due to their efficacy and tolerability (as hypnotic and anxiolytic), there is still a great concern on their ability to cause cognitive and motor impairement, dependence and abuse. This limits the clinical use of BDZ to 2 to 4 weeks only for the treatment of severe and disabling anxiety and insomnia. Thus, their long term use should as much as possible be avoided (Haw and Stubbs, 2007).

### Role of EEG in sleep

According to Lancel (1993), quantitative EEG analysis has been used to study the regulation of sleep-wake behavior. It has been shown that cortical EEG recorded during non-REM sleep (NREMS) is characterized by the occurrence of spindles and high voltage, slow waves (0.5-4.0 Hz). Furthermore, slow-wave activity (SWA) is low at the beginning of a NREM episode and it rises in the course of a NREM episode. The rise rate and the maximal level of SWA are a monotonic function of the duration of prior wakefulness. During REMS, cortical EEG typically exists of low-voltage, mixed frequencies and, in some animals, a prominent theta rhythm is superimposed. The EEG

activity during wakefulness depends considerably on the behavioral state, on the electrode location and on the species. On average, cortical EEG within wakefulness consists of low-voltage and mixed frequencies. The few studies done on subcortical EEG clearly show that the electrical activity differs highly between brain regions and between species. However, recent studies conducted in humans where a spectral analysis of subcortical EEG was made, showed that, the changes occurring in subcortical EEG associated with changes in sleep-wake behavior parallel the general characteristics of cortical EEG described above.

## CHAPTER THREE

## MATERIALS AND METHODS

### Materials, Equipments, Chemicals and Animals

### Materials and equipments

* Beaker
* Sample bottles
* Electroconvulsive machine (Ugo Basile, model no. 7801)
* Evaporation disc
* *Ficus vallis* stem bark
* Filter paper
* Flat ruler (80 × 3 cm)
* Funnel
* Metler balance (P162 Gallen Kamp, UK)
* Mortar and Pestle
* Plastic animal cages
* Spatula
* Stop watch
* Syringes (10, 5, 2 and 1 mls)
* Water bath
* Weighing balance (Ohio, New York, USA)
* Wooden beam rod (8 mm × 60 cm)
* Wooden hole board (40 × 40 cm)

### Chemicals

* 4-aminopyridine (Sigma Chemical Company, Louis Mo, USA)
* Diazepam (Roche Product Ltd. Welnyn Garden City)
* Pentylenetetrazole (Sigma Chemical Company, Louis Mo, USA)
* Phenobarbitone (Sigma Chemical Company, Louis Mo, USA)
* Phenytoin sodium (Sigma Chemical Company, Louis Mo, USA)
* Sodium chloride (Fisher Scientific Co. USA)

### Animals

* Day-old cockerels
* Adult Swiss albino mice (male and female)

### Preparation of the Plant Extract

### Collection and identification of the plant materials

The stem bark of *Ficus vallis* was collected from Samaru village, Zaria, Northern Nigerian; in the month of july (rainy season). It was taken to Herbarium section of Biological Sciences, Ahmadu Bello University Zaria for identification and authentication. A voucher number was collected (v/no 942) and the specimen deposited in the herbarium. Thereafter, the materials were allowed to dry for 2 weeks in an open air environment.

### Extraction of the stem bark using methanol

The dried stem bark was reduced to fine powder using mortar and pestle. The ground powder was then extracted by a successive cold maceration method using methanol as

an extraction solvent. The methanol extract was evaporated to dryness at a temperature of about 400C to get a concentrated extract.

### Study Animals

### Species

Two species of animals were employed in these studies, namely, mouse and chick. Young adult Swiss albino mice of both sexes with body weight range of 18 to 24 g bred in the Animal House, Department of Pharmacology and Clinical Pharmacy ABU Zaria, were used. Day old cockerels weighing 24 to 35 g were obtained from the National Agricultural Production and Research Institute (NAPRI) hatchery, ABU Zaria. These were used for experimental purposes between Days 2 and 3.

### Quarantine and acclimatization

The animals were isolated and kept for at least 1 week for mice and 1- 2 days for chicks in the experimental room for acclimatization prior to experimental use. Within this time the animals were closely monitored and observed for feeding and water intake, body weight changes and signs of ill health, so as to ascertain their health conditions. Only animals adjudged to be disease-free were used in experiments.

### Maintenance conditions

The acclimatized animals were maintained at a relative humidity and temperature of about 250C; on a 12/12 h light-dark cycle.

* + - 1. *Animal cages*

Plastic bodied cages with stainless steel wire mesh covers, floored with wood shavings to absorb urine, faecal matter and spilled water were used.

* + - 1. *Feeds*

Standard formulated feed was constantly available for each animal species.

* + - 1. *Water source*

Portable water supply was given using plastic water bottles, also *ad libitum.*

* + - 1. *Cleaning*

The experimental rooms were washed and cleaned to avoid dirt. The feed and water containers were washed regularly and beddings were replaced periodically.

### Experimental groupings

In grouping the animal, age, body weight and sex (in the case of mice) were taken into consideration. This was done to achieve approximately uniform condition among the groups and thereby minimize biological variations.

### Identification of animal and cages

Unique numbers were placed on individual animals using picric acid applied on cotton bud. Cages were labelled to indicate study number, group number, and dose levels for purposes of easy and accurate identification.

### Preliminary Studies

### Phytochemical screening

The methanol extract was subjected to phytochemical screening to test the presence or absence of alkaloids, saponins, flavonoids, tannins, glycosides and anthraquinones

according to standard procedures as outlined by Sofowora (1993); Trease and Evans (1996).

* + - 1. *Test for saponins*
1. General: 5 ml of the crude drug extract was vigorously shaken for two minutes with 10 ml of distilled water in the test tube; frothing occurred on addition of olive oil as an emulsion formed indicating the presence of saponins.
2. Saponin glycosides: To 2.5 ml of extract, 2.5 ml of Fehling‟s solution A and B were added. A bluish green precipitate shows the presence of saponin glycosides.
	* + 1. *Test for glycosides*
3. General: 2.5 ml of sulphuric acid was added to 5 ml of the extract in a test tube and boiled for fifteen minutes, cooled and neutralize with 10% sodium hydroxide. 5 ml of Fehling‟s solutions A and B were then added. A brick red precipitate of reducing sugar indicates presence of glycosides.
4. Digitalis glycosides: 2 ml of glacial acetic acid containing traces of ferric chloride was added to 1 ml of the extract. This was then poured over 2 ml of concentrated sulphuric acid. Formation of blue layer shows the presence of digitalis glycosides.
	* + 1. *Test for tannins*

Four (4) ml of water was added to the small portion of extract, followed by four drops of ferric chloride. An immediate green precipitate was formed as indicator of tannins. 4

ml of the extract was shaken again with 4 ml of 10% ammonia solution. Formation of an emulsion on shaking shows the presence of hydrolysable tannins.

* + - 1. *Test for flavonoids*

To 1 ml of the extract was added a small quantity of magnesium chips and drops of concentrated hydrochloric acid down the side of the tube. A reddish coloration indicates the presence of flavonoids.

* + - 1. *Test for alkaloids*

About 0.5 g of the extract was mixed with 10 ml of 1% aqueous hydrochloric acid on a water bath. 1 ml portions of the filtrate were treated with a few drops of the following reagents; Dragendorff‟s reagent (potassium bismuth iodide solution) and Wagner‟s reagent (solution of iodine in potassium iodine). Deep brown-colored precipitate and turbidity with Dragendoff‟s and Wagner‟s reagents was indicative of the presence of alkaloid.

* + - 1. *Test for anthraquinones (Borntrager’s test)*

Half (0.5) g of the crude extract was taken in a dry test tube. 10 ml of chloroform was added and the mixture shaken for 5 minutes. The mixture was filtered and an equal volume of ammonia solution was added to the filtrate and shaken. A bright pink color in the upper aqueous layer indicated the presence of free anthraquinones.

### Preparation and administration of drugs

To ensure stability of the preparations, freshly prepared drug solutions were used. Calculation of volumes to be administered was done based on the stock concentration of each solution, doses required and the body weights of the experimental animals. The frequency of drug administration followed was as prescribed by each specific method.

### Acute toxicity studies

The Lorke method (1983) was adopted using thirteen albino mice, and chicks; and involving two routes of administration (oral and intraperitoneal). The method was biphasic; the first phase utilized three groups of three animals each, which were administered with 10 mg/kg, 100 mg/kg and 1,000 mg/kg of the extract to ascertain the range of toxicity. The animals were observed for signs of toxicity for 24 hours. Depending on the outcome of first phase, other specific doses were administered to four different albino mice and chicks, and observed again for signs of toxicity for another 24 hours. From the outcome of this phase, LD50 value was determined by calculating the geometric mean of the doses for which 0/1 (lethal) and 1/1 (non lethal) were found i.e. using the lowest dose among all the doses used that killed the animal, and highest dose among all the doses used that did not kill; as shown by the formula below:

LD50= G.M. = √ XY

LD50= Median lethal dose

G.M. = Geometric mean X= Lowest lethal dose (0/1)

Y= Highest non lethal dose (1/1)

### Anticonvulsant Studies

### General study design

The studies were made using five groups, each containing twelve or six animals. Groups 2, 3 and 4 received graded doses of extract. Groups 1 and 5 served as negative and positive controls using normal saline and a standard drug respectively. Each administered agent was studied and recorded.

### Pentylenetetrazole-induced seizure test

The method of Swinyard (1969) was adopted. Sixty adult albino mice weighing 18 -24g were divided into five groups of twelve mice each. Mice in Group 1 were treated with normal saline (20 ml/kg i.p.) and Group 5 received phenobarbitone (30 mg/kg i.p.). Mice in Groups 2-4 were given three graded doses of the extract (20, 40 and 80 mg/kg). Thirty minutes later 85 mg/kg of freshly prepared solution of pentylenetetrazole was administered s.c. to each mouse. The mice were observed for presence or absence of threshold seizure, and episodes of clonic seizure of at least 5 second duration and death.

### Maximal electroshock test

* + - 1. *Maximal electroshock-induced seizures in mice*

The method of Swinyard and Kupferberg (1985) was adopted. Thirty mice were divided into five groups of six mice each. Mice in Group 1 were given normal saline (20 ml/kg i.p.) and Group 5 received phenytoin (20 mg/kg i.p.). Groups 2, 3 and 4 received graded doses of the extract i.p. Thirty minutes later, maximal electroshock was delivered to each mouse to induce seizure using an Ugobasile electro-convulsive machine (Model No. 7801) connected with corneal electrodes. The parameters used were 60 mA (current), 100 Hz (frequency), 0.2 s (shock duration) and 0.4 ms (pulse width) as

previously defined by Swinyard and Kupferberg (1985). Episodes of tonic extension of the hind limbs were regarded as full convulsion while lack of tonic extension of the hind limbs was considered as protection.

* + - 1. *Maximal electroshock-induced seizures in chicks*

The method of Swinyard and Kupferberg (1985) was adopted. Sixty chicks were divided into five groups of twelve chicks each. Chicks in Group 1 were given normal saline (20 ml/kg i.p.) and Group 5 received phenytoin (20 mg/kg i.p.). Groups 2, 3 and 4 received graded doses of the extract i.p. Thirty minutes later, maximal electroshock was delivered to each chick to induce seizure using an Ugobasile electro-convulsive machine (Model No. 7801) connected with corneal electrodes. The parameters used were 80 mA (current), 150 Hz (frequency), 0.6 s (shock duration) and 0.8 ms (pulse width) as previously defined by Swinyard and Kupferberg (1985). Episodes of tonic extension of the hind limbs were regarded as full convulsion while lack of tonic extension of the hind limbs was considered as protection.

### 4-aminopyridine-induced seizure test

The study was done according to method described by Yamaguchi and Rogawski (1992). Sixty mice of either sex were divided into five groups of twelve mice each. Group 1 was treated with normal saline (20 ml/kg i.p.) and served as control. Groups 2, 3 and 4 received 20, 40 and 80 mg/kg i.p. of the extract respectively, while Group 5 received phenobarbital (30 mg/kg). All mice then received 4-aminopyridine at a dose of 15 mg/kg s.c. 30 minutes after pretreatment. The mice were observed for presence or absence of hind limb tonic extension and onset of episodes of convulsion as well as death were recorded.

### Other Behavioral Studies

### Diazepam-induced sleeping time in mice

Mice of either sex were divided into four groups of six mice each. Animal in group 1 received normal saline (20 ml/kg) and served as control, while those in Groups 2, 3 and 4 received the extract doses of 20, 40 and 80 mg/kg (i.p.) respectively. Thirty minutes after treatment, all animals were given diazepam (30 mg/kg i.p.). Each mouse was observed for the onset and duration of sleep, with the criterion of sleep being loss of righting reflex (Wambebe, 1985; Amos *et al*., 2001b). The time from the loss of righting reflex to recovery was recorded as sleeping time (Soulimani *et al*., 2001).

### Exploratory behavior in mice

The study was done using the head-dip test on the hole-board (Ramirez *et al*., 1998). It was carried out using wooden board (40 × 40 cm) with four equidistant holes (1 cm diameter, 2 cm depth). Mice of either sex were divided into five groups of six mice each. Animals in Group 1 received normal saline (20 ml/kg i.p.) and served as control, while those in Groups 2, 3 and 4 received the extract at doses of 20, 40 and 80 mg/kg

i.p. respectively. The animals in Group 5 received diazepam (2 mg/kg i.p.). Thirty minutes after treatment, each mouse was placed at one corner of the board and allowed to move about and dipped its head into the holes indicating exploratory behaviour. The number of times the mice dipped their heads into the holes during the 5-minutes period was counted and recorded.

### Beam walking test for motor coordination deficits in mice

The study was done according to method described by Stanley *et al*., (2005). Mice were trained to travel from a start platform along a ruler (80 cm long, 3 cm wide) elevated 30 cm above the bench by metal supports to a metal box. Trials were performed for each mouse, and were designed such that the mice tested would be aware that there was a box that could be reached. Mice of either sex were divided into five groups of six mice each. Group 1 received normal saline (20 ml/kg i.p.) and served as control, while Groups 2, 3 and 4 received 20, 40 and 80 mg/kg i.p. doses of extract, respectively. Group 5 received diazepam at a dose of 2 mg/kg i.p. Thirty minutes after, each mouse was placed at one end of wooden beam (8 mm in diameter, 60 cm long and elevated 30 cm above the bench by metal supports), and allowed to walk to the box within a maximum of 60 s. The time taken on the beam, number of falls and the number of foot slips were counted and recorded.

### Statistical Analysis

Results were presented in tables and expressed as mean ± SEM. The level of significance between means was tested by the Students t-test and results were regarded as statistically significant from P<0.05. Also, the level of significance between control, test and standard groups were tested by the Chi-square test (wherever indicated), and results were regarded as statistically significant from P<0.05.

## CHAPTER FOUR

## RESULTS

### Preliminary Phytochemical Screening

The phytochemical screening of methanolic extract of *F. vallis* revealed the presence of alkaloids, tannins, glycosides, flavonoids and saponins. On the other hand, anthraquinones were found to be absent. These constituents may be responsible for the plant biological activities observed. (Table 4.1).

### Table 4.1: Result of Preliminary Phytochemical Screening of Methanol Extract of

***F. vallis***

Chemical Constituents Inference

Alkaloids +

Glycosides +

Tannins +

Flavonoids +

Saponins +

Anthraquinones -

Key= +: Present and -: Absent

### Pharmacological Studies

* + 1. **Results of acute toxicity studies**

The median lethal dose (LD50) of the *F. vallis e*xtract in mice was found to be 471.2 mg/kg intraperitoneally and above 5,000 mg/kg orally. In chicks, the LD 50 values were

774.6 mg/kg via intraperitoneal route and above 5,000 mg/kg orally (Table 4.2).

### Table 4.2: LD50 Values of *F. vallis* via Intraperitoneal and Oral Routes of Administration

**Species Routes of Administrations LD50 Values (mg/kg)**

Mice Intraperitoneal 471.2 (Moderately toxic)

Mice Oral **>**5,000 (Non-toxic)

Chicks Intraperitoneal 774.6 (Slightly toxic

Chicks Oral **>** 5,000 (Non-toxic)

### : Effect of Methanolic Extract of *F. vallis* (FV) and Phenobarbital (PHB) on Pentylenetetrazole (PTZ)-induced Seizures in Mice

The extract of *F. vallis* produced biphasic activities where 40 mg/kg has highest

protection (50%) against seizure induced by PTZ, while 80 mg/kg and 20 mg/kg offered

25 and 33.3% respectively as compared to standard antiepileptic drug used.

Phenobarbital (30 mg/kg) produced 100% protection against seizure and mortality as well. There was statistically significant difference in the mean onset of seizure between the extract (40 mg/kg and 80 mg/kg) and the control groups at p<0.005 and p<0.05 respectively (Table 4.3)

### Table 4.3: Effect of Methanol Extract of *F. vallis* (FV) and Phenobarbital (PHB) on Pentylenetetrazole (PTZ)-induced Seizures in Mice

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Treatment(mg/kg) | Mean Onsetof Seizures (min) | QuantalProtection | Protection againstSeizures (%) | Mortality rate(%) |
| N/saline | 6.18 ± 0.81 | 1/12 | 8.3 | 8.3 |
| FV 20 | 7.63 ± 0.96 | 4/12 | 33.3 | 25.0 |
| FV 40 | 13.0 ± 2.49\*\* | 6/12 | 50.0 | 8.3 |
| FV 80 | 9.56 ± 0.60\* | 3/12 | 25.0 | 25.0 |
| PHB 30 | 0.0 | 12/12a | 100.0 | 0.0 |
| Values are | presented as Mean ± | SEM, n = | 12 per group, FV= *Ficus* | *vallis, PHB* = |

phenobarbital, Significant difference between control (Saline) group at \*p<0.05 and\*\*p<0.005 (Student t-test); Significance difference between control (saline) and standard (PHB) at ap<0.001 (Chi-square test)

### Effect of Methanolic Extract of *F. vallis* (FV) and Phenytoin (PH) on

**Maximal Electroshock Test-induced seizures in Mice**

The extract of *F. vallis* did not exhibit protection against MEST-induced seizure in mice at the tested doses, while phenytoin (20 mg/kg) showed 100% protection against seizures as well as in the mortality. Mortality was observed in all the extract treated and control groups. There was no significant difference observed in the mean onset of seizures and mean recovery time of seizures between the extract treated and the control groups (Table 4.4).

### TABLE 4.4: Effect of Methanol Extract of F. vallis (FV) and Phenytoin (PH) on Maximal Electroshock Test (MEST)-induced Seizures in Mice

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatment (mg/kg) | Mean Onset of Seizures (s) | Mean Recovery from Seizures (min) | Quantal Protection | Protection against Seizures (%) | Mortality Rate (%) |
| N/saline | 2.50 ± 0.18 | 3.00 ± 0.18 | 0/6 | 0.00 | 66.7 |
| FV 20 | 2.13 ± 0.22 | 1.67 ± 0.52 | 0/6 | 0.00 | 50.0 |
| FV 40 | 2.17 ± 0.13 | 2.00 ± 0.45 | 0/6 | 0.00 | 50.0 |
| FV 80 | 2.35 ± 0.15 | 0.00 | 0/6 | 0.00 | 100.0 |
| PH 20 | 0.00 | 0.00 | 6/6a | 100 | 0.0b |

Values are presented as Mean ± SEM, n = 6 per group, FV = Ficus vallis, PH = Phenytoin, Significant difference between control (saline) and standard (PH) at ap<0.001 and bp<0.05 (Chi square test).

### Effect of Methanolic Extract of *F. vallis* (FV) and Phenytoin (PH) on Maximal Electroshock Test-induced seizures in Chicks

The extract of *F. vallis* exhibited 25% protection against MEST-induced seizure at the

highest dose (80 mg/kg) used, while phenytoin (20 mg/kg) showed 100% protection. No mortality was observed in all the groups. There was no significant difference observed in the mean recovery time of seizure between the extract treated and the control groups (Table 4.5).

### Table 4.5: Effect of Methanol Extract of *F. vallis* (FV) and Phenytoin (PH) on Maximal Electroshock Test (MEST)-induced Seizures in Chicks

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Treatment (mg/Kg) | MeanRecovery Time (min) | Quantal Protection | Protectionagainst seizures (%) | Mortality Rate (%) |
| N/saline | 6.13 ± 0.83 | 0/12 | 0.0 | 0.0 |
| FV 20 | 6.91 ± 0.93 | 1/12 | 8.3 | 0.0 |
| FV 40 | 7.64 ± 1.54 | 1/12 | 8.3 | 0.0 |
| FV 80 | 7.44 ± 2.73 | 3/12 | 25 | 0.0 |
| PH 20 | 0.0 | 12/12a | 100 | 0.0 |

Values are presented as Mean ± SEM, n = 12 per group, FV = *Ficus vallis*, PH = pheny- toin, Significant difference between control (saline) and standard (PH) at aP<0.001 (Chi-square test).

### Effect of Methanolic Extract of *F. vallis* (FV) and Phenobarbital (PHB) on 4- aminopyridine-induced Seizures in Mice

The extract of *F. vallis* produced a significant increase in the latency of seizure in 4-

aminopyridine-induced seizure at 20 mg/kg (p<0.001), while at 40 and 80 mg/kg was at p<0.05 compared to control group. The phenobarbital (30 mg/kg) gave 100% protection against the seizure (Table 4.6)

### Table 4.6: Effect of Methanol Extract of *Ficus vallis* (FV) and Phenobarbital (PHB) on 4-aminopyridine-induced Seizures in Mice

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Treatment (mg/kg) | Mean Onset of Seizures (min) | Quantal Protection | Protection againstSeizures (%) | Mortality Rate (%) |
| N/saline | 9.75 ± 1.30 | 0/12 | 0.0 | 100 |
| FV 20 | 16.33 ± 0.80\*\* | 2/12 | 16.7 | 83.3 |
| FV 40 | 14.00 ± 1.53\* | 2/12 | 16.7 | 83.3 |
| FV 80 | 14.58 ± 1.64\* | 0/12 | 0.0 | 100 |
| PHB 30 | 0.00 | 12/12a | 100 | 0.0a |

Values are presented as Mean ± SEM, n = 12 per group, FV = *Ficus vallis,* PHB = Phenobarbital, Significant difference between control (saline) group at \*p<0.05 and

\*\*p<0.001 (Student t test); significance difference between control (saline) and standard (PHB) at ap<0.001 (Chi-square test).

### Effect of Methanol Extract of *F. vallis* (FV) on Diazepam-induced Sleep in Mice

The extract of *F. vallis* produced a significant decrease in the mean onset of sleep in the

diazepam induced sleep at 20 mg/kg (p<0.05), while 40 and 80 mg/kg were at p<0.005 as compared to control group. Similarly, there was significant increase in the mean duration of sleep between the extract treated with 20 and 80 mg/kg (p<0.005) and 40 mg/kg (p<0.05) compared to control group (Table 4.7).

### Table 4.7: Effect of Methanolic Extract of *Ficus vallis* (FV) on Diazepam-induced

|  |  |
| --- | --- |
| **Sleep in Mice** |  |
| Treatment | Mean Onset | Mean Duration | Number of Animals |
| (mg/kg) | of sleep (min) | of Sleep (min) | Slept (out of 6) |
| N/saline | 4.50 ± 0.50 | 107.00 ± 33.00 | 6/6 |
| FV 20 | 2.67 ± 0.21\* | 221.00 ± 18.96\*\* | 6/6 |
| FV 40 | 2.50 ± 0.22\*\* | 224.00 ± 35.29\* | 6/6 |
| FV 80 | 2.17 ± 0.18\*\* | 278.17 ± 30.93\*\* | 6/6 |

Values are presented as Mean ± SEM, n = 6 per group, FV = *Ficus vallis,* Significant difference between control (Saline) group at \*p<0.05, \*\*p<0.005 (Student t-test).

### Effect of methanol Extract of *F. vallis* (FV) and Diazepam (DZ) on Exploratory Behaviour in Mice

The extract of *F. vallis* and diazepam (2 mg/kg) (the standard drug used) significantly

decreased the number of head dips in exploratory behaviour test in mice as compared to control (normal saline) group at p<0.001 (Table 4.8).

### Table 4.8: Effect of Methanol Extract of *Ficus vallis* (FV) and Diazepam (DZ) on Exploratory Behaviour in Mice

Treatment (mg/kg) Number of Head Dips

N/saline 17.50 ± 1.09

FV 20 6.50 ± 0.99\*

FV 40 7.50 ± 1.38\*

FV 80 4.00 ± 0.68\*

DZ 2 9.67 ± 1.05\*

Values are presented as Mean ± SEM, n = 6 per group, FV = *Ficus vallis*, DZ = diazepam, Significant difference between control (Saline) group at \*p<0.001 (Student t- test).

### Effect of Methanolic Extract of *F. vallis* (FV) and Diazepam (DZ) on Motor Coordination Deficit in Mice

The extract of *F. vallis* and diazepam (2 mg/kg) significantly increased the number of

hind limb slips in motor coordination test in mice where at 20 mg/kg of the extract and diazepam was p<0.001 and 20 and 80 mg/kg of the extract was at p<0.005 compared to control (normal saline) group. There was no statistical significant difference in the time taken to complete the task between the extract treated, diazepam and normal saline groups. Similarly, none of the animal felt down from the walking beam in all the groups (Table 4.9).

### Table 4.9: Effect of Methanolic Extract of *Ficus vallis* (FV) and Diazepam (DZ) on Motor Coordination Deficit in Mice

|  |  |  |  |
| --- | --- | --- | --- |
| Treatment (mg/kg) | Number of Hind Limb Slips | Time taken for the Task (s) | Number of Falls |
| N/saline | 0.00 | 10.00 ± 1.59 | 0/6 |
| FV 20 | 2.00 ± 0.37\*\* | 11.33 ± 1.26 | 0/6 |
| FV 40 | 2.00 ± 0.52\* | 12.17 ± 1.62 | 0/6 |
| FV 80 | 2.83 ± 0.65\* | 20.50 ± 5.71 | 0/6 |
| DZ 2 | 11.00 ± 1.21\*\* | 19.50 ± 4.64 | 0/6 |

Values are presented as Mean ± SEM, n = 6 per group, FV = *Ficus vallis*, DZ = Diazepam, Significant difference between control (saline) group at \*p<0.005,

\*\*p<0.001 (Student t-test).

## CHAPTER FIVE

### Discussion

The methanol extract of *F. vallis* revealed the presence of saponins, tannins, alkaloids, glycosides and flavonoids. The presence of glycosides and tannins in this plant was in compliance with an already documented literature that, plants from moraceae family are known to contain these compounds (Evans, 1996). The activity shown by the extract may be due to the presence of these secondary metabolites. For instance, saponins are known to have sedative property in experimental animals (Wagner *et al*., 1983). Alkaloid (nantenine derivatives) was found to be effective in inhibiting pentylenetetrazole- and maximal electroshock-induced seizures (Ribeiro, 2003). This showed that the effects observed may be due to the presence of these constituents.

The LD50 values of the extract in mice and chicks via oral route were above 5,000 mg/kg each. However, the values obtained for both mice and chicks via i.p. route were

471.2 mg/kg and 774.6 mg/kg respectively. The result showed that route of administration is a key factor in determining toxicity; as oral route was safer than the parenteral route, and species differences as well play a critical role in assessing toxicity of compounds.

Matsumura (1975) and Corbett *et al* (1970) had classified chemicals according to their oral LD50 values as follows:

* Extremely toxic LD50 ≤ 1 mg/kg
* Highly toxic LD50 = 1-50 mg/kg
* Moderately toxic LD50 50-500 mg/kg
* Slightly toxic LD50 0.5-5 g/kg
* Practically non toxic LD50 5-15 g/kg
* Relatively harmless LD50 > 15 g/kg

According to the above classification of oral LD50 of chemicals, the extract can be said to be practically non-toxic orally. However, through i.p. route it was moderately toxic in mice and slightly toxic in chicks.

The methanol extract of *F. vallis* afforded 33.3%, 50.0% and 25.0% protections against pentylenetetrazole-induced convulsion at doses of 20 mg/kg, 40 mg/kg and 80 mg/kg respectively. The extract also significantly delayed the onset of convulsion at doses of 40 mg/kg and 80 mg/kg. Phenobarbital afforded 100% protection against seizures as well as protected the mice against mortality. However, there was 25% mortality rate at doses that gave lowest protection as opposed to the less mortality found in normal saline treated group. This could probably be that some constituents at 20 mg/kg and 40 mg/kg were synergistic to the lethal effect of pentylenetetrazole, and hence, gave lower protection and higher mortality. On the other hand, the intermediate dose used (40 mg/kg) afforded higher protection both against seizures and mortality. Studies have shown that pentylenetetrazole induces seizures by blocking the major inhibitory pathways mediated by the predominant inhibitory neurotransmitter GABA, at all levels of the CNS (DeSarro et al., 1999). It has also been shown that seizures induced by pentylenetetrazole, can be blocked by drugs such as ethosuximide that reduces T-type Ca2+ currents (Rho and Saukar, 1999), and standard drugs such as diazepam and phenobarbital are thought to produce their effects by enhancing GABA-mediated inhibition in the brain (Rogawski and Porter, 1990). It has been reported that dopamine reduces the threshold of pentylenetetrazole convulsions in mice and pimozide (specific

dopamine receptor antagonist) protected experimental animals against pentylenetetrazole-induced seizures (Amabeoku, 1989). In addition, activation of N- methyl-D-aspartate (NMDA) receptor system appears to be involved in the initiation and propagation of pentylenetetrazole-induced seizures (Velisek *et al*., 1990). Drugs such as felbamate that block glutamatergic excitation mediated by NMDA receptors have shown anticonvulsant activity against pentylenetetrazole-induced seizures (White, 1997). It is therefore possible that the anticonvulsant effects shown by the crude extract of *Ficus vallis* against seizures produced by pentyletetrazole might be due to activation of GABA neurotransmissions, blockade of glutamatergic neurotransmission mediated by NMDA receptors, or blockade of the dopaminergic receptor system in the CNS. Anticonvulsant activity in scPTZ test identifies compounds that can raise the seizure threshold in the brain (White *et al*., 1998). Antiepileptic drugs effective in the therapy of generalized seizures of petitmal (absence or myoclonic) type such as phenobarbital, and benzodiazepines, are capable of raising seizure threshold induced by pentylenetetrazole (Loscher *et al*., 1991). Hence, the extract contains active compounds that may be useful in the management of absence or myoclonic seizures.

The Methanol extract of *F. vallis* did not show a significant activity against MEST in both mice and chicks; on the onset of seizures, recovery time, protection from electroshock as well in the mortality, while 100% protection against both the occurrence of seizure and mortality was achieved with the phenytoin. Mortality was observed in mice at all the extract treated groups as well as the control (saline) group. Highest mortality was recorded at the highest dose of 80 mg/kg, this could be associated to an increase in concentration of some constituents present in the crude extract which might be lethal in nature. The lethality could as well be synergistic to the electroshock and

resulted to death. Protection against hind limb tonic extension (HLTE) in the MEST predicts anticonvulsant activity of antiepileptic drugs that prevent the spread of seizure discharge from an epileptic focus during seizure activity (e.g phenytoin, carbamazepine, oxcarbazepine, and lamotrigine) (Browning, 1992). Thus, indicate the ability of the antiepileptic agent to serve in the treatment of generalized tonic-clonic and partial seizures (Raza *et al*., 2001). It has been suggested that, the inducement of seizures by electroshock machine is through inhibitory current breakdown and voltage-dependent sodium channels in these electrically induced stimuli (McNamara, 1996). Antiepileptic drugs that act via this pathway are able to limit the repetitive firing of action potentials by slowing the rate of recovery of voltage-activated sodium ions channels from inactivation and suppress hind limb tonic extension in maximal electroshock seizures (Rho and Sankar, 1999) Also, increased levels of noradrenaline, dopamine and serotonin (5-HT), have shown protective action against electro-convulsion in chicks and rats (Osuide and Wambebe, 1979). This showed that the extract did not contain a significant amount of biologically active compounds that may prevent the spread of the epileptic seizure discharge from an epileptic focus and hence, may not used in generalized tonic-clonic and partial seizures.

The extract showed a significant delay in the onset of convulsion in 4 -aminipyridine- induced seizures at all the doses. However, there was no significant protection against the occurrence of seizure, and the mortality rate was 100% at the highest dose (80 mg/kg) used. While the Phenobarbital used as control afforded 100% protection against seizure and mortality as well. 4-aminopyridine induces clonic-tonic convulsions by blocking potassium channels (Yamaguchi and Rogawski, 1992). Potassium channels play a vital role in the control of neuronal excitability and seizure susceptibility, and

would be of important for the suppression of seizure initiation and spread (Wickenden, 2002).

The extract has significantly decreased the onset of sleep, and increased the duration of sleep dose-dependently. This showed that the extract potentiated the diazepam-induced sleep, which may be attributed to an action on the central mechanisms involved in the regulation of sleep (N‟Gouemo *et al*., 1994; Amos *et al*., 2001) or an inhibition of diazepam metabolism (Kaul and Kulkarni, 1978). Sleep is also reduced when there is a decrease in serotonin concentration or destruction of the dorsal raphe nucleus in the brain stem, which contains most of the brain‟s serotonergic cell bodies. On the other hand, increased firing of norepinephrine-containing neurons in the locus ceruleus will result in a reduction of rapid eye movement sleep and increased wakefulness (Curtis and Jermain, 2002). It has also been reported that activation of GABAA receptor in the CNS is known to favour sleep (Gottesmann, 2002). Thus, the extract possessed bioactive compounds that may be used to achieve central depressive action.

The hole-board test is a measure of exploratory behaviour in mice (Crawley, 1985). The extract has significantly decreased the number of head dips by the mice. An agent that decreases this parameter reveals a sedative behaviour (File and Pellow, 1985). Also, agents that diminished the number of head-dips in this test are considered to have propensity for antipsychotic activities (Fielding and Lal, 1978). This suggests that the extract possessed central depressant activity and may have calming effect.

Similarly, the extract significantly increased the number of foot slips in mice; but no statistically significant effect on the time taken to complete the task on the beam; and

none of the animal felt down from the walking beam. Beam walking test assess benzodiazepine-induced ataxia as a predictor of sedative effects by measuring the extent of motor deficits caused by damage to the motor cortex. Out of the three parameters counted, number of foot slips is considered as best in determining the motor deficits. This showed that the extract has ability to induce motor deficits and hence, suggest sedative effect of the extract (Stanley *et al*., 2005).

## CHAPTER SIX

## SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

### Summary

The plant (*Ficus vallis*) is an indigenous plant found in Nigeria, and belongs to large family of moraceae. The result of phytochemical screening of methanol extract of *Ficus vallis* revealed the presence of some key secondary metabolites such as alkaloids, glycosides, tannins, flavonoids and saponins.

LD50 values in mice (via i.p. and p.o.) were found to be 471.2 mg/kg and >5,000 mg/kg respectively, while values found in chicks (via i.p. and p.o.) were 774.6 mg/kg and

>5,000 mg/kg respectively. The outcome of this result indicated that species variation and route of drug administration are important factor in the determination of toxicity profile of compounds.

The methanol extract of *Ficus vallis* gained its anticonvulsant activity in pentylenetetrazole-induced seizures in mice, where it protected a significant number of animals used (up to 50%) against seizures induced by pentylenetetrazole, as well as delayed the onset of seizures significantly. However, the extract did not protect the animals against seizures induced by both maximal electroshock-induced seizure and 4- aminopyridine-induced seizure tests. It is important to note from this experiment that, conventional antiepileptic agents used in clinical settings, were able to protect both the chicks and mice against the occurrence of seizures in all the three convulsive models used.

Moreover, the methanol extract of *Ficus vallis* showed a characteristic central depressant action closely similar to that of diazepam. It statistically decreased the mean onset of sleep and increased the mean duration of sleep in mice as compared to diazepam. Again, the extract possessed other centrally mediated effects such as change in the exploratory behaviour in mice as well as motor coordination deficit in mice; a typical characteristic of benzodiazepines. A statistically significant decrease in the number of head dips and an increase in the number of hind limb slips in mice were seen in the exploratory behaviour and motor coordination deficit tests respectively; for all the graded doses of the extract used.

### Conclusions

Based on the analysed data presented, the use of *Ficus vallis* in traditional medicine in Nigeria and other West African countries is to some extent justified scientifically. The pharmacological activities of the stem bark extract may be correlated with those of clinically useful drugs like benzodiazepines used as anticonvulsants as well as sedatives and muscle relaxants. Also, the crude extract can be said to be relatively safe especially via oral route; and this may justify the frequent use of the plant as food and medicines by local people in West Africa. Therefore, the plant material may be useful in the management of petit mal epilepsy with the tendency of causing sedation. Hence, there is need for further research geared towards isolating the bioactive component responsible for the observed effects through the bioassay guided fractionation.

### Recommendations

1. The plant (*Ficus vallis*) should be recollected from another part of Nigeria (outside Kaduna state), and the experimental protocols be conducted to make comparison on the same pharmacological activity.
2. Another method of extraction technique probably soxhlet method, using combination of two extraction solvents (methanol and water or ethanol and water) should be adopted, and the experimental procedures be repeated. This may enhance getting more concentrated extract with higher yield.
3. It is good to undertake both sub-acute and chronic toxicity studies of the plant extract to further evaluate its toxicity profile, and hence, ascertain its safety level.
4. The extract should be subjected to fractionation using specific extraction solvents (e.g. ethylacetate, n-butanol and chloroform).
5. Drug interaction studies should be conducted between the extract and other related conventional drugs with similar or opposite pharmacological effects, as well as other commonly popular drugs used in the management of common diseases. This may help to ascertain the tendency of the extract to cause synergistic or antagonistic characteristics on other orthodox drugs.

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APPENDIX A

Percentage (%) Yield of Crude Extract of *Ficus vallis*:

Extraction solvent: Methanol

Weight of crude drug extracted: 100 g Yield of methanol crude extract obtained: 6.78 g

% yield of methanol crude extract = 6.78/100 × 100

= 6.78%

APPENDIX B

### LD50 Determination in Mice and Chicks by Intraperitoneal (i.p.) and Oral (p.o.) Routes

**Mice (i.p.)**

1st PHASE

Number of animals Dose (mg/kg) Mortality

3 1,000 3/3 (all died)

3 100 0/3 (none died)

3 10 0/3 (none died)

2nd PHASE

Number of animals Dose (mg/kg) Mortality

1 600 1/1 (died)

1 370 0/1 (no death)

1 225 0/1 (no death)

1 140 0/1 (no death)

LD50 = G.M = √ XY

LD50= Median lethal dose G.M= Geometric mean

X= Lowest lethal dose (0/1) = 600 mg/kg

Y= Highest non lethal dose (1/1) = 370 mg/kg Therefore, LD50= √ 600×370

LD50= 471.2 mg/kg (i.p.) in mice

|  |  |
| --- | --- |
| **Mice (p.o.)**1st PHASE |  |
| Number of animals | Dose (mg/kg) | Mortality |
| 3 | 1,000 | 0/3 (none died) |
| 3 | 100 | 0/3 (none died) |
| 3 | 10 | 0/3 (none died) |

|  |  |
| --- | --- |
| 2nd PHASE |  |
| Number of animals | Dose (mg/kg) | Mortality |
| 1 | 5,000 | 0/1 (no death) |
| 1 | 2,900 | 0/1 (no death) |
| 1 | 1,600 | 0/1 (no death) |

Therfore, LD50> 5,000 mg/kg (p.o.) in mice

|  |  |  |
| --- | --- | --- |
| **Chicks (i.p.)**1st PHASE |  |  |
| Number of animals | Dose (mg/kg) | Mortality |
| 3 | 1,000 | 3/3 (all died) |
| 3 | 100 | 0/3 (none died) |
| 3 | 10 | 0/3 (none died) |
| 2nd PHASE |  |  |
| Number of animals | Dose (mg/kg) | Mortality |
| 1 | 600 | 0/1 (no death) |
| 1 | 370 | 0/1 (no death) |
| 1 | 225 | 0/1 (no death) |
| 1 | 140 | 0/1 (no death) |
|  | LD50 = G.M = √ XY |  |
| LD50= Median lethal doseG.M= Geometric mean |  |  |

X= Lowest lethal dose (0/1) = 1,000 mg/kg Y= Highest non lethal dose (1/1) = 600 mg/kg Therefore, LD50= √ 1,000×600

LD50= 774.6 mg/kg (i.p.) in chicks

|  |  |  |
| --- | --- | --- |
| **Chicks (p.o.)**1st PHASE |  |  |
| Number of animals | Dose (mg/kg) | Mortality |
| 3 | 1,000 | 0/3 (none died) |
| 3 | 100 | 0/3 (none died) |
| 3 | 10 | 0/3 (none died) |

|  |  |
| --- | --- |
| 2nd PHASE |  |
| Number of animals | Dose (mg/kg) | Mortality |
| 1 | 5,000 | 0/1 (no death) |
| 1 | 2,900 | 0/1 (no death) |
| 1 | 1,600 | 0/1 (no death) |

Therfore, LD50> 5,000 mg/kg (p.o.) in chicks