SOME NEUROPHARMACOLOGICAL AND TOXICOLOGICAL STUDIES OF THE ETHANOLIC LEAF EXTRACT OF *CUSSONIA BARTERI* SEEMAN (*ARALIACEAE*) IN LABORATORY ANIMALS

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DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS,

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OCTOBER, 2014

DECLARATION

I declare that the work in this thesis entitled "Some neuropharmacological and toxicological studies of the ethanolic leaf extract of C*ussoniabarteri*seeman (A*raliaceae*) in laboratory animals" has been performed by me in the Department of Pharmacology and Therapeutics under the supervision of Prof. J.A.Anuka and Prof. B.A.Chindo.The information derived from the literature has been duly acknowledged in the text and a list of references provided.No part of this thesis has been previously presented for another degree at this or any other Institution.

MUSA ITOPA YAKUBU October, 2014

Name of Student	**Signature**	**Date**

CERTIFICATION

This thesis entitled "SOME NEUROPHARMACOLOGICAL AND TOXICOLOGICAL STUDIES OF THE ETHANOLIC LEAF EXTRACT OF *CUSSONIA BARTERI* SEEMAN (*ARALIACEAE*) IN LABORATORY ANIMALS" by Musa Itopa YAKUBU meets the regulations governing the award of the degree of Masters of Science of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to the memory of my late father, MallamYakubu Oricha, for his belief in herbal medicines.

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All praises and gratitude are due to almighty Allah, the most Beneficent, the most Merciful and the most Knowledgeable for making this dream a reality.

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ABSTRACT

Cussonia barteri is used in traditional medicinefor the management of convulsion and epilepsy, especially in children in Ghana and Nigeria. Literature survey revealed that the safety and efficacy of such important medicinal plant have not been scientifically validated. In the present study, ethanolic leaf extract of the plant was subjected to toxicological and some neuropharmacological studies in laboratory animals (mice, rats and chicks) to determine its safety and justify its use in traditional medicine for the management of epilepsy. Theacute toxicity study was carried out in mice (*i.p*) and rats (oral) using method of Lorke (1983)and 28 days sub-acute toxicity study was performed in rats. Effects of the extract on body weight, relative organs weight, haematological and biochemical parameters were examined following 28 days of daily oral administration of graded doses of it in rats.The extract was evaluated for anticonvulsant activities using maximal electroshock test in chicks, pentylenetetrazole (PTZ), picrotoxin, strychnine, isoniazid and aminophylline-induced seizure in mice.The sedative and anxiolytic effect of the extract was tested on diazepam-induced sleeping time, hole-board, beam walking assay and open field test in mice. Preliminary phytochemical screening revealed that the extract suggested contains flavonoid, saponin, cardiac glycosides, tannins, steroids/terpenoids and glycosides. The oral median lethal dose (LD_{50}) of the extractwas estimated to be greater than 5,000 mg/kg body weight while the intraperitoneal median lethal dose (LD_{50}) was estimated to be 2,154.1 mg/kg in mice.The extract neither produces significant changes in body weights nor affected the haematological and biochemical parameters, but slightly increased the relative weight of the brain, liver and heart. Histopathological evaluation of the organs showed that

the morphology of kidney, liver and lungs were adversely affected, suggesting that the extract could be toxic to the liver, kidney and lungs.The extract did not protect the chicks against maximal electroshock seizure; neither didit shorten the mean recovery time. Similarly, the extract did not significantly protect against picrotoxin, isoniazid and aminophylline induced seizures in mice. The extract produced 66.67% and 83.33% protection against strychnine and pentylenetetrazole induced seizures respectively at the highest dose (400mg/kg) tested which suggests that the extract may contain compound(s) that may be beneficial in the management of absence or myoclonic seizures. The extract decrease the number of head dips in hole-board test, suggesting its sedative property which was confirmed by the ability of extract to prolonged diazepam sleeping time.The extract did not significantly increase the time spent on the beam but at the highest dose tested significantly increased the number of foot slips, an index of motor coordination deficit. The extract insignificantly decreased number of rearing, Total Square and Central Square crossedin an open field test.

These findings suggest that the ethanolic leaf extract of *Cussonia barteri* contain bioactive compound(s) that possesses anticonvulsant and sedative activities, thus supportingthe ethno-medical use of the plant for the management of epilepsy. However, the ethanolic leaf extract of *Cussonia barteri* may have some adverse effects on the liver, kidney and lungs and should be used with caution.

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ABBREVIATIONS AND SYMBOLS

AEDS	Anti-epileptic drugs
ALP	Alkaline phosphatase
ALT	Alanine Transaminase
ANOVA	Analysis of Variance
AST	Aspartate Transaminase
DZP	Diazepam
ELCB	Ethanolic Leaf extract of *Cussonia barteri*
FAB	Fast Atom Bombardment
GABA	Gamma aminobutyric acid
$GABA_A$	Gamma aminobutyric acid subtype A
H & E	Hematoxylin and Eosin Stain
HDL	High Density Lipoprotein
Hb	Haemoglobin
i.p	Intraperitoneal
INH	Isoniazid
IR	Infrared
LD_{50}	Median Lethal Dose
LDL	Low Density Lipoprotein
LYM	Lymphocytes
NEU	Neutrophils
NMR	Nuclear Magnetic Resonance

PBT	Phenobarbitone
PCV	Packed Cell Volume
PLT	Platelets
PTZ	Pentylenetetrazole
RBC	Red Blood Cells
S.c	Subcutaneous
SEM	Standard Error of Mean
SVP	Sodium valproate
TC	Total Cholesterol
TRI	Triglycerides
UV	Ultraviolet
WBC	White Blood Cells
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

Traditional medicinal practices have remained a component of health care system of many societies in spite of the availability of well-established alternatives (Oyeka, 1981; Ndoye, 2005). It is our socio-economic and socio-cultural heritage as well as the first-choice healthcare treatment for at least 80% of Africans who suffer from high fever and other common ailments (Elujoba *et al*., 2005). This is due to the fact that traditional medicine is the most affordable and accessible system of health cares (Stanley, 2004). Therefore, many people in the developing countries still rely on traditional healing practices and medicinal plants for their basic health care needs (Amos *et al.*, 2001).

In industrialized countries, adaptations of traditional medicine are termed "Complementary" or "Alternative" Medicine. The World Health Organization (WHO) estimates that in several African countries traditional birth attendants assist in the majority of births (WHO, 2003). In Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60% of children with high fever resulting from malaria is the use of herbal medicines at home (WHO, 2003).

The World Health Organization (WHO) encourages the inclusion of herbal medicines of proven safety and efficacy in the healthcare programs of developing countries (Amos *et al.*, 2001).The degree of sensitization and mobilization by the World Health Organization (WHO) has encouraged some African countries to commence serious development on Traditional African Medicine (Elujoba *et al*., 2005).

Epilepsy is a disease that affects about 40 million people worldwide (Njamshi *et al*., 2010), making it one of the most common chronic diseases affecting human beings. All the available conventional medical treatments for epilepsy are not uniformly effective, they are expensive, possess chronic side effects and do not meet the criteria of an ideal antiepileptic drug. These observations have led to a shift in focus to the use of herbal remedies in the management of epileptic seizures, probably because these measures fit into the cultures of people and are not usually as expensive as the more refined orthodox drugs (Balamurugan *et al.*, 2009).

Treatment of epilepsy with herbal drugs as adjuvant seems to be more beneficial and is gaining more popularity due to their fewer side effects. Herbal drugs are acting at the target site having same mechanism of action as that of synthetic drugs (Upadhyaya and Sharma, 2010). Medicinal plants used for the therapy of epilepsy in traditional medicine (TM) have been shown to possess promising anticonvulsant activities and can be invaluable source of new antiepileptic drugs (Senthil, 2013).

There are number of plants being used in traditional medicine for treatment of epilepsy and presently many of these plants are being explored scientifically to ascertain their anticonvulsant activity (Malvi *et al*., 2011). The long historical use of medicinal plants has demonstrated the safety and efficacy of traditional medicine. However, scientific research is needed to provide evidences of their safety and efficacy (WHO, 2000).

Regarding high incidence of epilepsy in human society and with respect to insufficient

therapies, there is need for more work to be done in order to develop an ideal antiepileptic drug that can effectively control epileptic seizures with none or minimal side effects.

1.1 STATEMENT OF RESEARCH PROBLEMS

Epilepsy is the most common non-infectious neurologic disease (Senanayake, and Roman, 1993) that it remains a major medical and social problem (Osuntokun, 1978) in developing countries, including Nigeria. It usually begins in childhood, potentially impeding education, employment, social relationships and development of a sense of self-worth (Dennis *et al.,* 2003). This neurological disorder is strongly associated with significant psychological and social consequences for everyday living (Warren, 2003), with a considerable source of stigma and misery for sufferers in Nigeria and other African Countries.

About 30,000 people develop epilepsy every year and the condition affects about one person in twenty at some time during their lives (Dhanasekaran and Palayan, 2010).

It affects approximately 50millions people worldwide (Fisher *et al*., 2005) and it is the second most common neurological disorder attended to by neurologists after stroke (Sridharan, 2002). The prevalence of epilepsy is particularly high in developing countries especially in Latin America and several African countries, notably Liberia, Nigeria and United Republic of Tanzania (Senanayake, and Roman, 1993). The estimated prevalence in sub-Saharan Africa was found to be two or three times higher than the rate in developed nations (Ngoungou *et al.*, 2006). In Nigeria, the prevalence has been estimated to be 8 to 13 per thousand people (Azubuike and Nkanginieme, 1996).

Approximately 80% of patients diagnosed with epilepsy respond to some form of pharmacologic and/or surgical intervention. The other 20%, however, do not respond to any treatment and continue to have seizure episodes (Turkmani, 2012).There are many classes of anticonvulsants that are of clinical usefulness with good prognosis for controlling seizures in most patients (Cockerel *et al*., 1995).

Most of these currently available antiepileptic/anticonvulsant drugs are synthetic molecules with serious adverse effects and interactions. Despite the availability of these several anticonvulsant drugs to treat convulsion, the treatment of epilepsy is still far from adequate. Besides, about 30% of epilepsy is refractory to treatment (WHO, 2001) and many people in developing countries may not receive basic treatment due to high cost, unavailability and untoward effects associated with the available antiepileptic drugs. Traditional herbs are therefore very useful and indispensable in the struggle for seizure management and future antiepileptic drugs (Adeyemi *et al*., 2007).

The interest in the use of herbal products has grown dramatically in the western world as well as in developed countries (Kraus *et al*., 1998). It is now becoming exceedingly apparent that the available psychotherapeutics do not properly meet therapeutic demands of a vast majority of patients with neurological disorders and that herbal remedies remain to be the ultimate therapeutic hope for many such patients in the western world and elsewhere (Herrero *et al.*, 2002).

Medicinal plants used for the therapy of epilepsy in traditional medicine have been shown

to possess promising anticonvulsant activities in animal models of anticonvulsant screening. *Coriandrum sativum* (Hosseinzadeh *et al*., 2000), *Nigella sativa* (Hosseinzadeh *et al*., 2004), *Ferula gummosa* (Sayyah *et al*., 1975), *Glycyrrhiza glabra*(Ambawade *et al*., 2002) are reported to have anticonvulsant activity. *Cussonia barteri* is reportedly use in the treatment of convulsion and epilepsy, especially in children in Ghana and Nigeria (Burkill, 1985). Literature survey has shown that no work has been carried out to scientifically scrutinize the claim of therapeutic benefit of *Cussonia barteri* in the management of epilepsy. It is in the light of these that this study is aimed at evaluating the ethanolic leaf extract of *Cussonia barteri* for anticonvulsant activity and also to determine its safety in order to provide scientific basis for its use traditionally in the management of epilepsy or otherwise.

1.2 THEORETICAL FRAMEWORK

1.2.1 Median lethal dose (LD_{50}) determination

The method used to determine the median lethal dose was as previously described by Lorke (1983). Using this method, it is possible to obtain adequate information on the acute toxicity and the median lethal dose (LD_{50}) of a compound with fewer experimental animals. It is also useful for every route of administration and applicable to drugs, industrial and agricultural chemicals.

1.2.2 Sub-acute/sub- chronic toxicity studies

The chronic toxicity study provides information on the possible health hazards likely to arise from repeated exposure over a considerable part of the lifespan of the species used.

The study will provide information on the toxic effects of the substance; indicate target organs and the possibility of accumulation. It can also provide an estimate of the no – observed -adverse effect level which can be used for establishing safety criteria for human exposure. The majority of chronic toxicity studies are carried out in rodent species, and the three main routes of administration used in chronic toxicity studies are oral, dermal and inhalation. The choice of the route of administration depends on the physical and chemical characteristics of the test substance and the predominant route of exposure of humans. The exposure via oral route is the most commonly used in chronic toxicity studies, OECD, (1995; 2009).

1.2.3 Models of Epilepsy

Epilepsy is a condition which causes seizures to occur. The use of animal seizure model is essential in the discovery and development of new drugs for the treatment of epileptic seizures. Discovery of a new therapeutic agent begins with the hypothesis that there is a relationship between the experimental seizure model and the initiation and propagation of the seizure, and that the experimental seizure approximates the pathophysiology underlying the human condition (Kupferberg, 2001).

1.3 AIM AND OBJECTIVES

1.3.1 Aim of the study

The aim of this study is to evaluate the neuropharmacological and safety property of ethanolic leaf extract of *Cussonia barteri*(Araliaceae) in laboratory animals.

1.3.2 Specific objectives

The specific objectives of the study are;

1. To carry out anticonvulsant studies using pentylenetetrazole (PTZ)-induced seizure testin mice, maximal electroshock (MES) test in chicks, strychnine, picrotoxin, aminophylline and isoniazid-induced seizure test in mice.

2. To carry out some behavioural studies using diazepam-induced sleeping test in mice, test for exploratory behaviour in mice, mouse beam walk assay and open field test in mice.

1.4 STATEMENT OF RESEARCH HYPOTHESIS

The ethanolic leaf extract of *Cussonia barteri* contain active constituents that possess neuropharmacological activity without significant toxicity.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Epilepsy

According to the World Health Organization dictionary of Epilepsy, Epilepsy is defined as a `chronic disorder of the central nervous system of various etiologies characterized by recurrent seizures due to excessive discharge of cerebral neurons (Ogunrin, 2006).

A seizure is defined as a sudden, excessive discharge of electrical activity in the brain that causes a change in a person's feelings, perception, or behavior. Epilepsy is a chronic process that causes a person to have recurrent seizures (Vincent *et al*., 2007).These seizures are transient signs or symptoms due to abnormal excessive or synchronous neuronal activity in the brain (Malvi *et al*., 2011).

As proposed by the International League against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE) in 2005, epilepsy is defined as a brain disorder characterized by an enduring predisposition to generate epileptic seizures and by the neurobiologic, cognitive, psychologic, and social consequences of this condition (Fisher *et al*., 2005). Anticonvulsants, more accurately called antiepileptic drugs (AEDs) are a diverse group of drugs used in the treatment of epileptic seizures. They are sometimes referred to as anti-seizure drugs. Available antiepileptic drugs control seizures in about two-thirds of patients (Chandradhar, 2001).

2.2 The Plant; *Cussonia barteri Seeman (*Syn:*Cussonia arborea* Hochst)

2.2.1 Plant description

Cussonia barteri is a 10 m high tree, with tortuous trunk, very thick corky bark, of the savanna from Guinea to Nigeria, and extending into East Africa. In the dry season the tree becomes completely defoliated, and the thick stumpy branches resembling amputated and deformed limbs sticking up into the sky invoke the Bambara name in Mali 'stump of an amputated limb' and in Upper Volta 'cut hand', and the Hausa name in Northern Nigeria 'leper's hand'(*hannum kuturuu*). From the likeness of the defoliated tree to deformed limbs, the plant is used, on the Theory of Signatures, in the treatment of leprosy (Burkill, 1985).

2.2.2 Chemical constituents

Phytochemical studies of *Cussonia barteri* methanol extract led to the isolation of 1-O-chlorogenoylchlorgenic acid and 1-O- chlorogenoylneochlorogenic acid, a new type of quinic acid esters, in addition to six known quinic acid esters, rutin, and a mixture of saponins (Papajewski *et al*., 2001).

2. 2.3 Ethnomedical uses

In Mali the stem is macerated and taken as a purgative and applied as a lotion. In Upper Volta and in Ivory Coast, the powdered stem-bark is used to dress leprous sores. The leafy twigs are also used with magical rites for 'yellow fever', oedemas, paralysis and sleeping-sickness. The plant is an emeto-purgative and diuretic by which actions cure is sought. Leaf-decoction is used as an eye-wash for conjunctivitis, pulped up young shoots is eaten for diarrhea in Ivory Coast. It is also prescribed as a poison-antidote.



Plate I: *Cussonia barteri (*Araliaceae) in natural Habitat

Root and stem-decoctions are used for painful menstruation, a root decoction as an emetic for biliousness (indigestion), the water in which leaves have been boiled is used as purgative for constipation, and a leaf-decoction is used in cases of epilepsy in Nigeria. The latter application is found also in Ghana for treating epileptic children.

The plant has a number of unspecified medicinal uses in Mali and unspecified parts are used to treat urethral discharge in women, and are taken by men as an aphrodisiac. The plant has use against fever and the root is prepared in vapour-baths in the treatment of gonorrhea in Tanganyika (Burkill, 1985).

2.2.4 Previous studies

Several medicinal plants have been studied for theirneuropharmacological activities. Some studies have been done at molecular level and several significant phytochemicals have been isolated. For example, several experiments with some natural and synthetic flavones and flavanones have shown that they can modulate GABA-generated chloride current, either po sitively or negatively (Medina *et al*., 1997). The evaluation of seed acetone extract of *Ferula gummosa Boiss* reported its anticonvulsant effect against PTZ and MES induced convulsions (Sayyah *et al*., 2002).The aqueous methanolic stem bark extract of *Paullinia spinnata*(Sapindaceae) is reported to contains bioactive constituents that may be beneficial in grand mal epilepsy and lend pharmacological credence to the ethnomedical claim for the use of the plant in the management of this epilepsy (Maiha *et al*., 2009).

Literature survey revealed that two major saponins have been isolated from the stem bark of *Cussonia barteri* Seem (Araliaceae). Structural elucidation employing mainly H- and C-

NMR, FAB-MS and GC-MS spectroscopic methods revealed a new hederagenin 28-*O*-[α-*L*-rhamnopyranosyl (1 → 4)-β-*D*-glucopyranosyl (1 → 6)]-β-*D*-glucopyranosyl ester (Cussonoside A), and the corresponding oleanolic acid analogue (Cussonoside B). Cussonoside A exhibits a pronounced sedative effect (Dubois, *et al*., 1986).

Phytochemical studies on methanolic extract of *Cussonia barteri* revealed the presence of a mixture of saponins, quinic acid esters, rutin and 1'-O-Chlorogenoylchlorogenic acid and 1'-O-chlorogenoylneochlorogenic acid, a new type of quinic acid esters. All these compounds were tested for inhibitory activity on 5-lipoxygenase and cyclooxygenase-1, for antimicrobial activity against *Bacillus subtilis*, *Pseudomonas fluorescens,* and *Cladosporium cucumerinum*, and for haemolytic activity (Papajewski, *et al*., 2001). In another studies, antimicrobial and antimalarial activity of *Cussonia* species (Araliaceae) were also carried out to find scientific evidence in support of its uses in African traditional medicine against pain, inflammation, gastro-intestinal problems, malaria and sexually transmitted diseases (De Villiers, *et al*., 2010). However, much work has not been done to evaluate the anticonvulsant activity of *Cussonia barteri* in order to provide scientific evidence in support of its uses traditionally in the treatment of epilepsy.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Drugs and Chemicals

Aminophylline ampoule (TianHe Pharm. Jiangsu, China)

Bromine water

Chips of magnesium metal

Chloroform (Sigma Chemical Co.USA)

Dragendorffreagent

Diazepam 10mg in 2ml (Roche Product Ltd, France)

Ethanol (Sigma Chemicals Co. USA)

Ferric chloride (BDH Ltd Poole, England)

Formaldehyde (Sigma-Aldrich LaborchemikaleinGmBH Germany)

Glacial acetic acid (Searle Essex, England)

Isoniazid tab.(MancarePharm.PVT Ltd. India)

Lead sub acetate (Strong) (BDH Ltd Poole, England)

Meyer reagent (BDH Ltd Poole, England)

Normal saline (0.9% NaCl Isotonic Solution)

Pentylenetetrazole (Sigma Chemical Co. USA)

Phenobarbitone (Lab.Renaudin, France)

Phenytoin sodium caps

Picric acid reagent (BDH Ltd Poole, England)

Picrotoxin (Sigma Chemical Co. USA)

Sodium valproate (Sanofi, France)

Strychnine hydrochloride (Sigma Chemical Co. USA)

Sulphuric acid (BDH Ltd Poole, England)

3.1.2 Equipment and Apparatus

Animal cages

Metler balance P165

Open Field apparatus

Pestle and mortar

Ruler (80 cm X 3 cm) with metal support

Syringes (1ml, 2ml, 5ml and 10 ml)

Tally counters

Stop watches

UgoBasile current electroshock machine (Model 7801 with corneal electrodes)

3.2 Methods

3.2.1 Preparation of plant material and extract

Fresh leaves of *Cussoniabarteri* was collected from Basawa area of Sabongari, Kaduna state and authenticated by Mallam U. S.Gallah of the Herbarium Section of the Department of Biological Sciences, Ahmadu Bello University (ABU), Zaria-Nigeria(voucher no.193). The plant material was air dried, crushed into coarse powder with pestle and mortar. 900g of the powdered plant was extracted with 2.5 Litres of 70% v/v ethanol using cold

maceration for 72 hours. The solvent was evaporated on a water bath to give a dry extract. Fresh aqueous solutions of the extract were prepared for each study.

3.3 Animals

Wistar rats of either sex, weighing 110 -130 g, obtained from animal house of Nigerian Trypanosomiasis Research (NITR), Kaduna were used for the toxicity studies. Fifty (50) day old white cockerelsused in the studies were obtained from National Animal Production Research Institute (NAPRI), Zaria while Swiss albino mice weighing 18-25 g of either sex used in the studies were procured and housed in the animal house of Pharmacology and Therapeutics department, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The animals were kept in standard cages and well maintained under standard hygienic conditions, at 27 ± 2^0c, humidity (60 ± 10 %) with 12 hours day and night cycle, with food and water ad libitum.

3.4 Phytochemical Screening of the plant extract

Phytochemical screening of the ethanolic leaf extract of *Cussoniabarteri* was carried out according to the methods described by Trease and Evans, (2002).

3.5 Toxicity studies

3.5.1Acute Toxicity studies: LD_{50}

The intraperitoneal median lethal dose of *Cussoniabarteri* was determined using the method of Lorke (1983). Briefly; the method was divided into two phases. In the initial phase, 3 groups of three mice each were treated with the ethanolic leaf extract of the plant at doses of

10,100 and 1000mg/kg body weight *i.p*and were observed for signs of toxicity and death for 24 hours. In the second phase, three groups each containing one mouse was injected with three more specific doses of the extract based on the result of the initial phase. The LD_{50} value was determined by calculating the geometric mean of the lowest dose that caused death (minimum toxic dose) and the highest dose for which the animal survived (maximum tolerated dose). That is, the geometric mean of doses that caused 0 and 100% mortality (if any) respectively Lorke (1983). This is represented with the formula below;

LD_{50} = √minimum toxic dose x maximum tolerated dose.

3.5.2 Sub-Acute Toxicity (28-Day) Studies

Sub-acute toxicity study was carried out in accordance to WHO (1992) and OECD (1995) guidelines. Forty wistar rats of either sex deprived of food for 24 hours, were randomly divided into four test groups of ten animals per group (n= 10). The test groups received graded doses (100, 200 and 400 mg/kg) of the extract, feed and water; while the control group received feed and water. The treatment continued for 28 days. All the animals were observed daily for clinical signs and mortality. The body weight of each rat was measured at specific times using a sensitive balance, once during the acclimatization period, once before commencement of dosing (Day 0) and once every 7 days (day 7, day 14, day 21 and day 28) during the dosing period. At the end of the treatment period (28 days), animals were humanely anaesthetized with chloroform and blood collected for sera preparation by cardiac puncture. Vital organs like the brain, liver, heart, kidney and lungs were surgically dissected out and weighed in grams. Blood samples obtained at the end of the 28-day treatment period were taken immediately to Haematology and Chemical Pathology Departments of

Ahmadu Bello University Teaching Hospital, Zaria for haematological and biochemical analysis while the organs were taken to Anatomy Department, A.B.U, Zaria for histopathological examination. The Relative Organ Weight (ROW) of each rat was calculated as follows;

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

The percentage changes in the body weights of the animals were also calculated by taking the mean percentage changes in their body weights between day 0 (before administering the extract) and day 28 (at the end of treatment period).

3.6 Anticonvulsant studies

3.6.1 Pentylenetetrazole-induced Seizure in mice

The method of Swinyard *et al.*, (1989) was employed. Twenty-five mice were divided into five groups each containing five mice. The first group received normal saline (10 ml/kg) *i.p.*; second, third and fourth groups were treated with different doses (100, 200 and 400mg/kg) of the plant extract, *i.p.* The last group was treated with sodium valproate 200mg/kg, *i.p.* (positive control). Thirty minutes post treatment, the mice in all the groups received 85mg pentylenetetrazole per kg *s.c.*and were observed over a period of 30 minutes. Absence of an episode of clonic spasm of at least 5 seconds duration indicated a compound's ability to abolish the effect of pentylenetetrazole on seizure threshold.

3.6.2 Maximum electroshock-induced convulsion in chick

The method described by Swinyard and Kufferberg (1985) as modified by Sayyah *et al.,*(2002) was employed in this study. Fifty (50) day old white cockerels were randomly divided into five groups each containing ten chicks. The first group received normal saline (10 ml/kg) *i.p.*; second, third and fourth groups were treated with different doses (100, 200 and 400 mg/kg) of the plant extract respectively. The last group was administered with phenytoin (20 mg/kg), *i.p.* Thirty minutes after pre-treatment, maximal electroshock was administered to induce seizure in the chicks using Ugobasile electroconvulsive machine (Model 7801) connected to Claude Lyons stabilizer with corneal electrodes placed on the upper eyelids of the chicks. The shock duration, frequency and pulse width were set and maintained at 0.80s, 200 pulse per second and 0.8ms respectively. A current of 90mA, which produced tonic seizures in 90% of the control chicks, was used throughout the study. Seizures were manifested as tonic hind-limb extension (THLE). The ability to prevent this feature or prolong the latency and or onset of the THLE was considered as an indication of anticonvulsant activity (Sayyah *et al.*, 2002).

3.6.3 Strychnine- induced convulsions in mice

The method of Porter *et al*., (1984) was employed. A total of thirty (30) mice divided into five groups of six mice each. The first control group received 10 ml normal saline per kg body weight *i.p.*, the second group (positive control) was given 200 mg valproic acid per kg body weight *i.p.*, while the third, fourth and fifth groups received 100, 200 and 400 mg/kg respectively doses of the plant extract *i.p.*Thirty minutes later, mice in all the groups received 1.0 mg strychnine per kg, *s.c.* Any mouse that did not convulse within 30 min after

strychnine administration was considered protected.

3.6.4 Isoniazid (INH)-induced convulsions in mice

Thirty (30) albino mice were randomly divided into 5 groups of six mice per group. The first group which served as negative control was treated with 10ml normal saline per kg body weight *i.p*. Groups 2, 3 and 4 received 100, 200 and 400 mg/kg *i.p* respectively doses of the extract. Group 5 which served as positive control was treated with 30 mg/kg *i.p.* phenobarbitone. Thirty minutes later, 400mg/kg of INH was administered subcutaneously to all the mice. The mice were observed for 60 minutes for characteristic behavioural signs such as hyperactivity, trembling, intermittent forelimb extension, clonic seizure and death. Lack of tonic-clonic seizure or delay in the onset of seizure during 60 minutes of observation was regarded as protection (Kale *et al*., 2005).

3.6.5 Picrotoxin-induced convulsions in mice

Mice were randomly divided into the different control and test groups. The first control group received 10 ml normal saline per kg body weight *i.p.*, the second group (positive control) was given 20 mg diazepam per kg body weight. The third, fourth and fifth group received 100, 200 and 400 mg/kg of the extract respectively. Picrotoxin (5 mg/kg, *i.p.*) was used to induce seizure using the same treatment procedure as described for strychnine. The absence of tonic extensor jerks of the hind limbs by the animals is an indication that the extract offered protection against picrotoxin-induced convulsions.

3.6.6Aminophylline-induced convulsions in mice

Mice were randomly divided into five groups of six each. Group 1 & 5 served as negative and positive control respectively while the remaining groups received different doses of extract as given above during picrotoxin treatment. After thirty minutes, aminophylline (350mg/kg, *i.p.*) was administered to all the mice to induce convulsion. The animals were observed for clonic convulsions and time taken to produce clonic convulsion for 60minutes. Lack of clonic convulsion or delay in the onset of clonic convulsion was considered as protection.

3.7Behavioural Studies

3.7.1 The effect of the extract on diazepam- induced sleeping time.

The method described by Beretz*et al*., 1978 and modified by Rakotonirina *et al*., 2001 was used in this study. Four groups of each containing six mice were used for the study. The first group served as control and was treated with normal saline 10ml/kg body weight. The remaining groups (2, 3 and 4) were treated with 100, 200, and 400 mg/kg of the plant extract respectively. Thirty (30) minutes after pretreatment with different doses (100, 200 and 400 mg/kg body weight) of the plant extract and normal saline, all the mice were administered with diazepam at a dose of 20mg/kg. The onset and duration of sleep was recorded for each mouse. The loss of straightening reflex was regarded as the onset of sleep while the time difference between the disappearing and the recovery of the rightning reflex was taken as the duration of sleep (sleeping time).

3.7.2 Test for exploratory behaviour in mice

The method for the hole-board test in mice was similar to those described previously (File, 1973). The apparatus consist of a wooden board (60 cm x 30 cm) with 16 evenly spaced holes (1cm x 2 cm depth). Thirty mice were randomly divided into five groups of six mice each. The first group was treated with normal saline (10ml/kg, *i.p.*), the fifth group was treated with 0.5mg/kg diazepam while the remaining groups were treated with different doses of the extract; 100, 200, and 400 mg/kg body weight *i.p.* respectively. Thirty (30) minutes post-treatment, each mouse was placed at a corner of the board and the number head dips on the hole was counted. A head dip was considered when the mouse dipped its head into the hole to the level of the eyes.

3.7.3 Beam walking assay in mice

The method used for this study was similar to that previously described by Stanley *et al.*, (2005). Adult mice were trained to walk from a start platform along a ruler (80 cm long, 3 cm wide) elevated 30 cm above the bench by metal supports to a goal box. The successful mice after three trials were randomly grouped into five (n=5). The first group received normal saline (10ml/kg), intraperitoneally. The second, third and the fourth groups were treated intraperitoneally with 100, 200 and 400 mg/kg body weight of the plant extract respectively while the fifth group received diazepam (1 mg/kg body weight).

Thirty minutes post-treatment, each mouse was placed on the beam at one end and allowed to walk to the goal box. Mice that fell were returned to the position they fell from, with a maximum time of 60 s allowed on the beam. The number of foot slips (one or both hind limb slipped from the beam) was recorded with the aid of a tally counter. The time taken to

complete the task was also recorded. The number of foot slip is a measure of motor coordination deficit (Stanley *et al.*, 2005).

3.7.4 Open field test in mice.

The study was conducted according to method previously described by Brown *et al*., (1999) with some modifications. The apparatus was made up of plywood measuring 72 cm x 72 cm x 36 cm. One of the walls was made of transparent Perspex glass to ensure that the mouse under investigation was visible to the observer. The floor, made of cardboard was divided into 16 equal squares (18 cm x 18 cm) with blue marker and a central square drawn with black marker. The cardboard was covered with a transparent Plexiglas. 25 mice divided into 5 groups (n=5). The groups were treated with normal saline (10ml/kg), the plant extract (100, 200 and 400 mg/kg) or diazepam (0.25 mg/kg). Thirty (30) minute post-treatment, each mouse was placed individually at the corner of the arena and its behaviour monitored for a period of 5 minutes with the aid of a video camera hung 2 m above the apparatus and connected to a monitor. The number of rearing, number of squares and number of Central Square crossed by each mouse was recorded. The apparatus was wiped between observations with 70% ethyl alcohol and allowed to dry to remove any olfactory cue.

3.8 Statistical Analysis

Data were expressed as mean ± SEM and presented as tables, charts and plates. The difference between the control and the test groups were analyzed for statistical difference using One Way ANOVA followed by Dunnett's post hoc t-test for multiple comparisons.

$P < 0.05$ was considered significant.

CHAPTER FOUR

4.0 Results

4.1 Plant Extract

4.1.1 Percentage yield

The 900g of powdered plant gave a yield of 158.4 g (17.6% w/w).

4. 1.2 Phytochemical constituents

Preliminary phytochemical screening of ethanolic leaf extract of *Cussonia barteri* revealed the presence of cardiac glycosides, flavonoids, saponins, steroids, tannins, glycosides and carbohydrates.

4.2 Results of Toxicological Studies

4.2.1 Median lethal dose (LD_{50})

Intraperitoneal administration of the ethanolic leaf extract of *C. barteri* (10-1,000 mg/kg) in mice did not produce any visible sign of toxicity or mortality over a period of 24 h for the first phase of the Lorke's method. In the second phase, there was mortality from a dose of 2,900mg/kg to 5,000mg/kg. The *i.p* median lethal dose (LD_{50}) of the ethanolic leaf extract of *Cussonia barteri* for mice was estimated to be 2,154.1 mg/kg in mice. Oral administration of the ethanolic leaf extract of *C. barteri* (10-1,000 mg/kg) in rats (for the first phase) and 1,600-5,000 mg/kg (for the second phase) did not produce any visible sign of toxicity or mortality over a period of 24 h in both phases. The oral median lethal dose (LD_{50}) of the extract was estimated to be greater than 5,000 mg/kg body weight in rats.

4.2.2 Sub-acute toxicity studies

4.2.2.1 Percentage change in body weight and relative organ weight

The animals in both control and treated groups appeared healthy at the end of the experiment as well as throughout the 28 day period. The percentage change in body weight and relative organ weight of the vital organs of the animals were recorded in Table 4.1 and Table 4.2 respectively. All values in the test groups were not significantly different from control group (Table 4.1). Oral administration of graded doses (100- 400 mg/kg daily) of *Cussonia barteri* for 28 days resulted in significant increase ($P<0.05$) in some relative organ weights; brain at 200 and 400 mg/kg, liver at 400 mg/kg and heart at 200 mg/kg of the doses tested (Table 4.2).

4.2.2.2 Effects of the extract on liver enzymes of wistar rats

All the values in the test groups were not significantly different from control group, thus sub-acute oral administration of the extract for 28 days did not significantly affect the liver enzymes of the animals (Table 4.3).

4.2.2.3 Effects of the extract on lipids and biochemical parameters of wistar rats.

The values obtained in the test groups were not significantly different from the control groups in both lipids profile analysis and biochemical studies. The extract neither produced significant changes in lipids parameters nor biochemical parameters (Table 4.4 and 4.5).

4.2.2.4 Effects of the extract on serum electrolytes of wistar rats.

There was no significant difference between the values obtained in test groups and control group, suggesting that the daily oral administration of extract for 28 days did not produce any significant changes in the serum electrolytes of the animals (Table 4.6).

4.2.2.5 Effects of the extract on haematological parameters

The daily administration of the extract for 28 days did not produce any significant changes in the haematological parameters of the animals (Table 4.7).

4.2.3Histopathological studies

The control group (Plates I, II, III and IV) showed normal sections of the heart, kidney, liver and lungs of a rat respectively. There were no lesions or pathological features of the organs of all the animals in the control group. Histopathological examination of the heart showed no pathological features or lesions at all the doses of the extract administered (Plates I, V, IX and XIII). Similarly, the sections of liver and kidney of a rat that received the extract at 100 mg/kg body weight showed no lesions or pathological features (Plates VI and VII), that is, normal hepatocytes/connective tissues (liver) and normal glomeruli/tubules (kidney). However, at a dose of 200 mg/kg of the plant extract, there was venous congestion, sinusoidal congestion with slight hepatic necrosis and moderate tubular necrosis while at a dose of 400mg/kg there was kupfer cell hyperplasia, slight hepatic necrosis and sinusoidal congestion and intense tubular necrosis with slight glomerular necrosis (Plates X and XI). Histopathological examination of the lungs showed intense

alveoli congestion with lymphocyte hyperplasia, moderate alveoli congestion with lymphocyte hyperplasia and intense alveoli congestion with lymphocyte hyperplasia respectively (Plates VIII, XII and XVI).

4.3 Anticonvulsant studies

4.4.1 Effect of the extract on maximal electroshock-induced seizures in chicks

The extract did not protect the chicks against tonic hind limb extension induced by maximal electroshock. There was a significant increase ($P<0.05$) in the mean recovery time at a dose of 100 mg/kg and at 200 and 400 mg/kg of the extract, but with no significant reduction in the mean recovery time. Phenytoin protected 80% of the chicks at the dose of 20 mg/kg (Table 4.8).

4.4.2 Effect of the extract on Pentylenetetrazole-induced Seizure in mice

The extract protected mice against PTZ seizures with the highest dose (400mg/kg) producing 83.33% protection, while at the doses of 200 and 100 mg/kg, the extract produced 33.33% protection. The standard agent, sodium valproate at the dose of 200 mg/kg produced 100% protection against PTZ-induced seizure in mice (Figure 4.1). The extract had no significant effect on the mean onset of seizure.

4.4.3 Effect of the extract of on Strychnine-induced seizure in mice

The extract at the highest dose (400 mg/kg) produced 66.67% protection against seizure, while at the doses of 100 and 200 mg/kg produced 50% protection against this seizure. Similarly, at the highest dose tested, the extract offered 66.67% protections against

mortality while there was 50% protection at the dose of 100 mg/kg and 83.33% protection at the dose of 200 mg/kg against mortality induced by strychnine at the dose of 1.2 mg/kg. However, there was no significant effect on the mean onset of seizure. Phenobarbitone (20 mg/kg) produced 100% protection against strychnine-induced seizure (Figure 4.2).

4.4.4 Effect of the extractagainst Picrotoxin-induced seizure in mice

The extract did not protect the animals against picrotoxin-induced seizure and had no significant effect on the mean onset of seizure compared with the normal saline treated group. Diazepam at the dose of 20 mg/kg produced 33.33% protection against picrotoxin-induced seizure and significantly increased the mean onset of seizure (Table 4.9).

4.4.5 Effect of the extract against Aminophylline-induced seizure in mice.

The extract did not significantly protect the animals against aminophylline-induced seizure. There was also no significant effect on the mean onset of seizure. The standard drug used, Phenobarbitone (30 mg/kg) did not protect against aminophylline-induced seizure and also did not significantly affect the mean onset of seizure. Phenobarbitone significantly ($P< 0.01$) increased the mean latency of mortality (Table 4.10).

4.4.6 Effect of the extract against Isoniazid-induced seizure in mice.

The extract did not significantly protect the mice against isoniazid-induced seizure.

There was also no significant effect on the mean onset of seizure at doses of 100 and 200 mg/kg but at the highest dose (400 mg/kg) tested, there was a significant increase in the mean onset of seizure (Appendix v). Phenobarbitone (30 mg/kg) did not protect against

isoniazid-induced seizure but significantly (P< 0.05) increase the mean onset of seizure and significantly (P< 0.001) increased the mean latency of mortality (Figure 4.3).

4.4 BEHAVIOURAL STUDIES

4.4.1 Effect of the extract on Diazepam induced-sleep in Mice

The extract significantly (P<0.01) at 200 mg/kg and(P<0.001) at 400 mg/kg increased the mean onset of sleep. Also, the extract significantly (P<0.05) at 200 mg/kg and (P<0.01) at 400 mg/kg increased the duration of sleep in mice (Figure 4.4).

4.4.2 Effect of the extract on exploratory activity of mice in hole -board test

The extract significantly (P<0.001) decreased the number of head dips in the hole board test. Diazepam at the dose of 0.5 mg/kg, also, significantly (P<0.001) decreased the number of head dips (Figure 4.5).

4.4.3 Effect of the extract on motor co-ordination in mice (Beam Walk Assay)

The extract did not significantly affect the time taken to complete the task on the beam but at the dose of 400 mg/kg it significantly (P<0.05) increased the number of foot slips. Diazepam at the dose of 1 mg/kg significantly (P<0.05) increased the number of foot slips but did not significantly affect the time taken to complete the task (Table 4.11).

4.4.4 Effect of the extract on behaviour of mice in Open field Test

The extract did not significantly affect the number of rearing, number of total square crossed and the number of Central Square crossed at all the doses tested. Diazepam at the

dose of 0.25 mg/kg did not significantly affect the number of total square crossed and the number of Central Square crossed but significantly decreased the number of rearing (Table 4.12).

Table 4.1: Effects of the extract on body weight variation in Wistar rats expressed as Percentage change in weight (28 days).

Treatment	Dose (mg/kg)	Mean weight in gram(g) ± SEM		Percentage change in weight ± SEM
		Day 0	Day 28	
Control		140.50 ± 4.60	195.00 ± 10.70	38.42 ± 5.43
ELCB	100	152.80 ± 4.06	203.70 ± 10.55	32.65 ± 4.08
ELCB	200	140.90 ± 5.04	193.50 ± 7.88	37.97 ± 5.25
ELCB	400	132.80 ± 5.51	184.10 ± 5.54	39.59 ± 4.07

Data presented as; Mean ± SEM (Standard Error of Mean), n=10, control= Distilled water 1ml/kg, ELCB= Ethanolic Leaf extract of *Cussonia barteri.* Statistical tool: ANOVA (one way).

Table 4.2: Effects of the extract on the Relative Organ Weight (ROW) of Wistar rats (28 days).

Treatment/ dose (mg/kg)	Relative Organ Weight (ROW) in grams (g) ± SEM				
	Brain	**Liver**	**Kidney**	**Heart**	**Lungs**
Control	0.57 ± 0.03	3.32 ± 0.06	0.70 ± 0.02	0.3 ± 0.05	0.83 ± 0.05
ELCB 100	0.64 ± 0.02	3.64 ± 0.07	0.68 ± 0.01	0.32 ± 0.01	0.93 ± 0.07
ELCB 200	0.87 ± 0.05*	3.50 ± 0.05	0.71 ± 0.02	0.37 ± 0.01*	1.00 ± 0.06
ELCB 400	0.92 ± 0.02*	3.72 ± 0.1*	0.63 ± 0.01	0.34 ± 0.02	0.91 ± 0.06

Data presented as; Mean ± SEM (Standard Error of Mean), n=10, control= Distilled water 1ml/kg, ELCB= Ethanolic Leaf extract of *Cussonia barteri.** P<0.05. Statistical tool: ANOVA (one way).

Table 4.3: Effects of the extract on Liver enzymes of Wistar rat (28 days).

Treatment	Dose mg/kg	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Control		89.80 ± 8.15	82.6 ± 9.00	61.6 ± 2.98
ELCB	100	61.00 ± 8.40	77.2 ± 7.83	72.6 ± 1.21
ELCB	200	78.20 ±3.90	71.0 ± 0.95	69.2 ± 0.80
ELCB	400	62.00 ± 4.30	70.2 ± 0.49	70.2 ± 0.49

Data presented as; Mean ± SEM (Standard Error of Mean), n=10, Control= Distilled water 1ml/kg, ELCB= Ethanolic Leaf extract of *Cussonia barteri,* ALT=Alanine Transaminase, AST=Aspartate Transaminase, ALP=Alkaline Phosphatase. Statistical tool: ANOVA (one way).

Table 4.4: Effects of the extract on Lipids parameters of Wistar rat (28 days).

Treatment	Dose mg/kg	TC (mg/dL)	TRI (mg/dL)	LDL(mg/dl)	HDL (mg/dL)
Control		2.36 ± 0.117	0.88 ± 0.086	1.14 ±0.093	0.80±0.126
ELCB	100	2.60 ± 0.055	1.02 ± 0.116	1.25±0.167	0.88±0.150
ELCB	200	2.6 ± 0.141	0.92 ± 0.146	1.37±0.223	0.80±0.114
ELCB	400	2.5 ± 0.084	0.76 ± 0.103	1.32±0.080	0.82±0.150

Data presented as; Mean ± SEM (Standard Error of Mean), n=10, control= Distilled water 1ml/kg, ELCB= Ethanolic Leaf extract of *Cussonia barteri*, TC=Total cholesterol, TRI=Triglycerides, LDL=Low density Lipoproein, HDL=High density Lipoprotein.

Statistical tool: ANOVA (one way).

Table 4.5: Effects of the extract on some serum biochemical parameters of Wistar rat (28 days).

Treatment	Dose mg/kg	Total protein (mg/dL)	Albumin (mg/dL)	Urea (mmol/L)	Creatinine (mg/dL)
Control		63.30±0.929	31.4±0.884	3.715±0.358	48.4±0.2.034
ELCB	100	65.30±0.989	33.4±0.653	3.33±0.178	48.5±2.040
ELCB	200	62.20±1.254	30.90±1.005	3.005±0.189	46.80±2.010
ELCB	400	63.90±0.823	32.80±0.712	2.92±0.245	46.50±1.408

Data presented as Mean ± SEM (Standard Error of Mean), n = 10. Control= Distilled water 1ml/kg, ELCB= Ethanolic Leaf extract of *Cussonia barteri.* Statistical tool: ANOVA (one way).

Table 4.6: Effects of the extract on selected serum Electrolyte parameters of Wistar rat (28 days).

Treatment	Dose mg/kg	Calcium ion (mg/dL)	Sodium ion (mg/dL)	Potassium ion (mmol/L)	Chloride ion (mg/dL)
Control		2.432±0.021	138.6±0.702	97.30±0.989	98.70±0.857
ELCB	100	2.416±0.045	140.0±1.043	98.00±1.265	99.2±0.892
ELCB	200	2.390±0.047	139.0±0.746	98.8±1.02	98.7±1.126
ELCB	400	2.426±0.0337	138.70±0.761	99.80±1.8	100.6±1.087

Data presented as Mean ± SEM =standard error of mean, n = 10. Control= Distilled water 1ml/kg, ELCB= Ethanolic Leaf extract of *Cussonia barteri.* Statistical tool: ANOVA (one way).

Table 4.7: Effects of the extract of on selected Haemotological Parameters of Wistar rats (28 days).

Treatment	Dose (mg/kg)	WBC (x 10^3/µL)	RBC (x 10^6/µL)	Hb (g/dL)	LYM (x 10^3/µL)	NEU (x 10^3/µL)	PLT (x 10^9/L)	PCV %
Control		6.798±0.360	7.052±0.233	13.365±0.381	65.30±1.58	33.6±1.5	328.2±14.4	39.30±0.95
ELCB	100	6.798±0.360	7.052±0.233	13.365±0.381	65.30±1.58	33.6±1.5	328.2±14.4	39.30±0.95
ELCB	200	6.798±0.360	7.052±0.233	13.365±0.381	65.30±1.58	33.6±1.5	328.2±14.4	39.30±0.95
ELCB	400	6.798±0.360	7.052±0.233	13.365±0.381	65.30±1.58	33.6±1.5	328.2±14.4	39.30±0.95

WBC = White Blood Cell, RBC = Red Blood Cell, HGb = Haemoglobin, LYM = Lymphocyte, NEU = Neutrophil, PLT = Platelet, PCV = Packed Cell Volume, ELCB=Ethanolic leaf extract of*Cussonia barteri*. Control= Distilled water 1ml/kg.Data presented as Mean ± SEM = Standard Error of Mean, *n* = 10. Statistical tool: ANOVA (one way).

Table 4.8: Effects of the extract on maximal electroshock-induced seizures in chicks

Treatment/Dose (mg/kg)	Mean Recovery Time (min) ± SEM	Quantal Protection Against Seizure	% Protection Against Seizure
Control	6.2 ± 0.79	0/10	0
ELCB 100	9.2 ± 0.67*	0/10	0
ELCB 200	6.3 ± 0.72	0/10	0
ELCB 400	6.1 ± 0.69	0/10	0
Phenytoin 20	5.9 ± 2.83	8/10	80

Data presented as; Mean ± SEM=Standard Error of Mean, Control =Distilled water 1ml/kg, ELCB = Ethanolic Leaf Extract of *Cussonia barteri*, * ($P<0.05$), n=10. Statistical tool: ANOVA (one way).

Table 4.9: Effect of the extract against Picrotoxin-induced seizure in mice

Treatment/Dose (mg/kg)	Mean onset of seizure (min) ±SEM	Quantal protection against mortality	Mean latency of mortality
Control	5.02 ± 0.28	0/6	7.83 ± 0.68
ELCB 100	5.0 2± 0.10	0/6	8.39 ± 0.36
ELCB 200	5.00 ± 0.83	0/6	8.39 ± 1.68
ELCB400	3.62 ± 0.14	0/6	5.97 ± 0.71
Diazepam 20	10.88 ± 0.32*	2/6 (33.33%)	18.71 ± 0.61

Data presented as Mean ±SEM=Standard Error of Mean, Protection against seizure expressed as quantal protection; Control = Distilled water 1ml/kg, ELCB = Ethanolic Leaf Extract of *Cussonia barteri*,*P<0.001, n=6. Statistical tool: ANOVA (one way).

Table 4.10: Effect of the extract against Aminophylline-induced seizure in mice

Treatment/Dose (mg/kg)	Mean onset of seizure (min) ± SEM	Quantal protection against mortality	% Quantal protection against mortality	Mean latency of mortality (mins) ± SEM
Control	13.3 ± 1.01	0/6	0	16.0 ± 1.49
ELCB 100	9.4 ± 0.84	0/6	0	11.6 ± 0.94
ELCB 200	13.2 ± 2.59	0/6	0	14.6 ± 1.72
ELCB 400	8.3 ± 0.45	1/6	16.67	9.6 ± 0.99
PBT 30	16.5 ± 2.18	0/6	0	30.3 ± 4.6**

Data presented as; SEM=Standard Error of Mean, Protection against seizure expressed as quantal protection; PBT: Phenobarbitone, Control = Distilled water 1ml/kg, ELCB = Ethanolic Leaf Extract of *Cussonia barteri*, **P<0.01; n=6. Statistical tool: ANOVA (one way).

Table 4.11: Effect of the extract on motor co-ordination in mice (Beam Walk Assay)

Treatment	Dose (mg/kg)	Time spent on the beam(mins) ± SEM	Number of foot slips ± SEM
Normal saline	10 ml/kg	19.4 ± 2.36	5.5 ± 0.96
ELCB	100	22.4 ± 4.71	4.8 ± 0.58
ELCB	200	27.7 ± 7.33	6.7 ± 1.80
ELCB	400	40.7± 9.69	14.5 ± 3.2*
Diazepam	1	30.0 ± 6.12	13.2 ± 3.94*

Data presented as Mean ± SEM= Standard Error of Mean, ELCB = Ethanolic Leaf extract of *Cussonia barteri*. *P< 0.05, Statistical tool: ANOVA (one way).

Table 4.12: Effect of the extract on behaviour of mice in Open field Test

Treatment	Dose (mg/kg)	Number of rearing ± SEM	Number of Total square crossed ± SEM	Number of central square crossed ± SEM
Normal saline	10ml/kg	11.0 ± 0.58	97.00 ± 13.00	3.0 ± 2.52
ELCB	100	14.3 ± 1.86	84.0 ± 11.27	2.3 ± 0.88
ELCB	200	5.7 ± 1.86	6.7 ± 1.20	0.67 ± 0.33
ELCB	400	7.33 ± 2.96	85.0 ± 30.39	2.33 ± 1.86
Diazepam	0.25	4.0 ± 0.00*	157.67 ± 1.45	0.67 ± 0.33

Data presented as; Mean ± SEM= Standard Error of Mean, ELCB = Ethanolic Leaf extract of *Cussonia barteri*. *P<0.05, Statistical tool: ANOVA (one way).

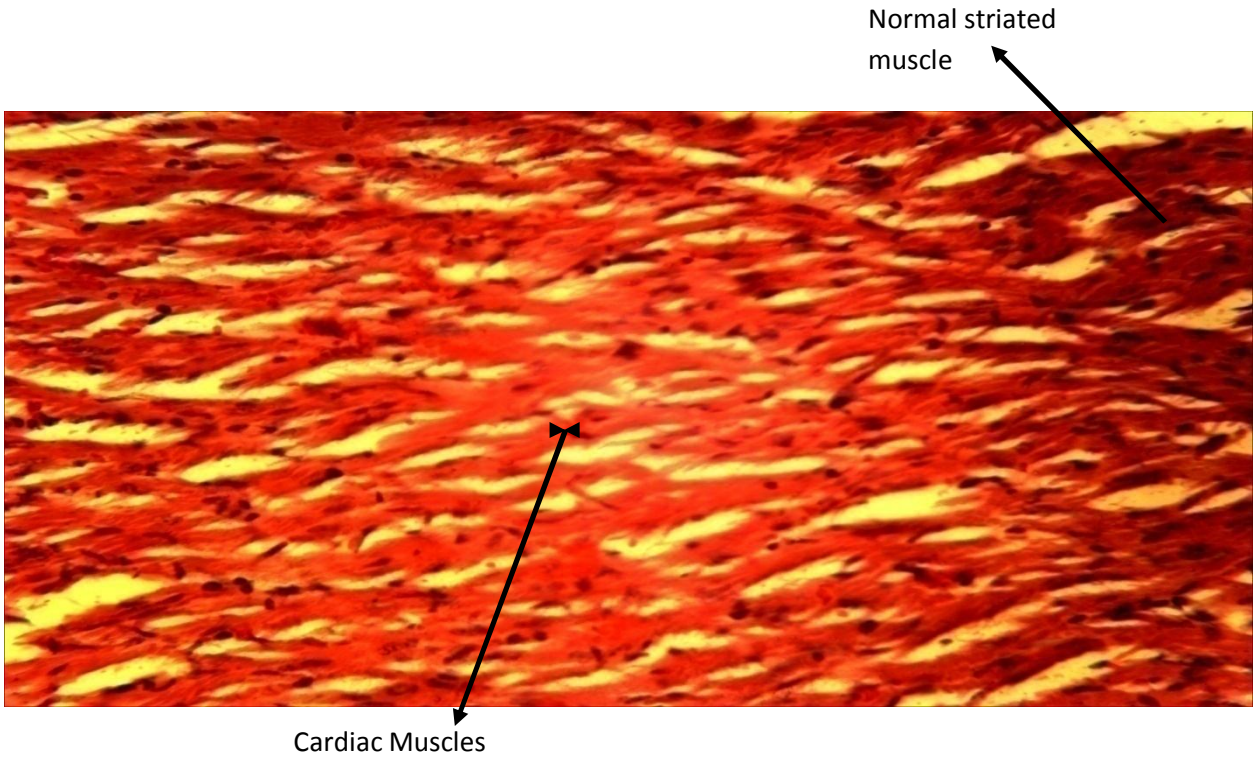


Plate II. Photomicrograph of a section of the Heart of a Wistar rat administereddistilled water 1ml/kg for 28 days (H & E x 250). Normal cardiac muscles.

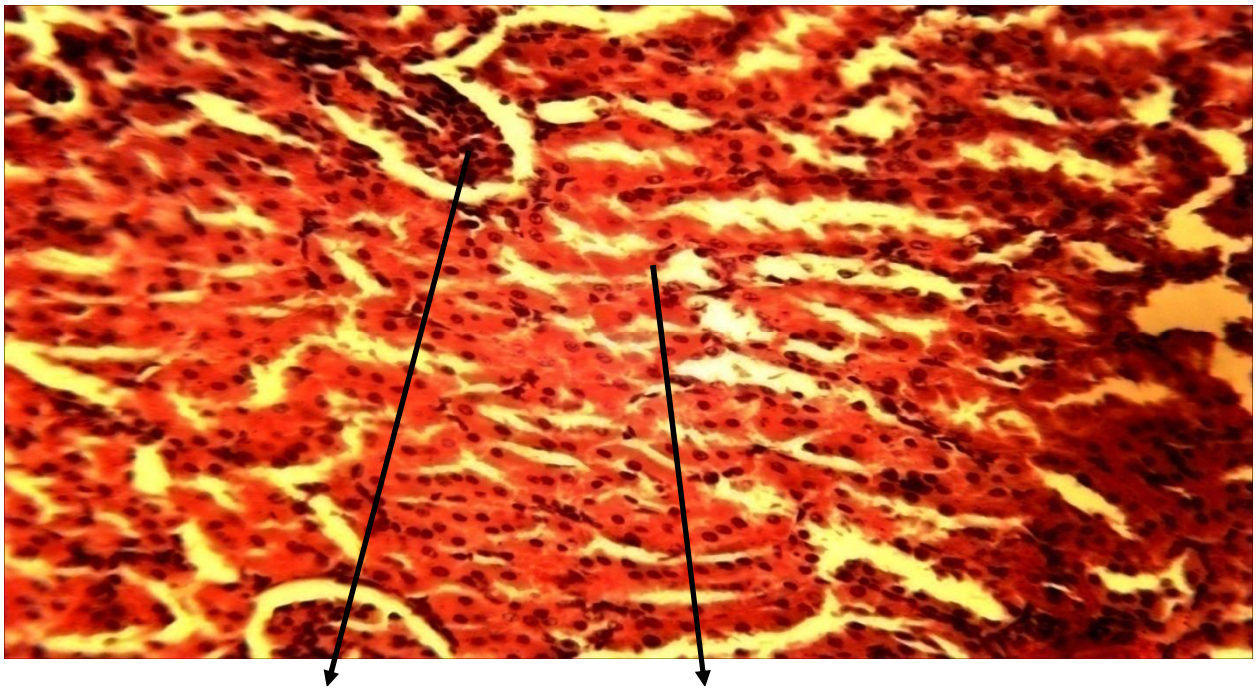


Plate III. Photomicrograph of a section of the Kidney of a Wistar rat administered distilled water 1ml/kg for 28 days (H & E x 250). Normal features.

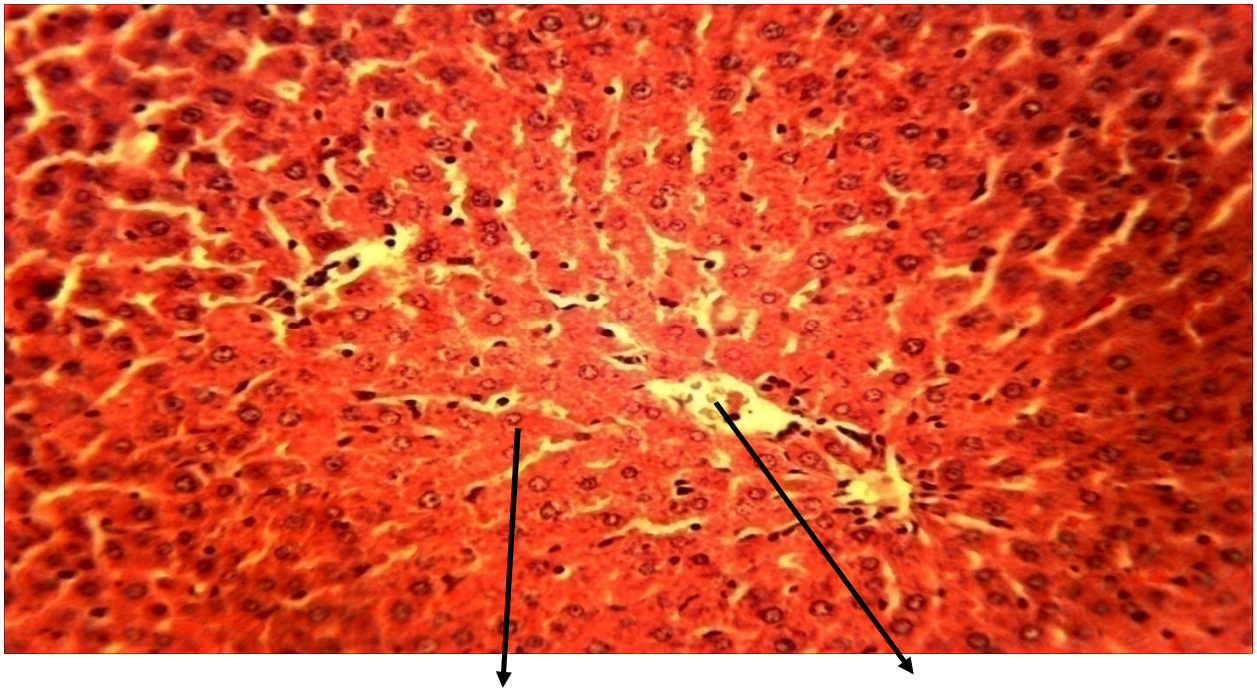


Plate IV.Photomicrograph of a section of the Liver of a Wistar rat administered distilled water 1ml/kg for 28 days (H& E x 250). Normal hepatocytes.

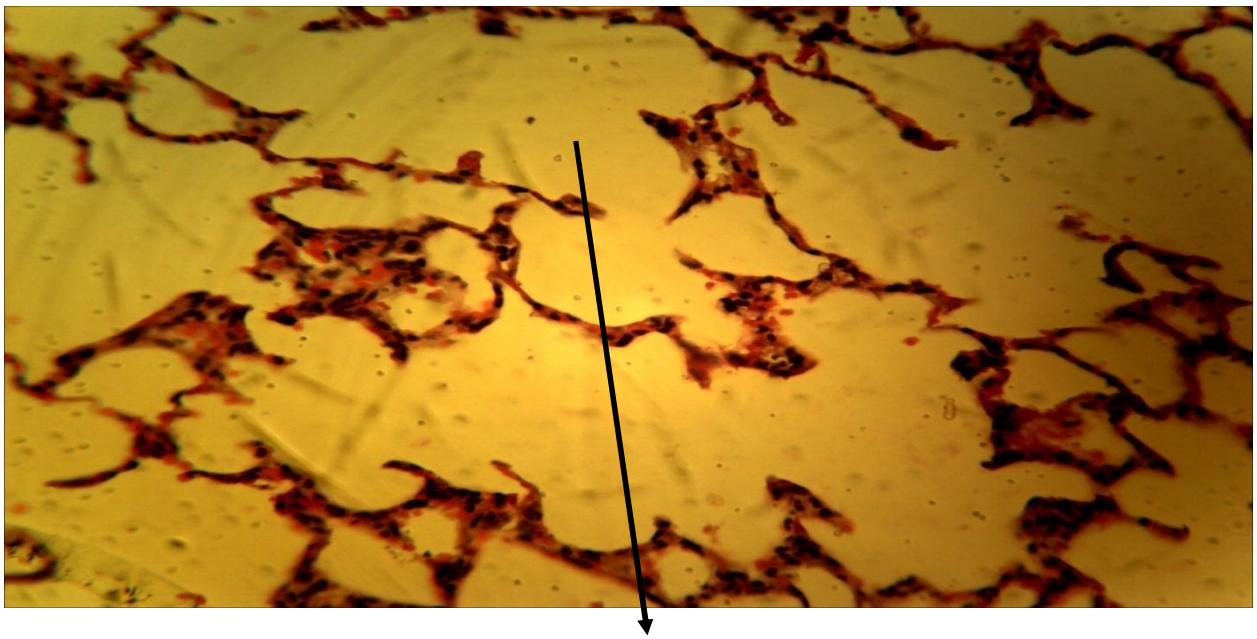


Plate V. Photomicrograph of a section of the lungs of a Wistar rat administered distilled water 1ml/kg for 28 days (H & E x 250). Normal features.

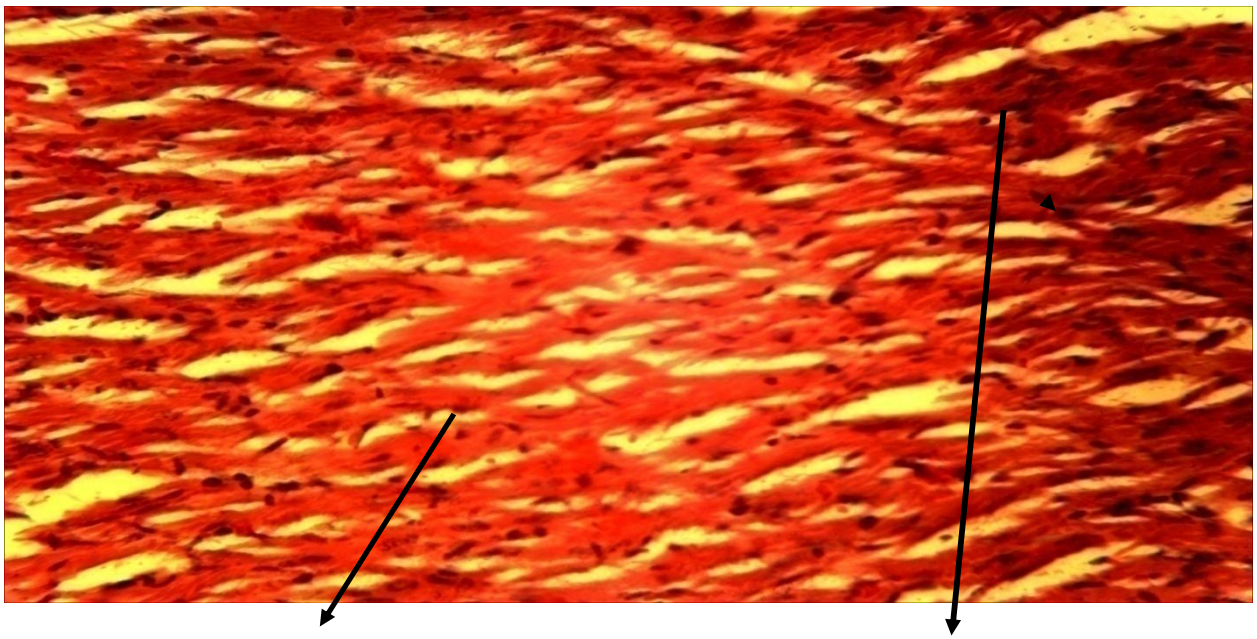


Plate VI. Photomicrograph of a section of the Heart of a Wistar rat administered Ethanolicleaf extract of *Cussoniabarteri*100 mg/kg for 28 days (H& E x 250). Normal features.

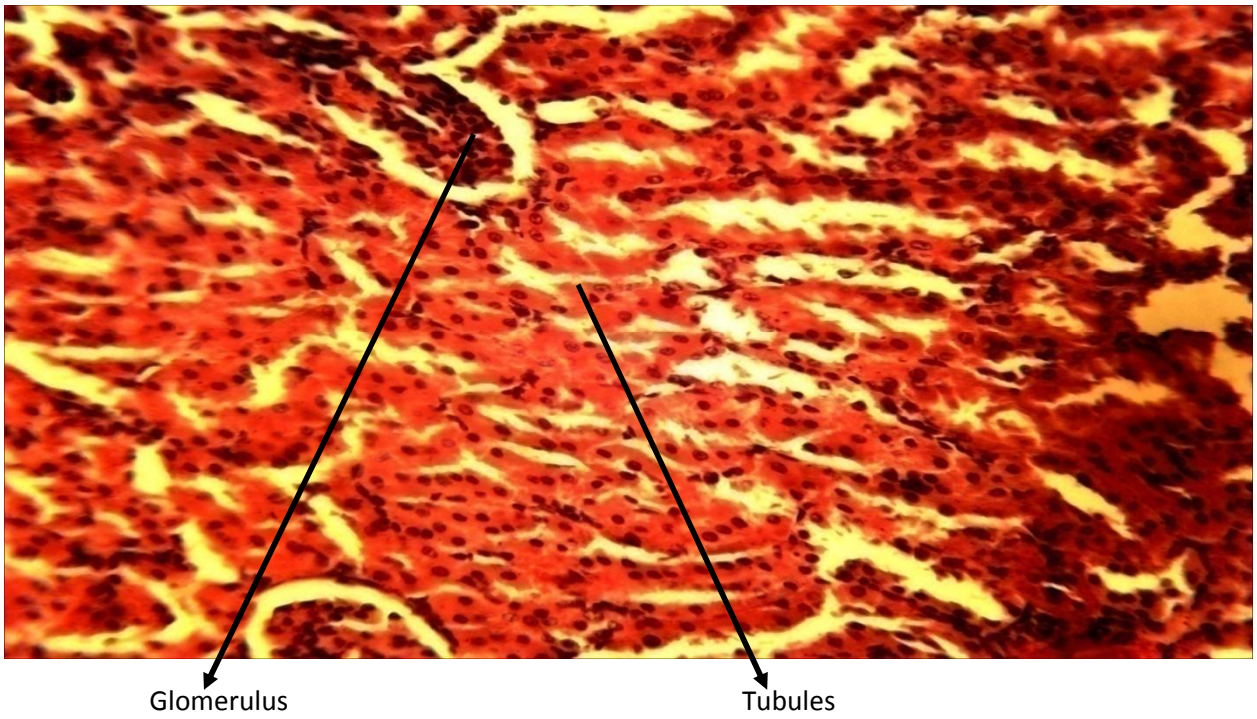


Plate VII. Photomicrograph of a section of the Kidney of a Wistar rat administered Ethanolicleaf extract of*Cussoniabarteri* 100mg /kg for 28 days (H & E x 250). Normal features.

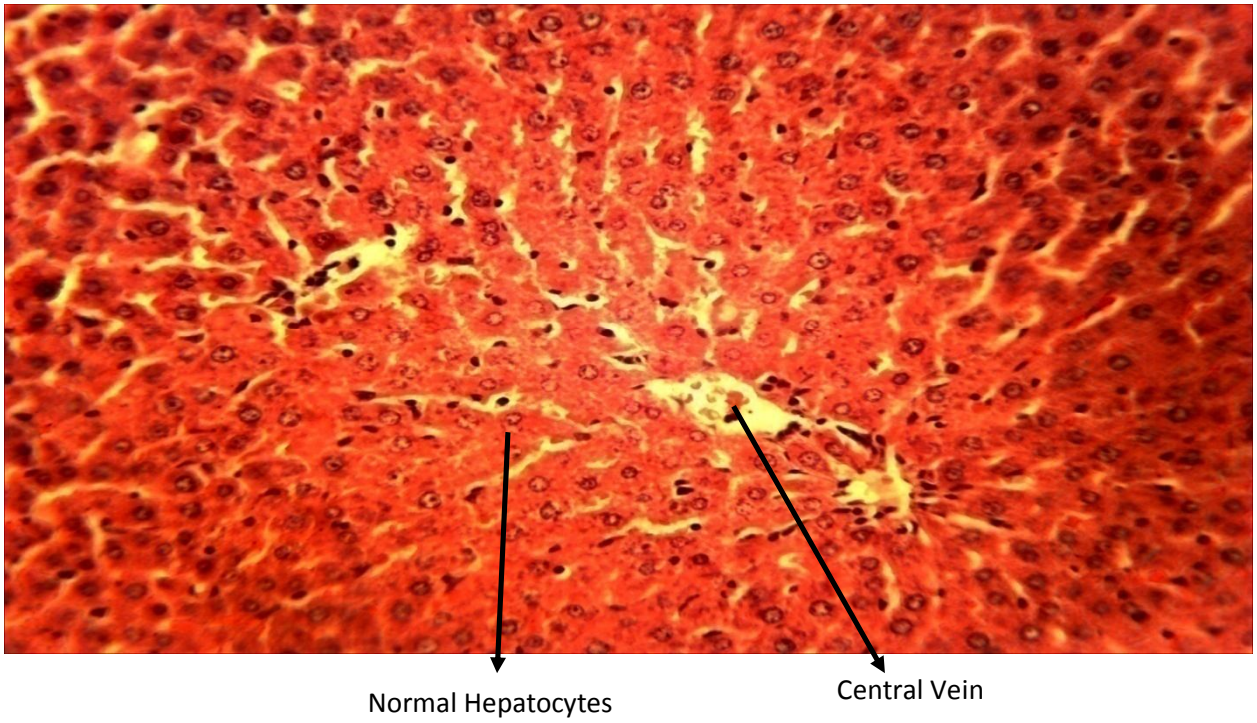


Plate VIII. Photomicrograph of a section of the Liver of a Wistar rat administered Ethanolic leaf extract of*Cussoniabarteri* 100mg/kg for 28 days (H & E x 250). Normal features.

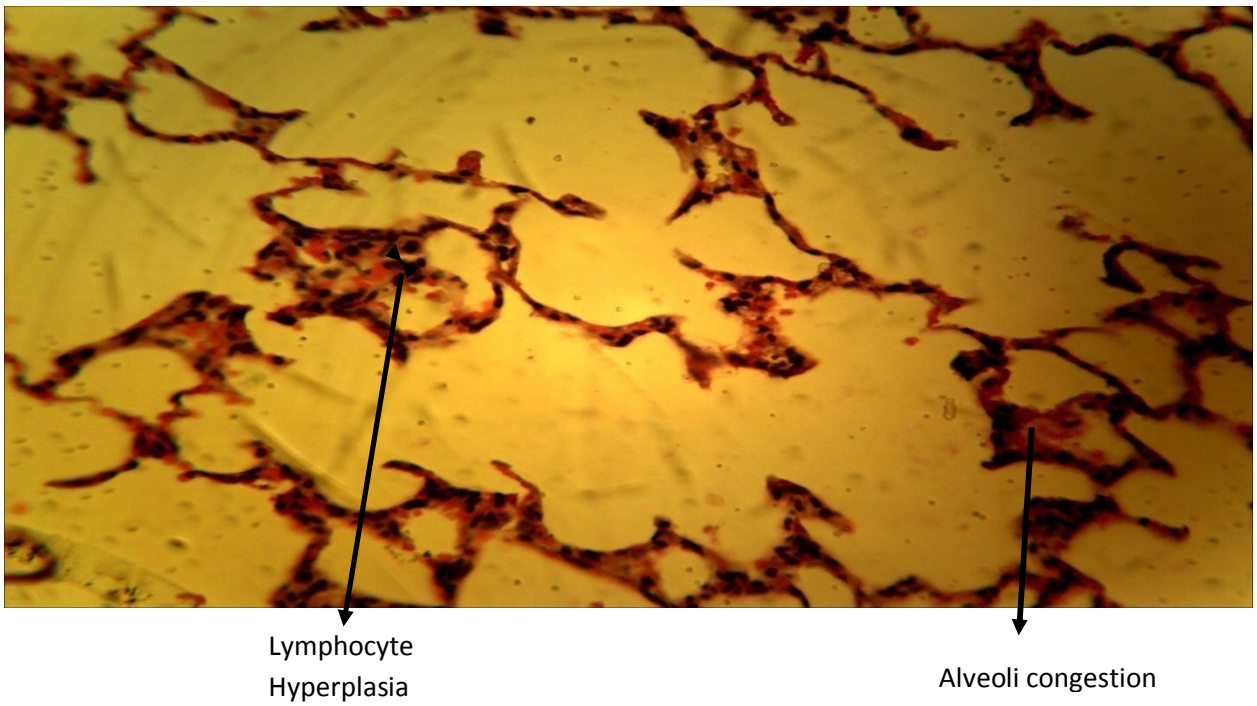


Plate IX. Photomicrograph of a section of the Lungs of a Wistar rat administered Ethanolicleaf extract of*Cussoniabarteri*100mg/kg for 28 days (H & E x 250). There was intense alveoli congestion with lymphocyte hyperplasia.

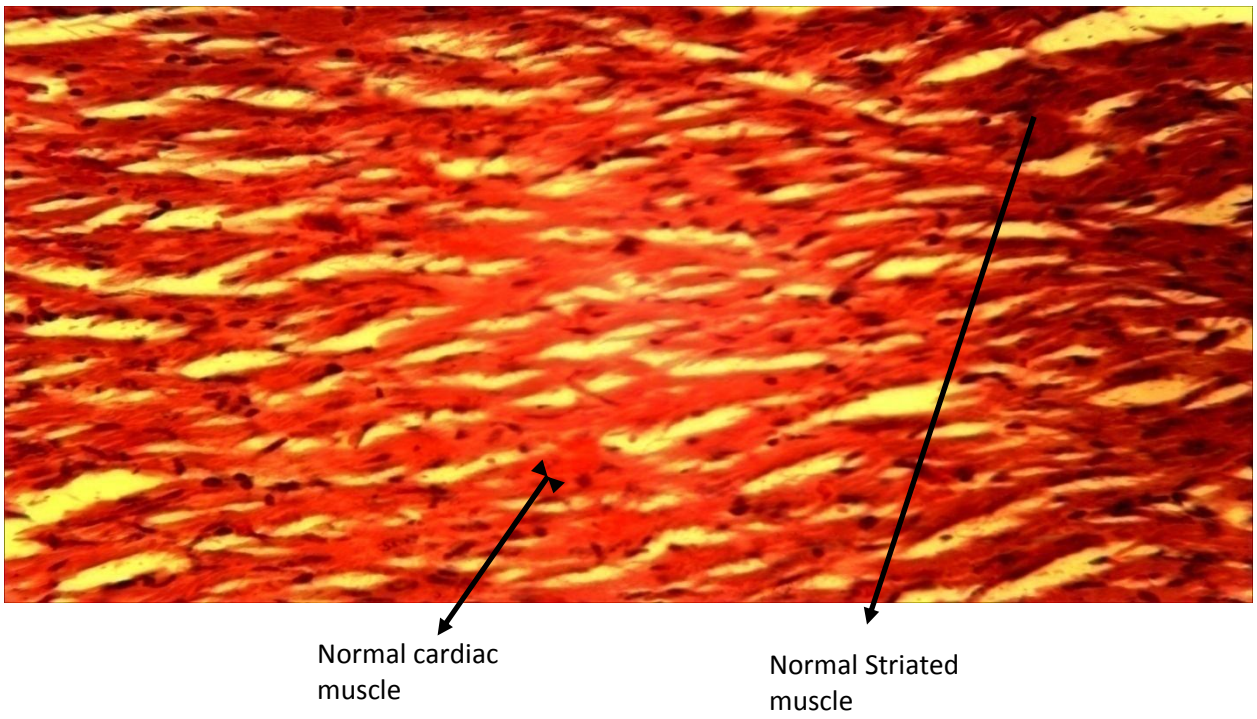


Plate X. Photomicrograph of a section of the Heart of a Wistar rat administered Ethanolicleaf extract of *Cussoniabarteri*200mg/kg for 28 days (H & E x 250). Normal cardiac muscles.

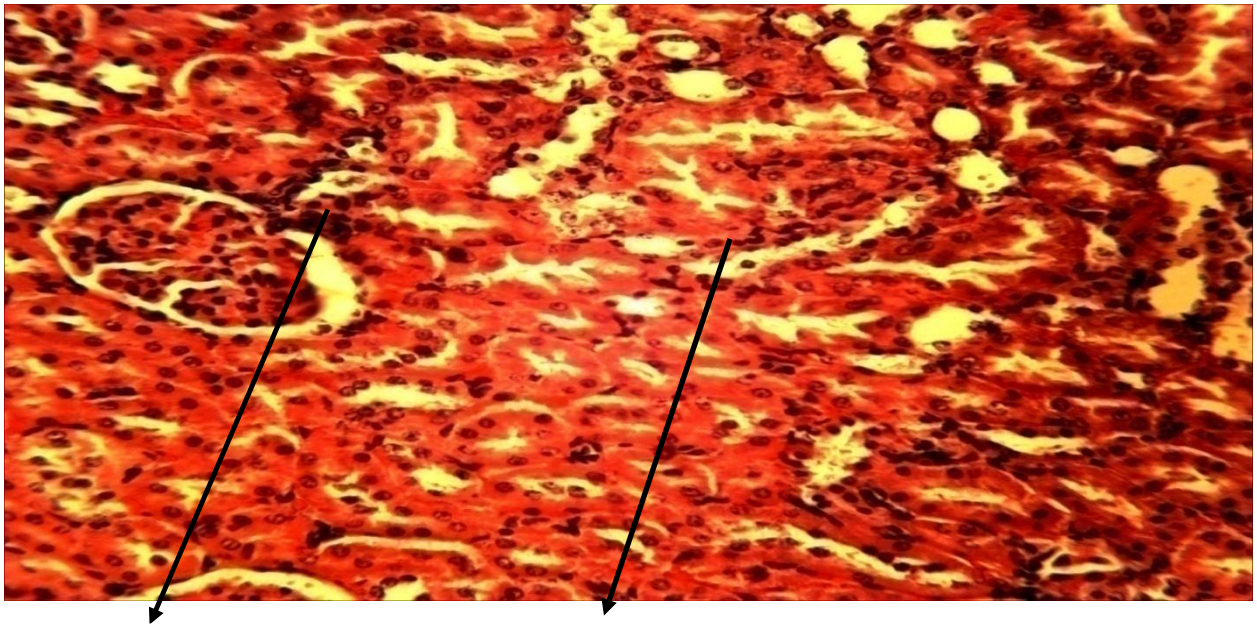


Plate XI. Photomicrograph of a section of the Kidney of a Wistar rat administered Ethanolicleaf extract of*Cussoniabarteri* 200mg /kg for 28 days (H & E x 250). There was tubular necrosis.

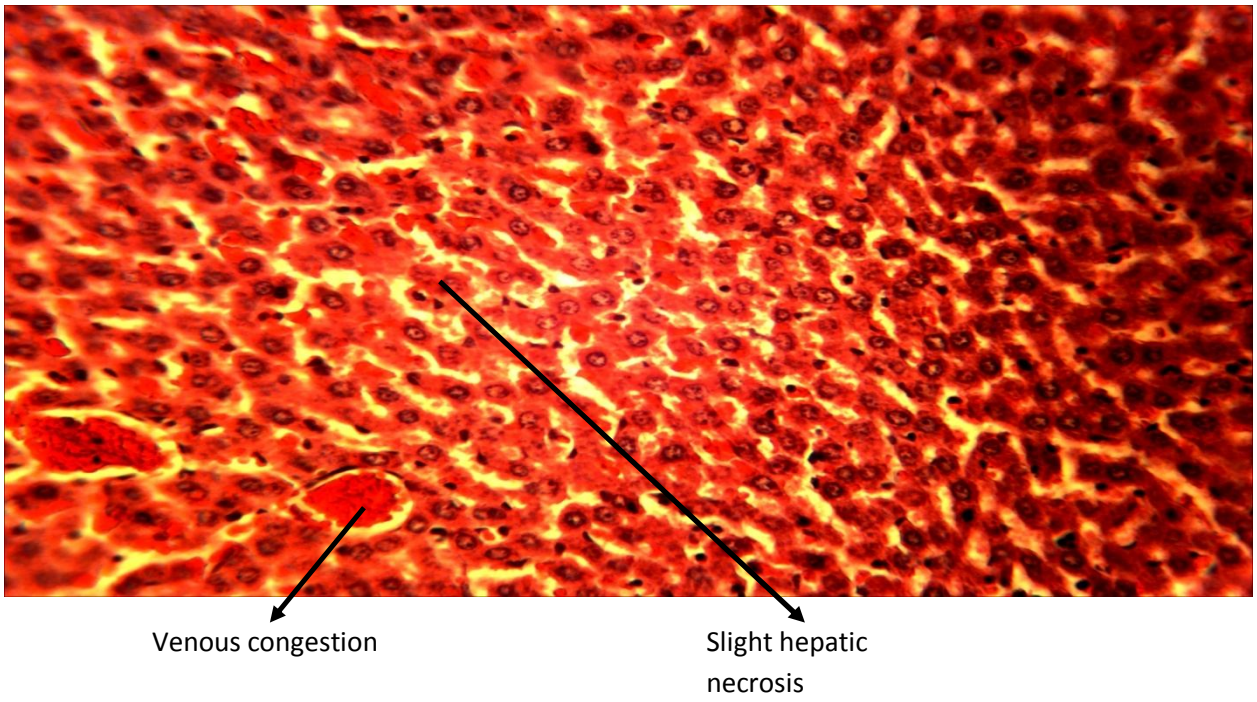


Plate XII. Photomicrograph of a section of the Liver of a Wistar rat administered Ethanolicleaf extract of*Cussoniabarteri*200mg/kg for 28 days (H & E x 250). There was venous congestion, sinusoidal congestion with slight hepatic necrosis.

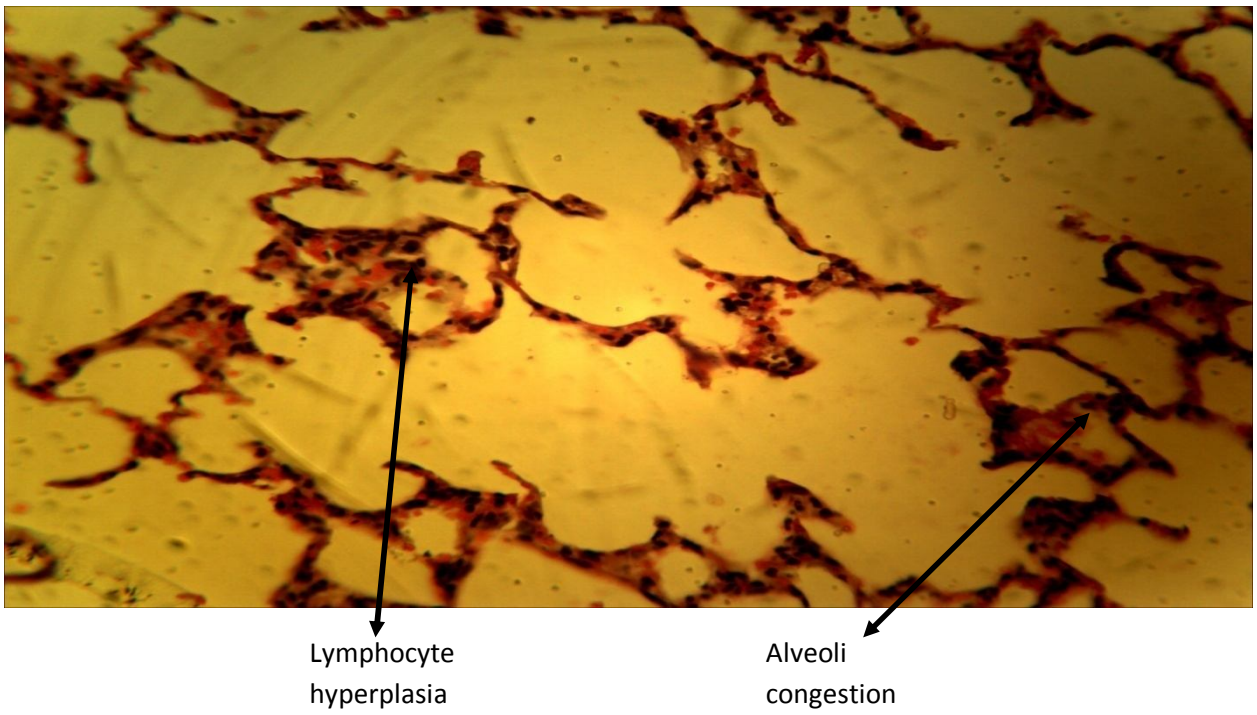


Plate XIII. Photomicrograph of a section of the Lungs of a Wistar rat administered Ethanolicleaf extract of*Cussoniabarteri*200mg/kg for 28 days (H & E x 250). There was moderate congestion with lymphocyte hyperplasia.

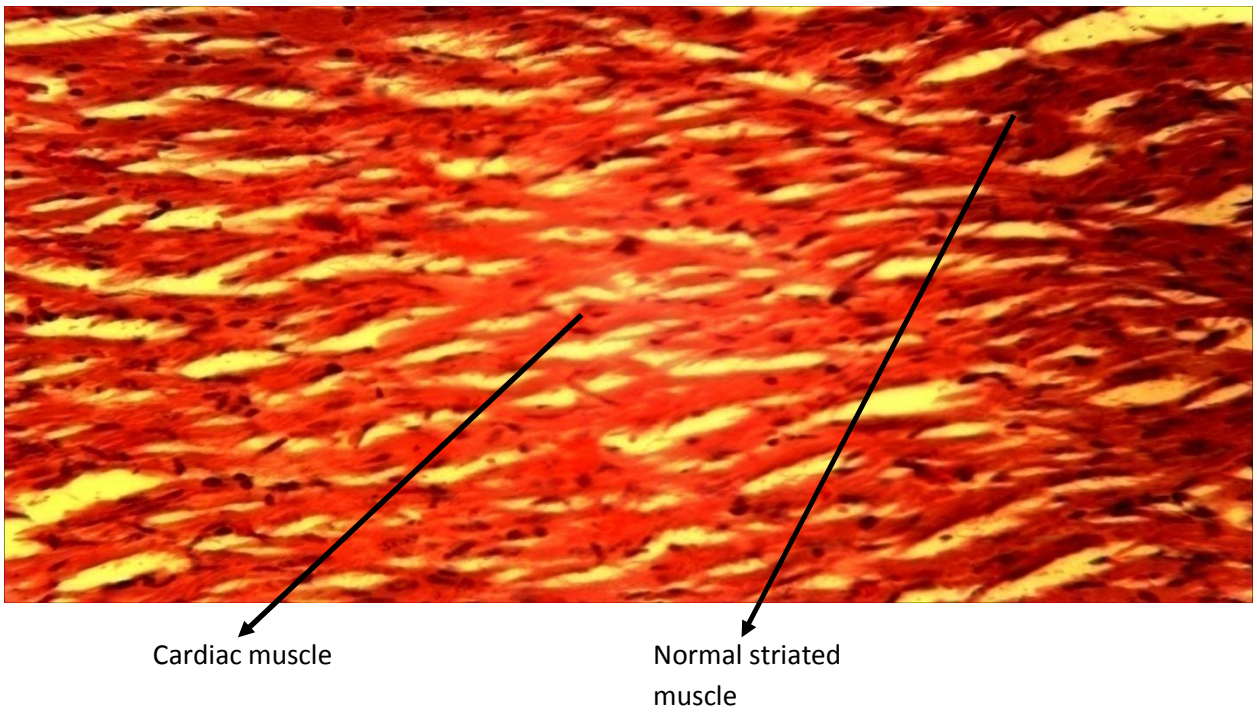


Plate XIV. Photomicrograph of a section of the Heart of a Wistar rat administered Ethanolicleaf extract of *Cussoniabarteri*400mg/kg for 28 days (H & E x 250). Normal features.

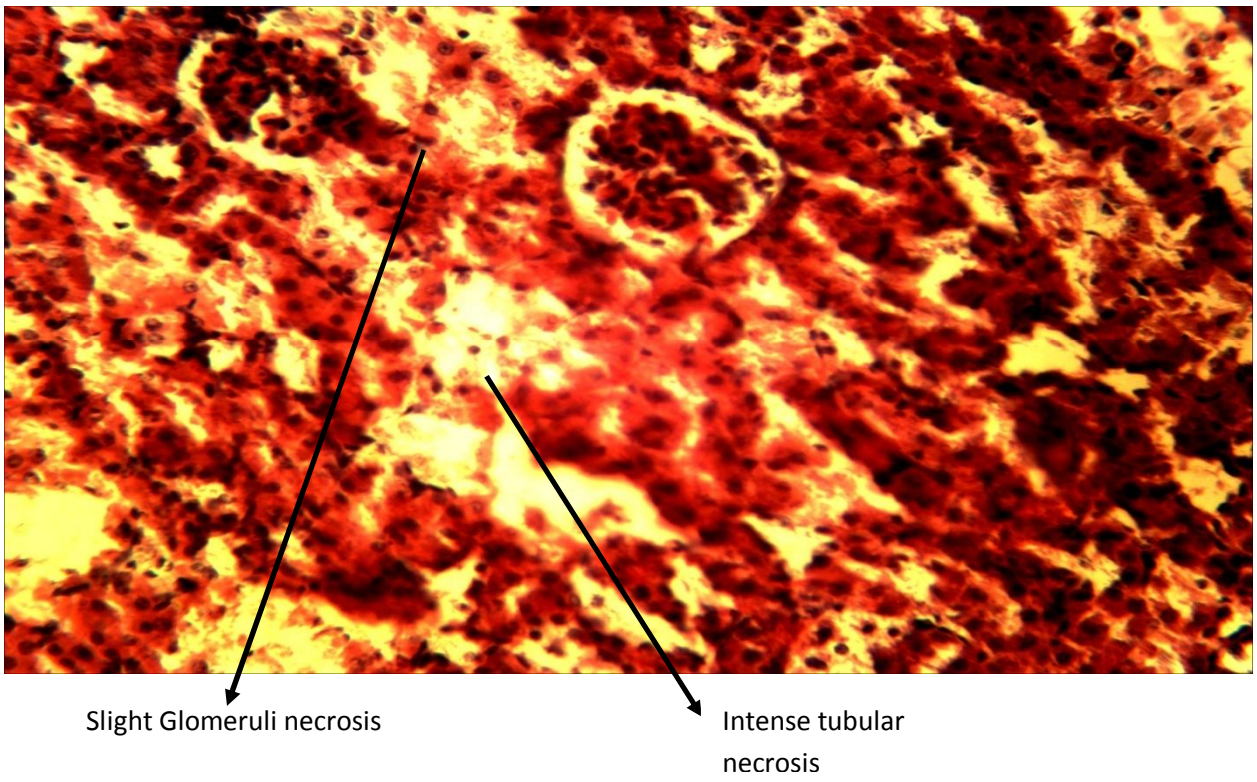


Plate XV. Photomicrograph of a section of the Kidney of a Wistar rat administered Ethanolicleaf extract of *Cussoniabarteri*400mg/kg for 28 days (H & E x250). The necrosis of tubules was intense with slight glomeruli necrosis.

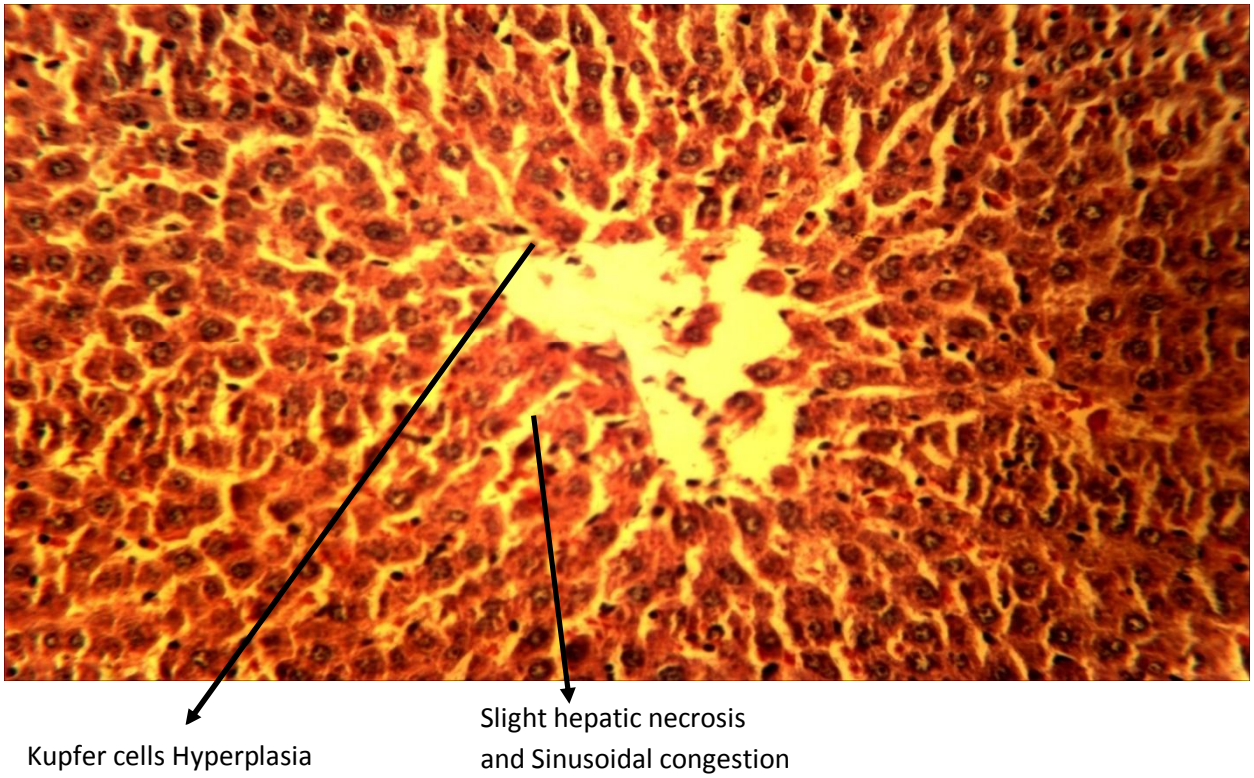


Plate XVI. Photomicrograph of a section of the Liver of a Wistar rat administered Ethanolicleaf extract of*Cussoniabarteri*400mg/kg for 28 days (H & E x 250). There was necrosis of the hepatic cells, sinusoidal congestion and kupfer cells hyperplasia.

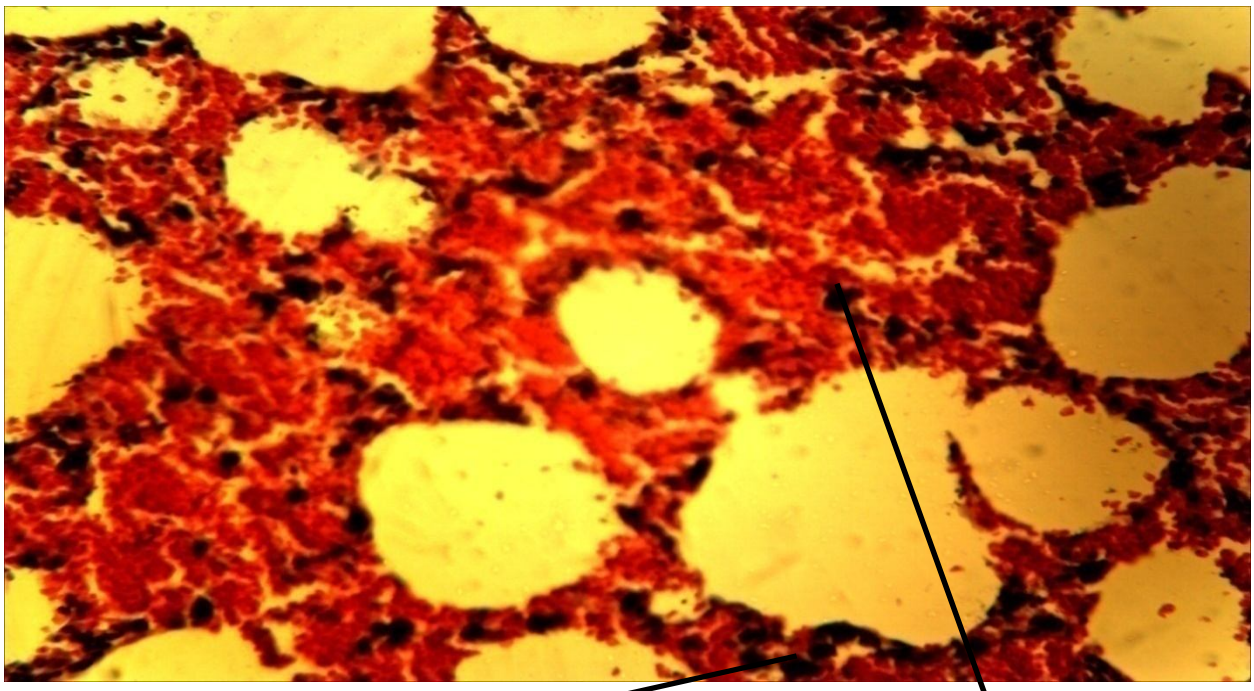


Plate XVII. Photomicrograph of a section of the Lungs of a Wistar rat administered Ethanolic leaf extract of*Cussoniabarteri*400mg/kg for 28 days (H and E X 250). Alveoli congestion.

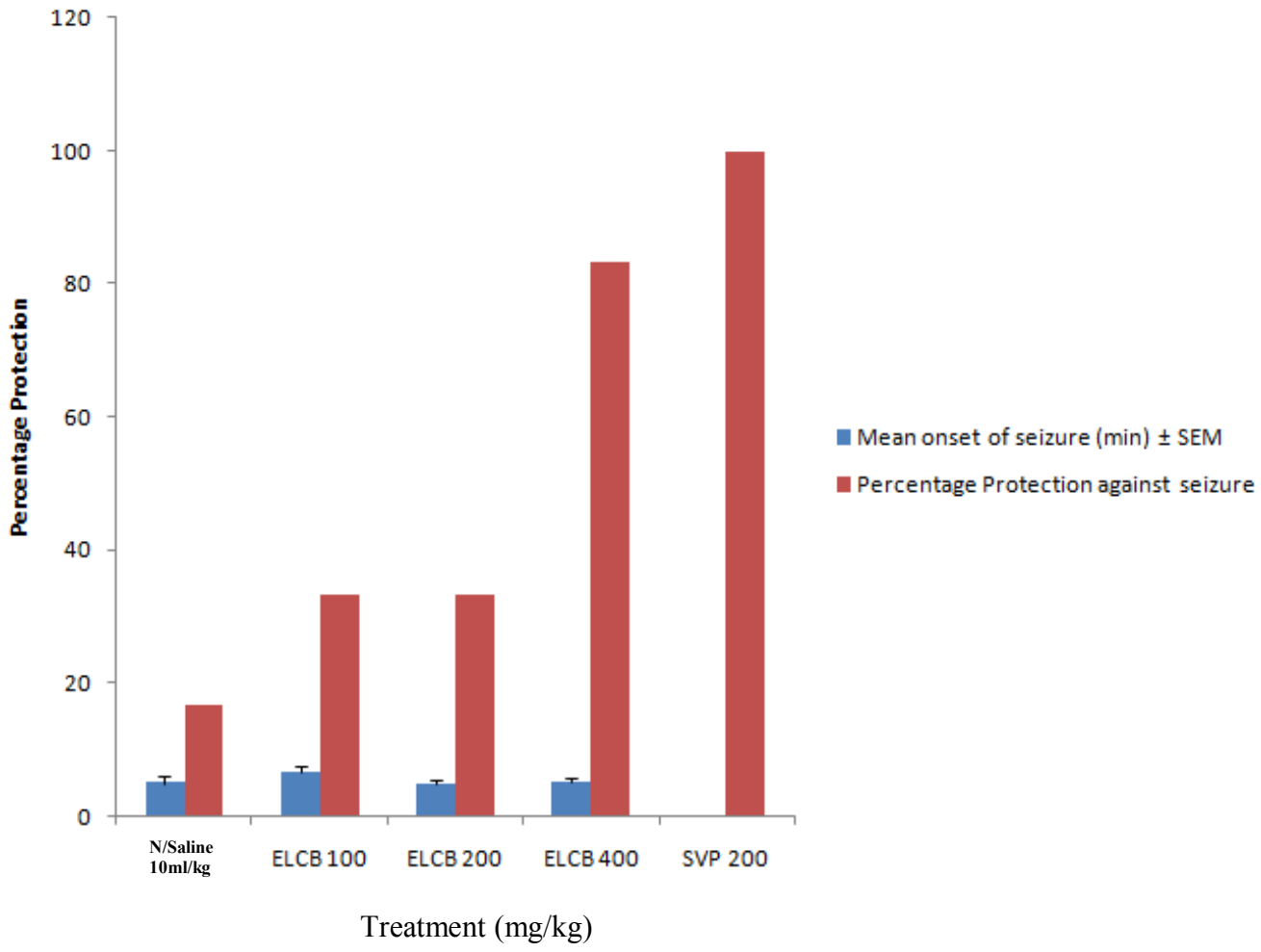


Figure 4.1: The effect of the extract against Pentylenetetrazole-induced seizure in mice; N/saline=Normal saline 10ml/kg, SVP=Sodium valproate, ELCB =Ethanolic Leaf extract of *Cussonia barteri*

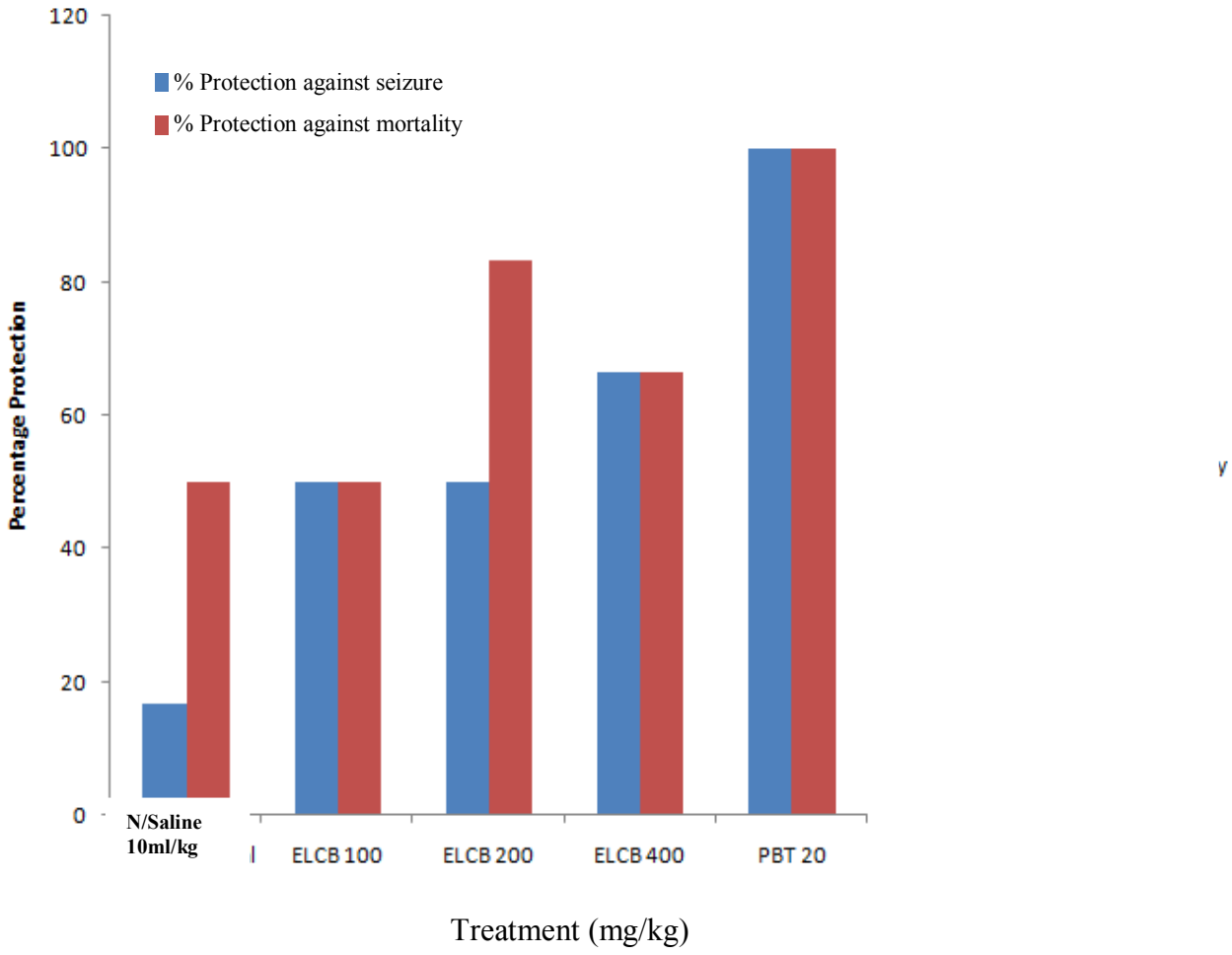


Figure 4.2: The effect of the extract against strychnine-induced seizure in mice; N/saline= Normal saline 10ml/kg,ELCB= Ethanolic Leaf extract of *Cussonia barteri,* PBT= Phenobarbitone. Data presented as Percentage Protection, n=6.

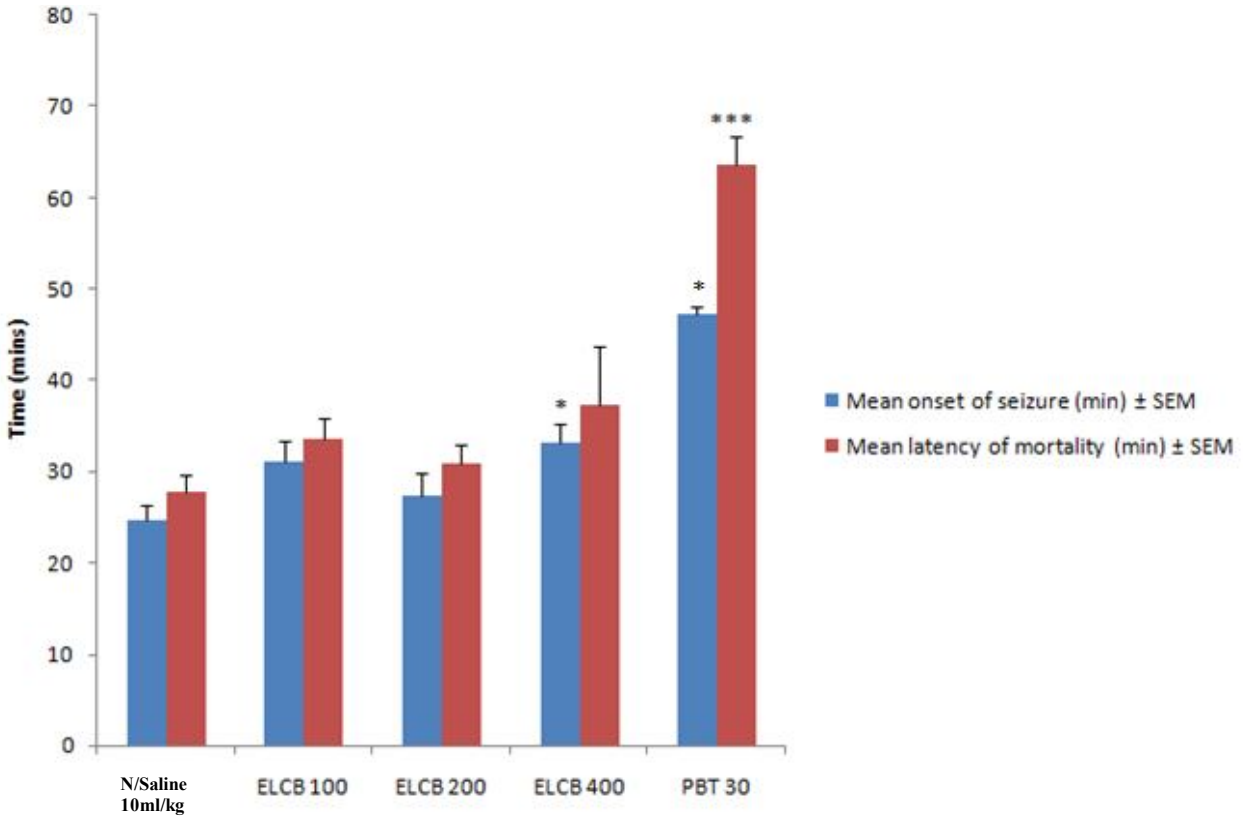


Figure 4.3: The effect of the extract against Isoniazid-induced seizure in mice; N/saline= Normal saline; ELCB= Ethanolic Leaf extract of *Cussonia barteri*; PBT= Phenobarbitone. *P<0.05, ***P<0.001, Statistical tool: ANOVA (one way).

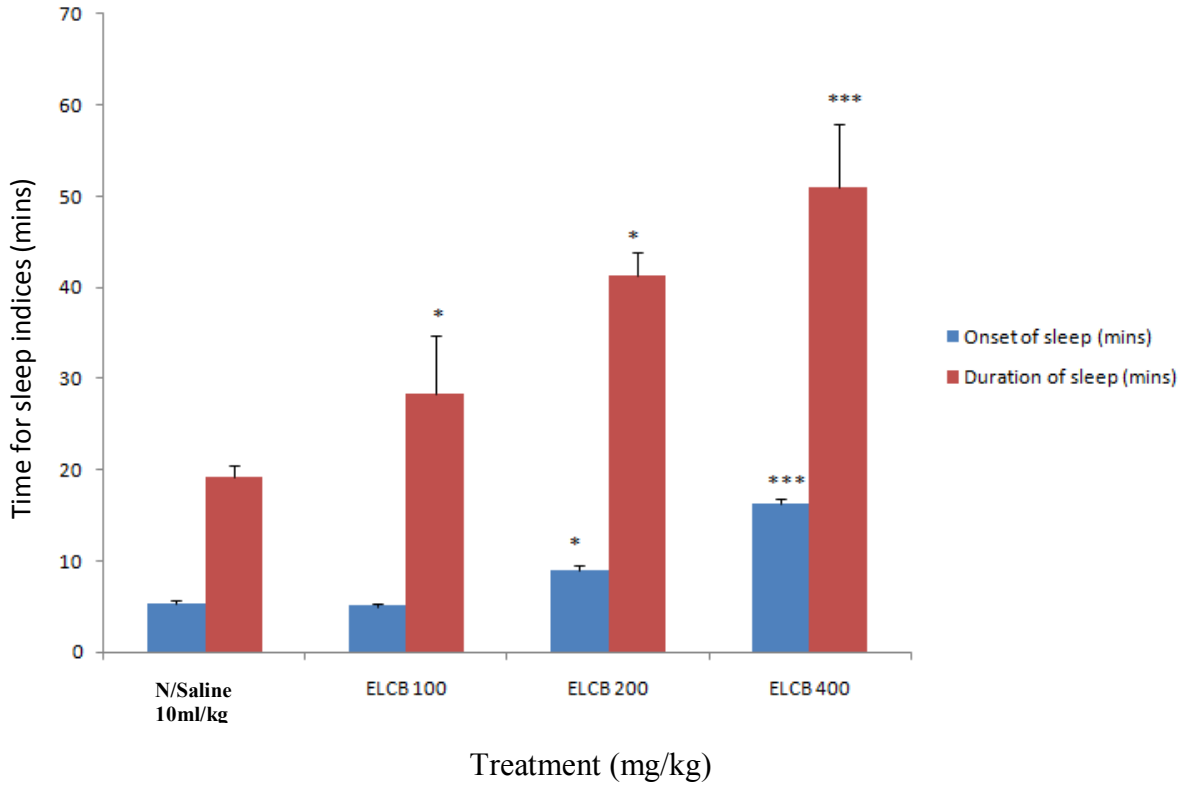


Figure 4.4: The effect of the extract on Diazepam-induced sleep in mice; onset and duration of sleep presented as mean ± SEM= Standard Error of Mean, N/saline= Normal saline; ELCB= Ethanolic Leaf extract of *Cussonia barteri*. *P<0.05, ***P<0.001; Statistical tool: ANOVA (one way).

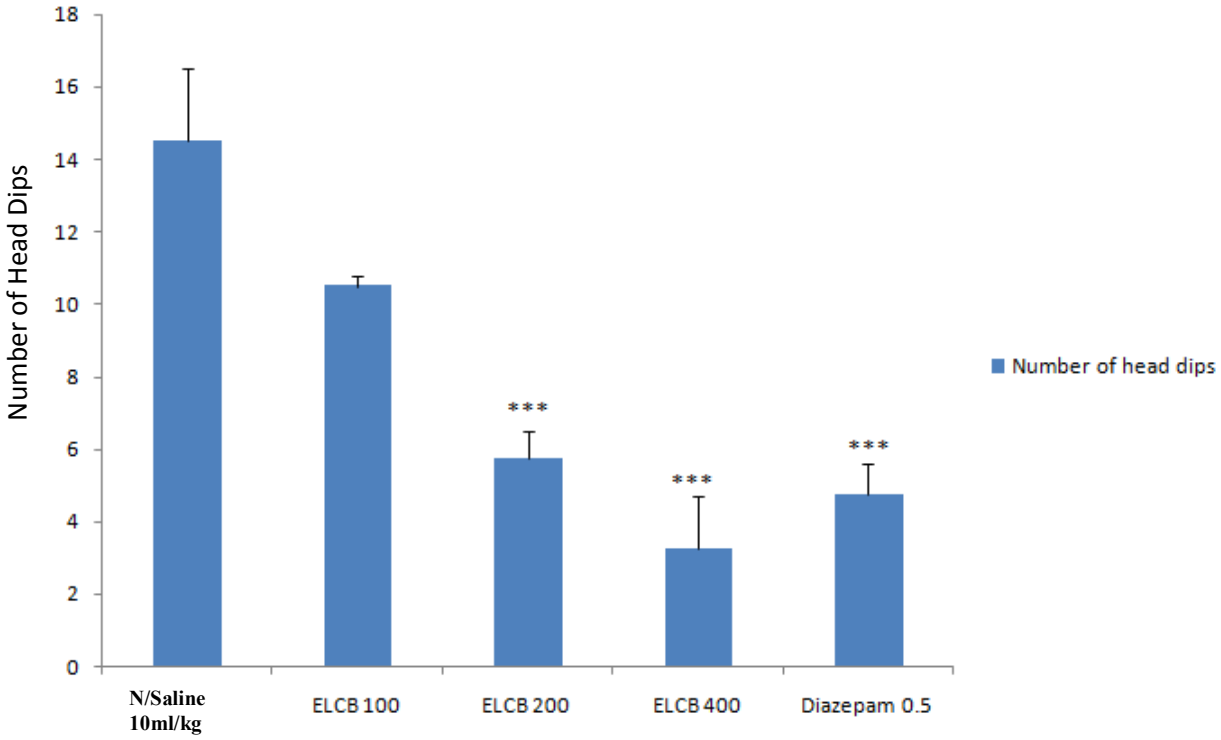


Figure 4.5: The effect of the extracton exploratory activity of mice in hole-board test; N/saline= Normal saline; ELCB= Ethanolic Leaf extract of *Cussonia barteri*. ***P<0.001, Statistical tool: ANOVA (one way).

CHAPTER FIVE

DISCUSSION

Generally, the data presented here suggest that the ethanolic leaf extract of *Cussonia barteri* may contain psychoactive substances that are sedative in nature, with potential anticonvulsant properties.

The preliminary phytochemical screening of the ethanolic leaf extract of *Cussonia barteri* revealed the presence of flavonoids, saponins, cardiac glycosides, steroids/terpenoids and tannins which might be responsible for the observed pharmacological activities of the extract.

The acute toxicity study indicated that the extract at a dose of 5,000 mg/kg caused neither visible signs of toxicity nor mortality in rats, suggesting its safety. The oral median lethal dose (LD_{50}) of the extract in rats is greater than 5,000 mg/kg and is of no practical interest based on level of safety by Lorke (1983).

Generally, reductions in body weight and internal organ weights are considered to be simple and sensitive indices of toxicity after exposure to toxic substances (Teo *et al*., 2002).
The results from the present study indicated that the extract did not significantly affect the body weight, suggesting that it did not hinder rat growth. However, the extract slightly increases the relative weight of the brain, liver and heart compared to the control group, which suggest that the extract might have caused hypertrophy of these organs.

The lack of significant alterations in the levels of ALT, AST, alkaline phosphatase, urea, and creatinine, which are good indicators of liver and kidney functions (El Hilaly *et al.*, 2004), suggests that sub-acute administration of ethanolic leaf extract of *Cussonia barteri* neither altered hepatocytes and kidney functions of rats nor the normal metabolism of the animals. However, the slight increase in the relative weight of the liver and the lesions or pathological changes observed in histopathological evaluation of the liver indicates that the extract might have toxic potential on liver with increasing dose. On the other hand, it could be suggested that these changes may not be toxicologically significant, as they were not corroborated by the biochemical findings (ALT, AST and ALP).

Histopathological evaluation of the sub-acute oral ingestion of the ethanolic leaf extract of *Cussonia barteri* in rats showed that the morphology of kidney, liver and lungs were adversely affected, suggesting the extract could be toxic to the hepatocytes, kidney and lungs. Alanine Transaminase (ALT) and Aspartate Transaminase (AST) are largely used in the assessment of liver damage by drugs or any hepatotoxin (Dobbs *et al*., 2003). The ratio of AST to ALT is sometimes used in differentiating causes of liver damage. Elevated AST levels are not specific for liver damage. Therefore, ALT is more specific to the liver, and thus a better parameter for detecting liver injury (Xu *et al*., 2002).Serum ALP level is related to the function of hepatic cell, thus increase in serum level of ALP is due to increased synthesis of the enzyme (Nyblom *et al*., 2006). In the present study, the pathological features observed in the liver was not supported by the plasma levels of aminotransferases (AST, ALT and ALP), thus further histological study and more specific

assays of toxicity could furnish more information regarding the hepatotoxicity of the extract.

Urea is a waste product of protein metabolism while creatinine is a waste product of muscle metabolism and both are excreted by kidney. The kidneys regulate the excretion of urea and reabsorption of electrolytes into the blood. Filtration occurs at the glomeruli while reabsorption takes place in the renal tubules. When there is compromise of normal glomerular function, substances normally cleared by the kidneys such as urea and creatinine accumulate in the biological fluid. The absence of any significant effect on the levels of serum urea, creatinine, potassium, sodium and chloride in this study suggests that the normal excretion of these biomolecules and electrolytes by the kidney was not adversely affected by the extract.

The haematopoietic system is one of the most sensitive targets of toxic compounds and is an important index of physiological and pathological status in man and animals (Adeneye *et al*., 2006). After 28 days of treatment, there were no treatment-related changes in haematological parameters between control and treated groups, indicating that the extract was not toxic to circulating red cells, nor interfered with their production and that of platelets.

The absence of protection against hind limb tonic-clonic extension (HLTE) in the maximal electroshock test (MEST) indicates that the extract cannot inhibit or prevent seizure discharge within the brain stem seizure substrate (Browing, 1992). This suggests that the

ethanolic leaf extract of *Cussonia barteri* may not be useful in the treatment of generalized tonic-clonic and partial seizures.

Pentylenetetrazole (PTZ) is a known convulsant and anticonvulsant activity in the subcutaneous PTZ test identifies compounds that can raise the seizure threshold in the brain (White *et al*., 1998).The moderate activity of the extract against PTZ-induced seizure suggests that it may have the potential of raising seizure threshold. It may, therefore be beneficial in the treatment of myoclonic and absence seizure (Loscher *et al*., 1991). Antiepileptic drugs like phenobarbitone, sodium valproate, ethosuximide and benzodiazepines effective in the treatment of generalized seizures of petit mal type (absence or myoclonic) suppress PTZ-induced seizures in a dose-dependent manner (Loscher, 2002). Sodium valproate which was used in this study as a reference anticonvulsant agent produced 100% protection against PTZ-induced seizures. According to De Sarro and Ferreri (2003), PTZ may be exerting its convulsant effect by inhibiting the activity of GABA at $GABA_A$ receptors. GABA is the major inhibitory neurotransmitter which is implicated in epilepsy. Thus, the inhibition of PTZ-induced seizures by the extract at the highest dose treated suggests that it may exert its anticonvulsant effect by enhancing GABAergic neurotransmission.

Strychnine (STN) is a competitive glycine receptor antagonist (Rajendra *et al*., 1997). The presence of anticonvulsant activity in the subcutaneous STN test by ethanolic leaf extract of*Cussonia barteri* indicates that the plant may contain compound(s) that interact with the glycine receptors probably as agonists or enhancing the binding of glycine to its receptors.

Picrotoxin (PIC) is a non-competitive GABA antagonist that blocks the chloride channel (Takeuchi and Takeuchi, 1969). The absence of protection against PIC induced seizures indicates that the extract may not exert its anticonvulsant activity by interacting with picrotoxin sites on $GABA_A$ –chloride ion channel complex.

Isoniazid (INH) induces seizure by interfering with GABA synthesis through inhibition of glutamic acid decarboxylase (GAD) activity, leading to rapid depletion of GABA.
The delay in the mean onset of INH-induced seizure at the dose of 400mg/kg of the extract suggests that the extract may exert its anticonvulsant activity through GABAergic neurotransmission.

Aminophylline has been reported to inhibit binding of GABA and diazepam to $GABA_A$ receptors in brain membranes, although the affinities are low (Boulenger*et al*., 1982: Daly, 2000). The absence of protection against aminophylline-induced seizure suggests that the extract may not act by inhibiting binding of GABA and diazepam to $GABA_A$ receptors. It could also be due to the strong convulsive action of aminophylline that are usually associated with repetitive generalized seizures relatively resistant to antiepileptic drugs (Ochi, *et al*., 2009) as even the standard drug used (Phenobarbitone 30mg/kg) could only significantly delay the mortality.

Diazepam binds to $GABA_A$ receptor and potentiates its activation. This enhancement of neuronal inhibition by GABA produces sedation (reduction of motor activity) which is mediated via α1 $GABA_A$receptors (Tobler *et al*., 2001). Many herbal preparations like

chamomile tea and valerian have been shown to enhance the positive allosteric modulating effects of benzodiazepines on $GABA_A$ receptors (Johnston, 2005). In the present study, ethanolic leaf extract of *Cussonia barteri* significantly prolonged the duration of sleep induced by diazepam. The ability of the extract to potentiate the sedative property of diazepam suggests that it may possibly act by interacting with GABA-mediated synaptic transmission. Previous works have reported the anticonvulsant and sedative properties of flavonoids (Johnston, 2005; Yao *et al*., 2010; Hanrahan *et al*., 2011). Flavonoids, found to be present in the ethanolic leaf extract of *Cussonia barteri* may therefore be responsible for the observed anticonvulsant and sedative activities.

The Hole-board test is a measure of exploratory behaviour in animals (File and Wardil, 1975).The head dipping behaviour is sensitive to changes in the emotional state of the animals and increase in head dipping behaviour is a reflection of anxiolytic activity (Takeda *et al*., 1998) while a decrease in the head dipping reveals sedative behaviour (File and Pellow, 1985). In this test, the extract at the doses of 200 and 400 mg/kg significantly ($P<0.01$ and $P<0.001$ respectively) produced a decrease in exploratory behaviour as indicated by decrease in the number of head dip. This finding supports the sedative property of the extract.

The mouse beam walking assay was employed to evaluate the effect of the plant extract on the motor coordination behaviour of the animals. It is a good predicator of drug producing clinical sedation and offers improved sensitivity over the mouse rota-rod in motor coordination deficit determination (Stanley *et al*., 2005). The increase in the number of foot

slips produced by the extract in this study is an index of motor coordination deficit. The above finding therefore suggests that the extract might cause clinical sedation at the highest dose tested.

The open field test (Walsh and Cummins, 1976) provides simultaneous measures of locomotion, exploration and anxiety. The open field model examines anxiety related behavior characterized by the normal aversion of the animal to an open, brightly lit area. Thus, animals removed from their acclimatized cage and placed in environment express anxiety and fear, by showing alteration in all or some parameters. Anxiolytic treatments reduce such fearful behavior of animals in open field (Mechan *et al*., 2002). Significant increase in number of rearing and number of square crossed by the animals in an open field during the 5 minutes interval is an indicative of anxiolytic activity. In the present study, the ethanolic leaf extract of *Cussonia barteri* did not significantly increase the number of rearing and number of square crossing and the animals were seen spending more time in the corners and the periphery than in the centre which suggests that the extract do not have anxiolytic activity.

CHAPTER SIX

6.0 Conclusion and Recommendation

6.1 Conclusion

In conclusion, the findings of these studies suggest that the ethanolic leaf extract of *Cussonia barteri* may contain bioactive compounds which possess significant anticonvulsant activity against pentylenetetrazole and strychnine-induced seizures therefore may be beneficial for the management of myoclonic and absence (petit mal) seizures. The use of *Cussonia barteri* in traditional medicine for the management of epilepsy in Nigeria and Ghana may be justifiable.

The anticonvulsant effects might be exerted through enhancement of GABAergic neurotransmission or interaction of the compounds with glycine receptors. The prolongation of duration of diazepam-induced sleep in mice is an indicative of sedative property and CNS depressant effect. However, prolong uses of the extract at high doses may be toxic to some organs and should be used with caution.

6.2 Recommendations

i. More toxicological studies should be conducted on the plant.

ii. Further purification and pharmacological investigations are needed to identify the active principle(s) responsible for the anticonvulsant and sedative properties of the plant extract.

References

Adeneye, A.A., Ajagbonna, O.P., Adeleke, T.I. and Bello, S.O. (2006). Preliminary toxicity and phytochemical studies of the stem bark aqueous extract of *Musangacecropioides* in rats. *Journal of Ethnopharmacology,* 105: 374-379.

Adeyemi, O.O., Omoniyi, Y.K., Olayemi, A.O. (2007): Sedative and anticonvulsant effects of the aqueous root extract of*Senevieri cagerome and Labroy. Journal of Ethnopharmacology*, 113: 111-114.

Ambawade, S.D., Kasture, V.S., Kasture, S.B., (2002). Anticonvulsant activity of roots and rhizomes of *Glyrrhizaglabra*.*Indian Journal of Pharmacology* 34: 251-255.

Amos, S., Kolarole, E., Akah, P., Wambebe, C. and Gamaniel, K. (2001).Behavioural effects of the aqueous extract of *Guierasenegalensis* in mice and rats.*Phytomedicine,* 8 (5): 356-361.

Azubike, J.C. and Nkanginieme, K.E.O. (1996).Paediatrics and child health in tropical region.Fourth Dimension publishers, Enugu, Nigeria, p.388.

Balamurugan, G., Muralidharan, P., and Selvarajan, S. (2009). Antiepileptic activity of polyherbal extract from Indian medicinal plants. *Journal of scientific Research,* 1(1): 153-159.

Beretz, A., Haag-Berrurie, R.M., Anton, R. (1978). Choix de methods pharmacologiques pour l'etude des activities de l'aubepine. *Medicinal plants and herbal medicine*, 4: 305-314.

Boulenger, J.P., Patel, J. and Marangos, P.J. (1982).Effects of caffeine and theophylline on adenosine and benzodiazepine receptors in human Brain.*Neuroscience letter*, 30:161-166.

Browing, R. (1992). The electroshock model, neuronal network and antiepileptic drugs. In: Faingold, C.L. and Fromn, G.H. (Eds). *Drugs for control of Epilepsy: Actions on Neuronal Networks in seizure Disorders*, CRC press. Boca Raton, FL. Pp.195-211.

Brown, R.E., Corey, S.C. and Moore, A.K. (1999).Differences in the measure of exploration and fear MHC-congenic C57BL/6J and B6-H-2K Mice.*Behaviour Genetics*, 26: 263-271.

Burkill, H.M. (1985). The useful plants of West Tropical Africa, 1: pp. 211-212.

Chandradhar, D. (2001): Antiepileptics; *American Journal of Pharmaceutical Education,* 65: 197-202.

Cockerel, O.C., Johnson, L., Sander, J.W.A., Hart, Y.M., Shorvon, D.S. (1995). Remission of Epilepsy: results from the National general practice study of epilepsy. *Lancet,* 346: 140-144.

Daly, J.W. (2000). Alkylxanthines as research tools. *Journal of Autonomic Nervous System*, 81: 44-52.

De Sarro, G., Ferreri, G. (2003). Comparative anticonvulsant activity of some 2, 3-benzodiazepine derivatives in rodents.*Pharmacological Biochemical Behaviour,* 74: 595-602.

De Villiers, B.J., Van Vuuren, S.F., Van Zyl, R.F. and Van Wyk, B.E. (2010).The antimicrobial and antimalarial activity of *Cussonia species (Araliaceae)*. *Journal of Ethnopharmacology*, 129: 189-196.

Dennis, K.L., Eugene, B., Anthony F. (2003).*Harrison's principle of internal medicine* (15th ed), New York, Mcgraw-Hill companies, 2542- 2543.

Dhanasekaran, S. and Palayan, M. (2010). CNS depressant and antiepileptic activities of the methanol extract of the leaves of *Ipomoea aquatic*Forsk,*E-Journal of Chemistry*, 7(5): 1555-1561.

Dobbs, N.A., Twelves, C.J., Gregory, A., Cruickshanka, C., Richards, M.A. and Reubens, R.D. (2003).Epirubicin in patients with liver dysfunction. Development and evaluation of a novel dose modification scheme. *European Journal of cancer,* 39: 580-586.

Dubois, M.A., Ilyas, M.A. and Wagner, H. (1986).Cussonosides A and B: The two Triterpene-saponins from *Cussoniabarteri. Plantamedica*,52(2): 80-83.

Elujoba, A.A., Odeleye, O.M. and Ogunyemi, C.M. (2005).Traditional medical development for medical and dental health care delivery system in Africa. *African Journal of Traditional, complementary and Alternative medicine*, 2(1), 46-61.

El-Hilaly, J., Israili, Z.H. and Lyoussi, B. (2004). Acute and chronic toxicological studies of *Ajugaiva* in experimental animals.*Journal of Ethnopharmacology*, 91: 43-50.

File, S.E. (1973).Effects of chlorpromazine on exploration and habitation in the rat.*British Journal of Pharmacology,* 49:303-310.

File, S. and Pellow, S. (1985). The effect of Triazolobenzodiazepines in two animal tests of anxiety and on the hole-board.*British Journal of Pharmacology*, 86:729-735.

File, S. and Wardil, A.G. (1975). Validity of head-dipping as a measure of explorating a modified hole-board.*Psychopharmacology* 44: 53-59.

Fisher, R.S., Van Emde Boas W., Blume W., Engel J. (2005). Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia,* 46(4):470-472.

Hanrahan, J.R., Chebib,M., Johnston, G.A.R. (2011). Flavonoid modulation of $GABA_A$ receptors. *British Journal of Pharmacology*, 163: 234–245.

Herrero, A.I., Del Olmo, N., Gonzales-Escalada, J.R. (2002). Two new actin topiramate: inhibition of depolarizing $GABA_A$ –mediated responses and activation of a potassium conductance. *Neuropharmacology,* 42: 220-221.

Hosseinzadeh, H., and Madanifare, M. (2000). Anticonvulsant effects of *Coriandrumsativum L*. seeds extract in mice. *Archives of Iranian Medicines,* 3: 182-184.

Hosseinzadeh, H., Parvadeh, S., (2004). Anticonvulsant effects of thymoquinone, the major constituents of Nigella sativa seeds, in mice. *Phytomedicine.* 11: 56-64.

Johnston, G.A.R. (2005). $GABA_A$ Receptor Channel Pharmacology.*Current Pharmaceutical Design,* 11: 1867-1885.

Kale, S.S., Shete, R.V., Kore, K.J., Patil, B.M., Bhutada, R.M. and Pattankuda, V.S. (2010). Anticonvulsant activity of Glycyrrhizic acid in mice.*International Journal of Pharmaceutical Research and Development,* 2(10): 123-128. Retrieved from https://www.ijprd.com.

Kraus, G.L., Johnson, M.A., Miller, N.R. (1998).Vigabatrin associated retinal cone system dysfunction; electroretinogram and ophthalmologic findings.*Neurology*, 50:614-8.

Kupferberg, H.J. (2001). Animal Models Used in the Screening of Antiepileptic Drugs, *Epilepsia*, 42 (Suppl. 4): 7–12.

Lorke, D.A. (1983). A new approach to practical acute toxicity testing.*Archives of Toxicology,* 54: 275-287.

Loscher, W., Honack, D., Fassbender, C.P., Nolting, B. (1991).The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs.III. Pentylenetetrazole seizure models.*Epilepsy Research*, 8: 171-189.

Loscher, W. (2002).Animal models of epilepsy for the development of antiepileptogenic and disease-modifying drugs. A comparison of the pharmacology of kindling and post-status epilepticus models of temporal lobe epilepsy. *Epilepsy Research*, 50: 105-123.

Maiha,B.B., Magaji, M.G., Yaro, A.H., Hamza, A.H., Ahmed, S.T., Magaji, R.A. (2009). Anticonvulsant studies on *Cochlospermuntintorium and Paulliniapinnata* extract in laboratory animals. Nigeria Journal of Pharmaceutical Sciences, 8(1): 102-108.

Malvi, R.K., Bigoniya, P., Sethi, S., Jain, S. (2011). Medicinal plants used in the treatment of Epilepsy. *International Research Journal of Pharmacy*, 2(2): 32-39.

Mechan, A.O., Moran, P.M., Elliott, M., Young, A.J., Joseph, M.H., Green, R. (2002). A comparison between dark agouti and Sprague-Dawely rats in therbehaviour on the elevated plus-maze, open field apparatus and activity meters and their response to diazepam. *Psychopharmacology* (Berl); 159: 188–95.

Medina, J.H., Viola, H., Wolfman, C., Marder, M., Wasowski, C., Calvo, D. (1997). Flavonoids: A new family of Benzodiazepine receptor Ligands. *Neurochemical Research*, 22(4): 419-425.

Ndoye, N.F., A.D. Sow, A.G. Diop, B. Sessouma, (2005). Prevalence of Epilepsy, Its Treatment Gap and Knowledge, Attitude and Practice of Its Population in Sub-Urban Senegal, an ILAE/IBE/WHO Study. Seizure - *European Journal of Epilepsy,* 14(2): 106-111.

Ngoungou, E.B., Dubreuil, C.M., Marin, B., Houinato, D.J., Nubukpo, P., Dalmay, F., Millogo, A., Nsengiyumva, G., KounaNdouongo, P., Diagana, M., Ratsimbazafy, V., Druet-cabanac, M., and Preux, P.M. (2006). Epidemiology of epilepsy in sub-Saharan Africa: a review. *Sante*, 16(4): 225-238.

Njamnshi, A.K., Bissek, A.C., Yepnjio, F.N., Tabah, E.N., Angafor, S.A., Kuate, C.T., Dema, F., Fonsah, J.Y., Acho, A., Kepeden, M.N., Azinwi, Y.H., Kuwoh, P.B., Angwafor, F.F. and Muna, W.F. (2010). A community survey of knowledge, perceptions, and practice with respect to epilepsy among traditional healers in the Batibo Health District, Cameroun.*Epilepsy and behaviour*, 17(1): 95-102.

Nyblom, H., Bjornsson, E., Simren, M., Aldenborg, F., Almer, S., Olsson, R. (2006). "The AST/ALT ratio as an indicator of cirrhosis in patients with PBC".*Liver International,* 26(7): 840-845.

Ochi, R., Katsuya, S., Hiromu, K. and Hiroaki, A. (2009): Effect of Brewer's yeast-induced pyrexia on Aminophylline-elicited convulsions in mice; *ActaMedica Okayama*, 63(5): 273-280.

OECD, 1995.Repeated dose 28-day oral toxicity study in rodents.OECD Guidelines for testing of chemicals, 407, pp.1-8.

OECD/OCDE 452; Guidelines for the testing of chemicals, September, 2009.

Ogunrin, A.O. (2006). Epilepsy in Nigeria: A review of etiology, epidemiology and management. *Benin Journal of Postgraduate medicine*, 8 (1): 27-51.

Osuntokun, B.O. (1978): Epilepsy in Africa. *Tropical Geographical Medicine*, 31: 24-31.

Oyeka, I.C. (1981). Lessons from Traditional medical and Health practices.*Interciencia* 6(3): 156-157.

Papajewski, S., Vogler, B., Conrad, J., Klaiber, I., Roos, G., Walter, C.U., Suessmuth, R., Kraus, W., (2001). Isolation from Cussoniabarteri of 1'-O-chlorogenoylchlorogenic acid and 1'- Ochlorogenoylneochlorogenic acid, a new type of quinic acid esters.*Planta Medical*, 67(8): 732-736.

Porter, R.J., Cereghino, J.J., Gladding, G.D. (1984). Antiepileptic drug development program. *Cleveland Clinic Journal of MedicineQuarterly*. 51: 293-305.

Rajendra,S., Lynch, J.W., and Schofield, P.R. (1997).The glycine receptor.*Pharmacological Therapeutics,* 73: 121-146.

Rakotonirina, S.V., Ngo Bum, E., Rakotonirina ,A., Bopelet, M. (2001). Sedative properties of the decoction of the rhizome of *Cyperusarticularis*.*Fitoterapia*, 72:22–29.

Sayyah, M., Kamalinejad, M., Rajab, B.H. and Rustaiyan, A. (1975).Antiepileptic potential and composition of the fruit essential oil of *Ferula gummosaBoiss.Indian Journal of medical Research,* 63 (3): 462-468.

Sayyah, M, Valizadeh, J., Kamalinejad, M. (2002).Anticonvulsant activity of the leaf essential oil of *Laurusnobilis* against pentylenetetrazole and maximal electroshock-induced seizures.*Phytomedicine*, 9:212-216

Senanayake, N and Roman, G.C. (1993).Epidemiology of epilepsy in developing countries.*World Health Organization Bulletin*; 71(2): 247-58.

Senthil, N.D. (2013). Effect of *Fagoniacretica (L)* extracts on biogenic amines concentrations in rat brain after induction of seizure. *International Journal of Preclinical & Pharmaceutical Research*, 4(2): 81-84

Sheorajpanday, R.V., De Deyn, P.P. (2007). Epileptic fits and epilepsy in the elderly: General reflections, Specific issues & therapeutic implications. *Clinical Neurology & Neurosurgery*, 109(9): 727-743.

Sridharan, R. (2002). Epidemiology of epilepsy.*Current Science,* 82:664-670.

Stanley, B. (2004). Recognition and respect for African Traditional Medicine. *International Development Research Center Bulletin*, 55582.

Stanley, J. L., Lincoln, R.J., Brown, T.A., McDonald, L.M., Dawson, G.R. and Reynolds, D.S. (2005). The mouse beam walking assay offers improved sensitivity over the mouse Rota-rod on determining motor coordination deficits induced by benzodiazepine. *Journal of Psychopharmacology*, 19 (3): 221 – 227.

Swinyard, E.A., Kupferber, H.J. (1985). Antiepileptic drugs: detection, quantification and toxicity and phytochemical studies of the stem bark aqueous extract of *Musangacecropioides* in rats. *Journal of Ethnopharmacology*, 105: 374-379.

Swinyard, E. A., Woodhead, J.H., White H.S and Franklin, M.R, (1989): General principles; experimental selection, quantification and evaluation of anticonvulsants In: R. Levy, R. Mattson, B. Meldium, J.K. Penry and F.E. Dreifuss (Eds.), *Anti-epileptic Drugs* (3rd ed., pp. 85-102). New York, NY: Raven Press Ltd.

Takede, N., Tanji, M., and Matsumiya, T. (1998). Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. *European Journal of Pharmacology*, 350: 21-29.

Takeuchi,A., Takeuchi,N.*(*1969*)*.Astudy of the action of picrotoxin on the inhibitory neuromuscular junction of the crayfish. *Journal ofPhysioogyl (London)* 205*:*377.

Teo, S., Stirling, D., Thomas, S., Hoberman, A., Kiorpes, A. and Khetani, V. (2002).A 90-day oral gavage toxicity study of D-methylphenidate and D, L- methylphenidate in Sprague-Dawley rats.*Toxicology*, 179: 183-196.

Tobler, I., Kopp, C., Deboer, T. and Rudolph, U. (2001). Diazepam-induced changes in sleep, Role of α1GABA$_A$receptor subtypes. *Proceedings of the National Academy of Sciences*, 98(11): 6464-6469.

Trease, G.E. and Evans, M.C. (2002).Phytochemistry In: T*extbook of Pharmacognosy*. Fourth edition. WB Sanders Company Ltd. London, UK, pp 224-343.

Turkmani, A. (2012). Treatment of Epilepsy: More and more accurate in developed countries and deep gap in developing countries.*North Africa and Middle East Journal*, 1(5): 4.

Upadhyaya, G. and Sharma, M.C. (2010).Ayurvedic Principles for the Treatment of epilepsy. *PlantaMedica,* 76: 109

Vergnes, M., Boehrer, A., Reibel S, Simler S, Marescaux, C. (2000).Selective Susceptibility to Inhibitors of GABA Synthesis and Antagonists of GABA$_A$ Receptors in Rats with Genetic Absence Epilepsy, *Experimental Neurology,* 161(2), 714-723.

Vincent, Q., Anthony, Q., and David, Q. (2007).The role of New Antiepilepic Drugs, *Pharmacy Times*, 07/2007/07-6657.

Walsh, R.N, Cummins, R.A. (1976). The open-field test: a critical review. *Psychological Bulletin*, 83: 482-504.

Warren, B.T. (2003). Diagnosis and management of epilepsy.*Canadian Medical Association Journal*, 168 (4): 441-448.

White, H.S., Wolf, H.H., Woodhead, J.H. and Kupferberg, H.J. (1998). The National Institute of Health anticonvulsant drug development program: Screening for efficacy. In: French, J., Leppik, I.E., and Dichter, M.A. (Eds.). Antiepileptic Drug Development.*Advances in Neurology*, (vol.76, pp.29-30). Philadelphia, USA, Lippincott-Raven publishers.

WHO, (1992).Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicine.WHO Regional Office for Western Pacific Manila, Philippine, p.38.

WHO, (2000).General guidelines for methodologies on research and evaluation of Traditional medicine.*WHO/EDM/TRM/2000.1.*

WHO, (2001); Epilepsy: aetiology, epidemiology and prognosis. Fact sheet No 165. Retrieved from http://www.who.int/inf-fs/en/fact165.html.

WHO, (2003).Traditional Medicine. *Fact sheet No.134.*Retrieved from http://www.who.int/topics/traditional_medicine/en/

Xu, Q., Lu, Z. and Zhang, X. (2002).A novel role of alkaline phosphatase in protection from immunological liver injury in mice liver.*Journal of Ethnopharmacology,* 22: 8-14.

Yao, Y., Jia, M., Wu, J.G., Zhang, H., Sun, L.N., Chen, W.S., Rahman, K. (2010). Anxiolytic and sedative-hypnotic activities of polygalasaponins from *Polygala tenuifolia* in mice, *Pharmaceutical Biology*, 48(7): 801-807.

APPENDIX I: Effects of ethanolic leaf extract of *C. barteri*on PTZ-induced seizure in mice

Treatment/Dose (mg/kg)	Mean onset of seizure (min) ± SEM	Quantal protection	% Protection
N/Saline 10 ml/kg	4.97 ± 1.01	1/6	16.67
ELCB 100	6.65 ± 0.84	2/6	33.33
ELCB 200	4.81 ± 0.78	2/6	33.33
ELCB 400	5.1 ± 0.50	5/6	83.33
SVP 200	-	6/6	100

Data presented as; SEM=Standard Error of Mean, n=6. PTZ= Pentylenetetrazole, Protection against seizure expressed as quantal protection; N/Saline=Normal Saline, SVP=Sodium Valproate, ELCB= Ethanolic Leaf extract of*Cussoniabarteri*.

APPENDIX II: Effect of ethanolic leaf extract of *C. barteri*on Strychnine-induced seizure in mice

Treatment/ Dose (mg/kg)	Mean onset of seizure (min)	Quantal protection against seizure	% Protection against seizure	Quantal protection against death	% protection against death
N/Saline	9.40 ± 0.87	1/6	16.67	3/6	50
ELCB 100	8.78 ± 0.18	3/6	50	3/6	50
ELCB 200	12.96 ± 2.08	3/6	50	5/6	83.33
ELCB 400	10.09 ± 1.49	4/6	66.67	4/6	66.67
PBT 20	-	6/6	100	6/6	100

Data presented as; SEM=Standard Error of Mean, Protection against seizure expressed as quantal protection; N/Saline=NormalSaline 10 ml/kg, ELCB= Ethanolic Leaf extract of *Cussoniabarteri*, PBT= Phenobarbitone, n=6.

APPENDIX III: Effect of ethanolic leaf extract of *Cussoniabarteri* against Isoniazid-induced seizure in mice.

Treatment/Dose (mg/kg)	Mean onset of seizure (min) ± SEM	Mean latency of Mortality (mins) ± SEM	Quantal protection against seizure	% Protection against seizure
N/Saline 10ml	24.73 ± 1.51	27.8 ± 1.90	0/6	0
ELCB100	31.01 ± 2.33	33.5 ± 2.34	0/6	0
ELCB200	27.45 ± 2.31	30.9 ± 1.96	0/6	0
ELCB 400	33.18 ± 2.02 *	37.4 ± 6.37	1/6	16.67
PBT 30	47.29 ± 0.81*	63.7 ± 2.99***	0/6	0

Data presented as; SEM=Standard Error of Mean, Protection against seizure expressed as quantal protection; N/Saline=Normal Saline, PBT: Phenobarbitone, ELCB= Ethanolic Leaf extract of *Cussoniabarteri,* * P< 0.05, ***P<0.001; n=6.

APPENDIX IV: Effect of Ethanolic Leaf extract of *Cussoniabarteri* on Diazepam induced-sleep in mice.

Treatment	Dose (mg/kg)	Onset of Sleep (mins)	Duration of sleep (mins)
N/Saline	10ml/kg	5.33 ± 0.35	19.17 ± 1.39
ELCB	100	5.07± 0.32	28.37 ± 6.31
ELCB	200	8.93 ± 0.17*	41.30 ± 2.65*
ELCB	400	16.27 ± 0.64 ***	50.97 ± 6.97**

Data presented as; Mean ± SEM=Standard Error of Mean, N/Saline=Normal Saline,ELCB= Ethanolic Leaf extract of *Cussoniabarteri**P<0.05; **P< 0.01; ***P< 0.001; n=5.

APPENDIX V: Effect of Ethanolic Leaf extract of *Cussoniabarteri*on exploratory activity of mice in hole -board test

Treatment	Dose (mg/kg)	Number of head dips ± SEM
Normal saline	10 ml/kg	14.3 ± 2.01
ELCB	100	8.5 ± 1.28
ELCB	200	4.8 ± 0.79*
ELCB	400	4.1 ± 1.68**
Diazepam	0.5	4.0 ± 1.0**

Data presented as; Mean ± SEM=Standard Error of Mean, *P<0.05, **P<0.01; ELCB= Ethanolic Leaf extract of *Cussoniabarteri*n=6.

APPENDIX VI: Effect of Leaf extract of *Cussoniabarteri*on motor co-ordination in mice (Beam Walk Assay)

Treatment	**Dose (mg/kg)**	**Time spent on the beam(mins) ± SEM**	**Number of foot slips ± SEM**
Normal saline	10 ml/kg	19.4 ± 2.36	5.5 ± 0.96
ELCB	100	22.4 ± 4.71	4.8 ± 0.58
ELCB	200	27.7 ± 7.33	6.7 ± 1.80
ELCB	400	40.7± 9.69	14.5 ± 3.2*
Diazepam	1	30.0 ± 6.12	13.2 ± 3.94*

Data presented as; Mean ± SEM=Standard Error of Mean, ELCB= Ethanolic Leaf extract of *Cussoniabarteri,* *P< 0.05, n=5.