EVALUATION OF ANTICONVULSANT ACTIVITY OF DATE (*PHOENIX DACTYLIFERA)* IN MICE AND CHICKS

BY

RIMAMTATSO MATTHEW JORO JOSHUA

DEPARTMENT OF HUMAN PHYSIOLOGY

AHMADU BELLO UNIVERSITY,

ZARIA, NIGERIA

JUNE, 2017

DECLARATION

I declare that the work in this Dissertation entitled "Evaluation of Anticonvulsant Activity of *Phoenix dactylifera* in Mice and Chicks" has been carried out by me in the Department of Human Physiology, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

Rimamtatso Matthew Joro JOSHUA ……. ……..

Signature Date

CERTIFICATION

This dissertation entitled EVALUATION OF ANTICONVULSANT ACTIVITY OF *PHOENIX DACTYLIFERA* IN MICE AND CHICKS by RIMAMTATSO MATTHEW JORO JOSHUA meets the regulations governing the award of the degree of Masters in Human Physiology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Dr. R. A. Magaji	.....	……..
Chairman, Supervisory Committee	Signature	Date
Dr. M. G. Magaji	.....	…….
Member, Supervisory Committee	Signature	Date
Prof. A. Mohammed	……..	……..
Head of Department	Signature	Date
Prof. S. Z. Abubakar	….....	……
Dean School of Postgraduate Studies	Signature	Date

ACKNOWLEDGEMENTS

I thank, praise, glorify and honour God Almighty, the giver of life and and all good things for sustaining me with good health and the ability, wisdom, strength and encouragement to complete this onerous research work. May all glory and praise be attributed to the all-knowing God. I thank my parents, siblings for their support and prayers which has been my mainstay during this research, financially and morally. God bless you all. I love you.

My sincere appreciation goes to my supervisors Dr. R. A. Magaji and Dr. M. G. Magaji for their moral and scientific advice which has made this research a reality amidst their tight schedule. May the Almighty God be there always to help them too in their day to day activities.

To my Head of Department, Human Physiology, Ahmadu Bello University, Prof Aliyu Mohammed and to all my lecturers, I say thank for imparting me with knowledge that will make me attain my chosen field. May the Almighty continue to support you too in your careers.

My unalloyed appreciation goes to my friends which I may not be able to mention one after the other for being there for me throughout this research. God bless you all. My appreciation also goes to the members of Staff of Pharmacology and therapeutics Department (the animal house, laboratory) and members of Staff of Pharmacognosy and Drug development Department for their technical support.

Table of Contents

List of Tables

List of Plate

List of Figures

Abbreviations

4-AP	4-Aminopyridine
AEDS	Antiepileptic Drugs
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
BZD	Benzodiazepine
CCL_4	Carbon Tetrachloride
CCS	*Carum copticum* Seeds
Cl	Chlorine
CNS	Central Nervous System
DRESS	Drug Rash with Eosinophilia with Systemic Symptoms
EPM	Elevated plus maze
FSH	Follicle Stimulating Hormone
GABA	Gamma aminobutyric Acid
GI	Glycemic Index
GTCS	Generalized Tonic-clonic Seizures
H_2SO_4	Hydrogen Tetraoxosulphate (IV)
HTLE	Hind Limb Tonic Extension
INH	Isoniazid
IP	Intraperitoneally
LDL	Low Density Lipoprotein
LH	Luteinizing Hormone
MDA	Malonaldehyde

MEST	Maximum Electroshock Test
MCS	Minimal Clonic Seizure
NaOH	Sodium Hydroxide
NAPRI	National Agricultural Production and Research Institute
NMDLA	N-methyl-DL-aspartic Acid
NMDA	N-Methyl-D-aspartate
PO	Per oral
PTZ	Pentylenetetrazole
ROS	Reactive Oxygen Species
SEM	Standard Error of Mean
SPSS	Statistical package for Social Sciences
STR	Strychnine

ABSTRACT

Phoenix dactylifera contains some phytochemical constituents which have been reported to be associated with different pharmacological activities. Triterpenes and steroids, flavonoids, cardiac glycoside among other phytochemicals have been reported to possess anticonvulsant activity. This study therefore is aimed at evaluating the anticonvulsant activity of aqueous extract of *Phoenix dactylifera* fruits using Pentylenetetrazole (PTZ), Maximum electroshock test (MEST), Strychnine, Picrotoxin, 4-Aminopyridine (4-AP) and Isoniazid (INH)-induced seizure. The acute toxicity study carried out on the aqueous extract revealed that the LD_{50} is above 5000 mg/kg i.e the extract is practically non-toxic. In each model of seizure, the animals were divided into 5 groups. Group 1 was administered Normal Saline (10 ml/kg, *p.o*), groups 2, 3 and 4 were administered graded doses of the aqueous extract 250 mg/kg, 500 mg/kg and 1000 mg/kg (*p.o*) one hour before seizure induction while group 5 was treated with the respective standard antiepileptic drug (*i.p*) thirty minute before induction of seizure. In MEST model, the extract exhibited a significant ($p<0.05$) decrease in recovery time from seizure at all the tested doses. In the Strychnine (2 mg/kg, *i.m*) seizure test, the extract significantly ($p<0.05$) delayed the onset of hind limb tonic extensor jerks which was dose-dependent. Aqueous extract of *Phoenix dactylifera* at the tested doses did not exhibit any form of delay or protection against seizure and mortality in Pentylenetetrazole (90mg/kg, *s.c*), 4-AP (13.3 mg/kg, *s.c*), picrotoxin (3.5 mg/kg, *i.p)* and INH (300 mg/kg, *s.c*)-induced seizures. It can therefore be concluded that the aqueous extract of *Phoenix dactylifera* posseses constituents which may be beneficial in the management of generalized tonic- clonic seizure and is possibly

mediated via the augmentation of inhibitory neurotransmitters, possibly glycinergic system.

CHAPTER ONE

1.0 Introduction

The role of medicinal plants in the solution of health problems is invaluable on a global level. Medicinal plants continue to provide valuable therapeutic agents, both in modern and in traditional medicine (Krentz and Bailey, 2005). With the associated side effects of the modern medicine, traditional medicines are gaining importance and are now being studied to find the scientific basis of their therapeutic actions.

Traditional medicines, especially with medicinal plants (herbal) have been practiced for long time in most parts of the world. In Saudi Arabia, there are many medicinal plants with claim of neurological potential. But these claims of treatment successes are often made without any scientific basis (Khoshnood-Mansoorkhani,and Moein, 2010). Date palm which is commonly grown in Saudi Arabia and many parts of the World including Nigeria is believed to have beneficial effects in the management or treatment of neurological disorders.

Although its place of origin is unknown because of long cultivation, date probably originated from lands around the Persian Gulf ((Walid and Marshall, 2003). The species is widely cultivated and is reportedly naturalized in Australia, Spain, North Africa, the Canary Islands, Madeira, Cape Verde, the Sahel region of Africa, Mauritius, Réunion, Afghanistan, Pakistan, India, China (Fujian, Guangdong, Guangxi, Yunnan), Fiji, New Caledonia, the United States (California, Nevada, Florida), Puerto Rico, northern Mexico, El Salvador, the Leeward Islands, the Cayman Islands, and the Dominican Republic (Kenoyer and Heuston, 2005).

Fruits of the date palm (*Phoenix dactylifera*) are very commonly consumed in many parts of the world and a vital component of the diet and a staple food in most of the Arabian countries. Dates are rich in certain nutrients and provide a good source of rapid energy due to their high carbohydrate content (70–80%). Most of the carbohydrates in dates are in the form of fructose and glucose, which are easily absorbed by the human body (Faqir *et al.,* 2012).

When used in baking they provide superb taste to the final product. Dates are also used as a component in food preparations like sweets, snacks, confectionery, baking products, institutional feeding and health foods (Allaith and Abdul, 2007). Muslims believe that ‘‘He who eats seven dates every morning will not be affected by poison or magic on the day he eats them’’ (Al-Fasir and Lee, 2008).

The fruits of the date palm (*Phoenix dactylifera*) are sweet berries with a sugar content of more than 50%. Because of its high nutritional value, great yields and its long life, the date palm has been mentioned as the “tree of life” (Faqir *et al.,* 2012). Date pulps hold easily digestible sugars (70%), mostly glucose, sucrose and fructose; dietary fibers and enclose less proteins and fats (Al-Farsi and Lee, 2008; Ali and Khamis, 2004). The fleshy tissues of dates contain 0.2-0.5% oil, while the seed contains 7.7-9.7% oil (Walid and Marshall, 2003).

Dates are especially delicious as a fresh fruit. Beside direct consumption of the whole dates, the fruits are traditionally used to prepare a wide range of different products such as date juice concentrates (spread, syrup and liquid sugar), fermented date products (wine, alcohol, vinegar, organic acids) and date pastes for different uses (e.g. bakery

and confectionary). Also the by-products arising from date processing can be used for different purposes (Hong *et al.,* 2006).

Plate 1.1 Date fruits (Medjool type from Niger Republic)(Snapped on September, 2015).

The date pulp is wealthy in phytochemicals like sterols, phenolics, carotenoids, procyanidins, anthocyanins and flavonoids. The concentrations and ratio of these constituents depend on the stage of fruit picking, type of the fruit, location and soil conditions. The nutritional value of *Phoenix dactylifera* per 100 g (3.5 oz) are calories (77.5 calories), calcium (17.9 mg), protein (0.5 g), potassium (195 mg), magnesium

(15.1 mg), phosphorous (17.4 mg), vitamin A (41.7 IU), carbohydrates (21.0g), vitamin C (0.4 mg), dietary fibres (8 g), beta-carotene (75 µg), thiamine B_1 (0.052 mg), niacin B_3 (1.274 mg), sodium (2 mg), iron (1.02 mg), fat (0.4 mg), folate B_9 (19 µg) and alpha-tocopherol vitamin E 0.05 mg (Faqir *et al*., 2012).

Chemical constituents of the *Phoenix dactylifera* fruits have since been identified (Al-Yahya 1986, Mikki *et al.,* 1988). However, their pharmacological activities have not been scientifically evaluated. Based on the phytochemical studies, date fruits contain anthocyanins, phenolics, sterols, carotenoids, procyanidins and flavonoids. These natural compounds are known to function as free radical scavenger, antioxidant, antimutagenic, anti-inflammatory, hepatoprotective and nephroprotective agents (Baliga *et al*., 2011). They are also associated with lower incidence and mortality rates of degenerative diseases in individual that consumes date (Javanmardi *et al.,* 2003). These phytochemicals also add to the nutritional and organoleptic properties of the fruits (Mansouri *et al.,* 2005; Abdul and Allaith, 2008).

Phoenix dactylifera provides a physiological benefit that is important to the brain, also known as neuroprotective or cerebroprotective effect. In general, this neuroprotective effect protects the brain from the destructive activity of the reactive oxygen species (ROS) that could come from the cell metabolism or from other exogenous sources.

Hence, date fruit extracts have been recently identified as promising neuroprotective agents in several models of neurodegeneration. This neuroprotective effect is basically an extension from the previously known antioxidant effect of palm date as it operates based on similar concept. Date palm fruit is an excellent antioxidant agent and this is

due to the high concentrations of phenolic compounds, flavonoids and anthocyanins as well as the presence of selenoproteins (Asadi *et al.,* 2008; Wan and Mohammed, 2013). The neuroprotection provided by date fruits might be as a result of strong antioxidants present in them. Recent findings suggest that antioxidant agents might exert neuroprotective effects and may be promising in therapy of neurological deficits and this might be from melatonin present in it (Nasser *et al.,* 2011).

1.1 Statement of Research Problem

The problem of antiepileptic drugs (AEDs) arise from their inability to control seizure efficiently and adverse effects which have not been circumvented completely (Gates, 2000), inability to affect epileptogenesis, drug interactions and high cost implication. Hence, search for more effective and better-tolerated antiepileptic drugs (AEDs) with new mechanisms of actions are needed due to drug resistance and AED side-effects profiles. The search for new compounds with more selective activity and lower toxicity should continue to develop newer agents for the treatment of epilepsy.

1.2 Justification of the Study

Epilepsy is one of the world's oldest recognized disorders which affect all ages, sex and race. About 75%-80% of epileptic patients may be provided with adequate seizure control with the help of conventional antiepileptic drugs. However, over 30% of people with epilepsy do not have seizures control even with best available medications (Musa *et al.,* 2014). Antiepileptic drugs available currently do not affect epileptogenesis and are associated with serious side effects, including teratogenicity, chronic toxicity and adverse effects on cognition and behaviour (Musa *et al.,* 2014).

Almost all the currently available antiepileptic drugs are associated with drug interaction making it difficult to attain easy seizure control (Hela *et al*., 2013). There is an urgent need for the development of newer antiepileptic agents with better safety and efficacy profile. There is also a reawakening interest in traditional medicine in the management of epilepsy, especially in developing countries (Magaji *et al.*, 2012).

In vitro studies have exposed that the aqueous fruit extract of date is a powerful scavenger of hydroxyl radicals and superoxide and to restrain protein oxidation and iron-induced lipid peroxidation in the rat brain homogenate in a concentration dependent manner (Vayalil, 2009). Afterward, other investigators have confirmed these explanations with different varieties of date (Abdul and Allaith, 2008; Al-Farsi and Lee, 2008). The existence of a variety of phenolic compounds particularly the coumaric acid and ferulic acid derivatives may have been accountable for the observed free radical scavenging effects.

Phoenix dactylifera has been traditionally claimed to be useful in treatment of various nervous disorders including epilepsy. However, these have not been scientifically documented. This present study therefore evaluated the activities of Aqueous fruit extract of *P. dactylifera* on the central nervous system (CNS) activities particularly epilepsy (because of its menace), paucity of data *vis a vis* its antiepileptic activity and to lend scientific basis for its use in traditional medicine for the treatment of epilepsy.

Researches are needed to validate the folkloric use of these medicinal plants in order to provide evidence of their safety and efficacy (Maiha *et al.*, 2009). One of such medicinal plants used in the traditional management of many disorders (memory

disturbances, fever, inflammation, paralysis, loss of consciousness) and neurological disorders but with paucity of scientific verification literature is *Phoenix dactylifera fruit*.

Aim and Objectives of the Study

1.3.1 Aim

This study is aimed at providing some scientific rationale for the traditional use of Date (*Phoenix dactylifera)* in the treatment of seizure.

1.3.2 Objectives

(i) To establish the phytochemical and chemical profiles of the aqueous fruit extract of *P. dactylifera*.

(ii) To establish the anticonvulsant activity of aqueous fruit extract of *P. dactylifera*

(iii) To establish the possible mechanism of anticonvulsant action of Aqueous fruit extract of *P. dactylifera*.

1.4 Research Hypothesis

Aqueous fruit extract of *Phoenix dactylifera* do not possess significant anticonvulsant activity.

CHAPTER TWO

2.0 Literature Review

Nervous system is a complex collection of nerves and specialized cells known as neurons that transmit signals between different parts of the body. Structurally, the nervous system has two components which are the central nervous system made up of the brain and the spinal cord and the peripheral nervous that is made up of the sensory neurons, ganglia and nerves that connect to one another and the central nervous system.

The brain is the control centre of the body consisting of three main components which are the forebrain (thalamus and hypothalamus) responsible for a variety of functions including receiving and processing sensory information, thinking, perceiving, producing and understanding language and controlling motor function. It also contains the largest part of the brain, the cerebrum. The brainstem (midbrain and hindbrain) contains pons, cerebellum and medulla which are responsible for regulation of balance and equilibrium, movement coordination, auditory and visual responses, autonomic functions such as breathing heart rate and digestion.

Epilepsy is a common neurological disorder characterised by paroxysmal dysrhythmia, seizure, with or without body convulsions and sensory or psychiatric phenomena (Raza *et al*., 2008). Epilepsy may also be defined as a neurological disease arising from abnormal and uncontrollable electrical firings of a group of neurons present in the central nervous system. Seizures occur when the brain cells, which communicate through electrical signals, send out abnormal signals (seizures are not considered to be epilepsy if they occur once or are correctable) (Osuntokun *et al*., 2005).

Seizures are caused by overexcited nerve cells in the brain (cerebral cortex) that fire abnormally (Jackson and Turkington, 2006). In many cases (about half), the cause is unknown. Some factors implicated in the occurrence of seizures include head injury, stroke, heart attack, meningitis, injury to the brain before birth. Researchers have also linked specific genes to epilepsy (Lukasiuk and Pitkanen, 2009).

Incidence of epilepsy in developed countries is approximately 50 per 100,000 while in developing countries including Nigeria and it's 8 to 13 per thousand people (Osuntokun *et al.,* 2005). Epidemiological studies on epilepsy have shown a prevalence of 5.2 to 74.4 per 1,000 person-years in sub-sahara Africa including Nigeria (Dada and Odeku, 2010).

The clinical manifestations range from a major motor convulsion to a brief period of lack of awareness. Epilepsy can occur at any age, but it is most common in the elderly (Duraisami *et al.,* 2009). There are many mechanisms by which seizures can develop in either normal or pathologic brains. Three common mechanisms include diminution of inhibitory mechanism (especially synaptic inhibition due to GABA) (Raza *et al.,* 2008), enhancement of the excitatory synaptic mechanism (especially those mediated by N-Methyl-D-aspartate) and enhancement of endogenous neuronal burst firing (usually by enhancing voltage dependent calcium currents).

Anticonvulsants also known as antiepileptic drugs or antiseizure are a diverse of pharmacological agents used in the treatment of epileptic seizures. Conventional antiepileptic drugs (AEDs) may block sodium channels or enhance α-aminobutyric acid (GABA) function. Several AEDs have multiple or uncertain mechanisms of action

(Rogwaski and Loscher, 2004). Additional targets are voltage –gated channels and components of the GABA system which include GABA receptors GAT-1 GABA transporter and GABA transaminase, voltage-gated calcium channels. By blocking the sodium or calcium channels, AEDs reduce the release of excitatory glutamate whose release is considered to be elevated in epilepsy.

Since AEDs have a narrow therapeutic index and their adverse effects can affect any organ and apparatus, their widespread use has significant safety implication. Overall, 10-30% of people with epilepsy discontinue their initially prescribed AEDs due to intolerance (Emilo, 2014). Among patients chronically treated with AEDs, the prevalence of adverse effects varies between 10-40% if tolerability is evaluated by means of spontaneous reports or non-structured interviews and between 60-95% when adverse effects are evaluated using a checklist (Wesnes *et al*., 2009).

Since AEDs act by modulating the activity of cerebral neurons, it is no surprise that the majority of their adverse effects affect the central nervous system. The most frequently observed include sedation, fatigue, dizziness coordination disturbances (ataxia, dysarthria, diplopia), tremor, cognitive deficits, mood alterations, behavioural changes and sexual disorders (loss of libido, erectile dysfunction) (Wesnes *et al*., 2009).

These effects are often dose-dependent, tend to appear in the early stages of the treatment, can be minimized sometimes by gradual dose titration and some may regress spontaneously during continuation of therapy. Their frequency varies in relation to type of drug and its dose (e.g sedation and cognition effects are more frequent with barbiturates, benzodiazepines and topiramate), patients characteristics (e.g elderly

patients are more susceptible to cognitive effects and motor coordination disturbances whilst children are more often develop behavioural effects) and comedication with specific agents (e.g co-administration of two or more AEDs which act by blocking sodium channels such as carbamazepine, oxcarbazepine, lamotrigine and lacosamide increases the risk of side effects secondary to this mechanism of action such as dizziness and coordination abnormalities (Hessen *et al*., 2009). Among effects on the central nervous system, the possibility of paradoxical worsening of seizures has been reported (Hessen *et al*., 2009).

Antiepileptic drugs (AEDs) in particular lamotrigine, carbamazepine, oxcarbazepine phenytoin, barbiturates and falbamate are among the drugs most frequently associated with cutaneous reactions (Glauser, 2010). Manifestations from simple morbilliform rashes to potentially fatal reactions such as Steven-Johnson syndrome, toxic epidermolysis and DRESS (Drug Rash with Eosinophilia with Systemic Symptoms). In general, these reactions appear within few days or weeks of initiation of therapy. Potentially fatal idiosyncratic reactions can affect other organs and tissues e.g aplastic anaemia induced by felbamate and pancreatitis caused by valproate.

For some of these effects, important risk factors are known. Example valproate hepatotoxicity is more frequent in paediatrics patients and in the presence of certain congenital metabolic defects or concomitant therapy with enzyme inducing AED (Glauser *et al*., 2010).

Some adverse of AEDs develop insidiously and may become apparent only after months or even years of therapy (von Stulpnagel, 2010). Examples include hirsuitism

and gingival hyperplasia induced by phenytoin, shoulder-hand syndrome induced by barbiturates, weight gain induced by valproate, gabapentin, pregabalin, perampanel and vigabatrin, weight loss induced by topiramate, zonisamide and felbamate and metabolic alterations secondary to enzyme induction (vitamin D deficiency, endocrine disorders, blood lipid abnormalities) in patients chronically treated with cabamazepine, phenytoin and barbiturates (von Stulpnagel, 2010). Some serious chronic effects have resulted in prescription of certain AEDs as in the case of irreversible visual field defects induced by vigabatrin and abnormal pigmentation of skin, lips, nails and retina induced by retigabine.

The risk for congenital malformations in newborns of mothers treated with AEDs during pregnancy is about 2-6%, versus 1-2% for the general population. The risk varies in relation to the type of drug, the dose and the number of administered drugs (risks are higher with polytherapy than with monotherapy) (Long, 2003). Valproate is associated with the highest risk : in a recent study, malformation rates among newborns exposed to valproate during gestation was 5.6% with maternal doses 700 mg/day, 10.4% with doses between 700 and 1,500 mg/day and 24.2% with doses greater than 1,500 mg/day (Ornoy, 2009). Prenatal exposure to high doses of valproate also increases the risk of postnatal deficits.

Modern drugs therapy of seizure/epilepsy is complicated by the inability to control seizures in some patients and side effects that range in severity from minimal impairment of the central nervous system to death from aplastic anaemia or hepatic failure (Muazu and Kaita, 2008). Medicinal plants used in traditional medicine for the treatment of epilepsy have been scientifically shown to possess promising

anticonvulsant activities in animal models for screening for anticonvulsant activity and other mental disorders (Igoli *et al*., 2005).

There are few records of psychotropic plants which have been used by indigenous people for medicinal purposes (Igoli *et al*, 2005). Researchers have attempted severally to evaluate the rationale or scientific basis for their usage by screening them in various biological assays for psychotropic activity and the ethnobotanical information on these plants used by traditional healers in Africa to treat mental illnesses, specifically epilepsy, depression age-related dementia and debilitative mental disorders have been shared among researchers (Stafford *et al*., 2008).

Methalonic extract of *Phoenix dactylifera* fruit and pits have effectiveness on inhibition of the selective serotonin reuptake, prevent the hyperactivity of the immune system, oxidative stress profile, lipid profile and improved the sucrose consumption, which play a role in improving the aetiology of depression (Ragaa *et al*., 2014). The dates are believed to boost immunity, relief against pains and ailments including fever, memory disturbances, nervous disorders and abdominal disorders (Vyawahare *et al.,* 2009). In a study on rats in which pain threshold was measured with warm container, it was demonstrated that dates had a distinct improving effect on memory, but not on weight and pain threshold.

Dates are one of the best natural sources of potassium, an essential mineral needed by the body to maintain muscle contractions and smooth functioning of the heart muscles (Al-Shahib and Marshall, 2003). Diabetics have to keep in consideration the glycemic index (GI) of food items, because the lower the GI, the better it is for their bodies. On

the average, dates have a glycemic index of 103 (Elleuch *et al*., 2008). Dates are a high source of energy, with 100 grams of dates yielding about 300 kcal of energy. They are packed with 29 grams of natural sugars (glucose, sucrose, and fructose), 3 grams of dietary fiber, and 31 grams of carbohydrates (Miller *et al*., 2003). Dates consist of 20 different kinds of amino acids which facilitate the digestive process. This is why dates are easily digestible and furnish the body with required energy for physical activities in about half an hour after consumption (Al-Shahib and Marshall, 2003).

Pretreatment with date ethanolic and aqueous extracts at a dose of 4 mg/kg for 14 days markedly ameliorated the ulcer index, histological indices such as necrosis, haemorrhage, congestion and oedema in stomach sections and biochemical levels of some enzymes such as gastrin in plasma and mucin and histamine in gastric mucosae of ethanol-induced gastric ulceration in rats (Al- Shahib and Marshall, 2003; Al-Qarawi *et al.,* 2005). *Phoenix dactylifera* aqueous extract at doses of 3, 6 and 12 mg/kg produced a statistically significant reduction in both castor oil induced intestinal transit and frequency of diarrhoea in rat (Abdullah and Al-Taher, 2008).

Pre and post treatment with aqueous extract of date flesh or pits significantly reduced CCl_4 induced elevation in plasma activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) enzymes and bilirubin concentration and ameliorated morphological and histological liver damage in rats (Al-Qarawi *et al.,* 2004). Moreover, Date extract have been shown to increase sperm count in guinea pigs and to enhance spermatogenesis and increase the concentration of testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) in rats (Barhmanpour *et al.,* 2006). Dates are also seen to strengthen the uterine muscles and

facilitates childbirth. A study (2007-2008) (Al-Kuran *et al.,* 2011) was conducted at Jordan University of Science and Technology, on pregnant women to analyze the link date has on labour. The crude acetone extracts of date at doses of 100 and 1000 mg/kg showed a rapid, strong and dose-dependent ability to inhibit the infectivity of Pseudomonas phage to Pseudomonas aeruginosa by binding to the phage, with minimum inhibitory concentration and completely prevented bacterial lysis (Sabah *et al.,* 2007).

In a study, *Phoenix dactylifera* fruit pulp was evaluated for its effect on the lipid metabolism in high cholesterol diet induced hypercholesterolemic hamsters wherein it significantly reduced elevated levels of plasma lipids including cholesterol, triglycerides and low density lipoprotein (LDL) in the treated animals as compared to high cholesterol-diet supplemented animals indicating its possible beneficial effects in atherosclerosis development in humans (Salah and Al-Maiman, 2005). Al-Qarawi *et al.* (2008) studied the effect of the flesh and pit of *Phoenix dactylifera* on gentamicin induced nephrotoxicity in rats was investigated in which it significantly reduced the increase in plasma creatinine and urea concentrations induced by gentamicin induced nephrotoxicity and ameliorated the proximal tubular damage (Al-Qarawi *et al.,* 2008).

Free radicals formed after metabolic activities, damage body cells, thereby causing cancer. However, antioxidants stabilize these free radicals and prevent them from causing damage to the cells, thereby helping in preventing cancer, heart diseases, etc (Javanmardi *et al.,* 2003). Methanolic extract of *Phoenix dactylifera* seeds showed a significant increase in plasma levels of vitamins C, E and A, β-carotene and significant decrease in the elevated malonaldehyde (MDA) levels due to the lipid peroxidation in

adjuvant arthritis in rats (Mohamed and Al-Okbi, 2004). The fruit of *Phoenix dactylifera* (Dates) is also used as a detersive and astringent in intestinal troubles, treatment for sore throat, colds, bronchial asthma, to relieve fever, cystitis, gonorrhoea, oedema, liver and abdominal troubles and to counteract alcohol intoxication (Barh and Mazumdar, 2008).

The neurobehavioural effects of *Phoenix dactylifera* was investigated by Vyawahare *et al.* (2009) in mice. In the results obtained, it revealed that the methanolic extract of *Phoenix dactylifera* considerably reduced the explorative time in closed arms with an increased exploration in open arms, increased time spent in mirrored chamber, increased the discrimination index, potentiated the haloperidol-induced catalepsy, reduced the onset of death in sodium nitrite-induced respiratory arrest. These results suggest that methanolic extract of *Phoenix dactylifera* possess a wide range of central nervous system activities which need further investigation (Vyawahare *et al*., 2009).

The research on the leaf aqueous and ethanolic extract of *Melanthera scandens* (MS) was conducted by Silvano *et al.* (2016) to investigate its anticonvulsant and anxiolytic activity using animal models of seizure (Pentylenetetrazole) and anxiety model (Elevated plus maze test). In the results obtained, there was significant increase in the time taken to see the first signs of pentylenetetrazole-induced convulsions with MS water extract (MS WE) compared to distilled water while there was no significant effect on the time taken for seizure onset in the MS ethanolic extract (MS EE) treated rats compared to distilled water. Both MS WE and EE did not have any significant effect on seizure duration. In elevated plus maze test, MS WE and MS EE at the lowest doses significantly increased the time spent in the open arm and increased entries or

time spent in the open arms respectively compared to distilled water. Animal treated with MS EE spent more time in the open arms compared to those treated with MS EE indicating the MS EE had better anxiolytic activity than MS WE. Since the extracts showed anticonvulsant effect against PTZ-induced seizures, it is possible that they possess constituents that interfere with GABA transmission. Reduction in the locomotor activity by both MS WE and MS EE in the elevated plus maze test may be correlated with central nervous depression (Silvano *et al*., 2016).

The anticonvulsant activity of *Cotyledion orbiculata* was investigated by studying the effects of both aqueous and methanol extracts of the plant species on seizures induced by pentylenetetrazole, bicculline, picrotoxin and N-methyl-dlaspartic in mice by Amabeoku *et al.* (2007). Aqueous extract of *Cotyledon orbiculata* and methanol extract significantly prolonged the onset of tonic seizures induced by pentylenetetrazole. Methanol extract also significantly reduced incidence of the seizures. The aqueous extract of *Cotyledon orbiculeta* significantly delayed the onset of the tonic seizures induced by bicuculline, Picrotoxin and N-methyl-dl-aspartic acid-induced seizures. Methanolic extract significantly reduced the incidence of the seizures induced by bicuculline. Phenobarbitone and diazepam effectively antagonized only seizures induced by pentylenetetrazole, bicuculline and picrotoxin. Phenytoin did not affect any of the seizures to any significant extent. The data obtained suggest that both aqueous and methanol extracts of *Cotyledon orbiculeta* have anticonvulsant property and may be probably be affecting both gabaergic and glutaminergic mechanisms to exert its effect (Amabeoku *et al*., 2007).

Aqueous and methanolic leaf extracts of *Nylandtia spinosa* were evaluated for anticonvulsant activity by Amabeoku (2008) against tonic seizures produced in mice by pentylenetetrazole (PTZ), bicuculline, picrotoxin and N-methyl-DL-aspartic acid (NMDLA). Aqueous and methanolic leaf extract of *N. spinosa* significantly attenuated PTZ-induced tonic seizures. The aqueous extract of *N. spinosa* significantly delayed the onset of tonic seizures elicited by bicuculline and picrotoxin respectively. Both aqueous and methanolic leaf extracts of *N. spinosa* did not affect NMDLA-induced tonic seizures. Phenytoin did not alter the tonic seizures produced by either PTZ, bicuculline or picrotoxin-induced seizures. The results obtained indicate that both aqueous and methanolic leaf extracts of *N. spinosa* possess anticonvulsant property, thus justifying the use of the plant by traditional medicine practitioners in the treatment of epilepsy (Amabeoku, 2008).

The anticonvulsant activity of *Teucrium polium* was investigated by Khoshnood-Mansoorkhani and Moein (2010). In the study, the protective effect of *T. polium* ethanolic aqueous extracts and related fractions on seizure induced by pentylenetetrazole (PTZ) and maximum electroshock stimulation (MES). It was found that the aqueous extract and related n-butanol fraction have antiseizure effects compared to the control groups. There was no difference between preventing of PTZ-induced death and MES-induced hindlimb tonic extension (HTLE) in ethanolic extract comparing to control groups (Khoshnood-Mansoorkhani and Moein, 2010).

The anticonvulsant activity of the ethanolic extract of *Punica granatum* seed was carried out by Mehrzadi *et al.* (2015) using strychnine (STR) and pentylenetetrazole (PTZ) induced seizures. In the study, the seed ethanolic extract did not protect animals

against seizures but demonstrated a significant increase in seizure latency in both STR and PTZ seizure models. It also showed a significant reduction in seizure duration in the STR seizure model and in the PTZ seizure model compared with the control group (Mehrzadi *et al*., 2015).

The effects of aqueous extracts of *Carum copticum* seeds (CCS) were evaluated in kindling models of epilepsy (Pentylenetetrazole), sedative (Open-field and anxiolytic (Elevated plus maze) models by Rezvani *et al.* (2011). The results indicate that aqueous extracts of CCS have a significant anticonvulsant effect. Different doses of the extract significantly delayed the incidence of every seizure stage in the pentylentetrazole (PTZ) model of kindling. Moreover, CCS extract suppressed after discharge duration, latency to the onset of bilateral forelimb clonus and stage five in the electrical kindling model. These results suggest that CCS extract has remarkable antiepileptic and central depressant effects (Rezvani *et al*., 2011).

The anticonvulsant activity of the aerial parts of *Satureja hortensis* was evaluated by Zolfagharian *et al.* (2016) using pentylenetetrazole (PTZ) and maximal electroshock (MES) models of seizure. In the study, aqueous and ethanolic extracts significantly increased minimal clonic seizure (MCS) and generalized tonic-clonic seizures (GTCS) latencies in PTZ model. Three doses of the extracts also decreased the total duration of seizure. These extracts did not show any protective effects on seizure induced by MES model. In PTZ model, flumazenil, an antagonist of benzodiazepine (BZD) site in the GABA-BZD receptor complex and 7-nitroindazole (7.NI), a selective neuronal nitric oxide synthase (Nnos) inhibitor, reduced the prolongation of seizure latency (Zolfagharian *et al.*, 2016).

The anticonvulsant effects of thymoquinone the major constituents of *Nigella sativa* were evaluated using pentylenetetrazole (PTZ) and maximal electroshock (MES)-induced seizure models by Hosseinzedah and Parvardeh (2004). There was also evaluation of thymoquinone effects on pentobarbitone-induced hypnosis, locomotor activity and motor coordination. In the data obtained, it showed that intraperitoneal injection of thymoquinone prolonged the onset of seizures and reduced the duration of myoclonic seizures in PTZ-induced seizure. In MES model, thymoquinone failed to reduce the duration of seizure, whereas exhibited a complete protection against mortality. In PTZ model, flumazenil, an antagonist of benzodiazepine (BZD) site in the GABA-BZD receptor complex, inhibited prolongation of seizure latency, but did not show any effect on the duration of myoclonic seizures. Also pretreatment with naloxone inhibited the prolongation of myoclonic seizure latency and antagonized the reduction myoclonic seizure duration induced by thymoquinone in PTZ-induced model. Moreover, thymoquinone did not have any hypnosis effect in the pentobarbital-induced hypnosis, but impaired the motor coordination and reduced motor activity. These results indicate that thymoquinone may have anticonvulsant activity in the *petit mal* epilepsy probably through an opiod receptor-mediated increase in GABAergic tone (Hosseinzedah and Parvardeh, 2004).

The anticonvulsant, anxiolytic and sedative activity of the aqueous root extract of *Securidaca longepedunculata fresen* was investigated by Adeyemi *et al.* (2010) using strychnine (STR) and picrotoxin-induced models in anticonvulsant activity, elevated plus maze (EPM) and Y maze in anxiolytic activity while the hexobarbitone induced sleep and the hole board models were used to investigate the sedative and exploratory activities in mice respectively. In the result obtained, the extract produced a significant

dose-dependent increase in onset of convulsion compared to the control for STR- and picrotoxin-induced seizures. It also produced a significant dose-dependent prolongation of the cumulative time spent in the open arms of the EPM and Y maze control with the control. The extract produced a significant reduction in the time of onset of sleep induced by hexobarbitone. The prolongation of hexobarbitone sleeping time by the extract was comparable to that produced by diazepam. The extract produced a dose-dependent decrease in exploratory activity of the mice. The reduction in exploratory activity produced by the extract was greater than of chlorpromazine. These findings justify the use of the extract in traditional medicine for the management of convulsion and psychosis (Adeyemi *et al*., 2010).

CHAPTER THREE

3.0 Materials and Methods

3.1 Materials

3.1.1 Fruits collection

Date fruits (*Medjool* type from Niger Republic) were purchased from open market in Samaru, Zaria, Kaduna State on the 28th September, 2015. They were confirmed and authenticated at the Herbarium, Biological Sciences Department, Faculty of Sciences, Ahmadu Bello University, Zaria, Nigeria, by comparing with existing specimens and was given a Voucher Number 3252.

3.1.2 Chemical/Reagents

Diazepam (NRN 04-5648, Micro Labs Ltd, India), phenobarbitone (Sigma-Aldrich, ATC: N03AA02, CAS: 0000050-06-6), phenytoin (Elition Forte, Elite Pharmaceutical Pvt, Ltd, Sodium valproate (NRN A7-0223L, Valnex CR-200, Next Labs Pvt, Ltd), Strychnine, Isoniazid (NDC 52126-0961-02, LOT B0028321-052314, Barr Laboratoris, Inc. Pomona, NY 10970), 4-Aminopyridine (Sigma-Aldrich, CAS number: 504-24-5, Beilstein Registry Number: 105782, A78403), pentylenetetrazole (Sigma-Aldrich, CAS number: 54-95-5, EC number: 200-219-3, P6500) , picrotoxin (Sigma-Aldrich, CAS number: 124-87-8, Beilstein Registry Number: 3894406, P44706).

3.1.3 Equipment/Apparatus

Digital weighing balances, cages, water trough, animal feed, distilled water, syringes, stop watch, Ugo Basile electroconvulsive machine (Model 7801).

3.1.4 Experimental animals

3.1.4.1 Mice

One hundred and fifty mice of both sexes of weight range 18-25g were procured from the Animal House facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. Kaduna State, Nigeria. The animals were housed in standard cages with free access to food and water *ad libitum* for 14 days.

3.1.4.2 Chicks

Fifty (50)-day old Ranger Cockerels were procured from the National Agricultural Production and Research Institute (NAPRI), Zaria.

3.1.4.3 Animal grouping

After acclimatization period of 14 days, the mice were divided into 25 groups of 6 (n = 6) each and housed in standard cage. These were used for pentylenetetrazole, strychnine, picrotoxin, 4-Aminopyridine and isoniazid models while the chicks were divided into five groups of 10 (n = 10), they were used for maximum electroshock model. The animals were divided into five groups for each of the models of seizure. Animals in group one were pretreated with normal saline (*p.o)* while animals in groups two, three and four were pretreated with the graded dose of the extract at 250 mg/kg, 500 mg/kg and 1000 mg/kg (*p.o)* (in order to establish dose dependency) one hour before seizure induction and animals in group five were pretreated with the respective standard Antiepileptic drugs (*i.p)* thirty minutes prior to induction of seizure. They were evaluated for onset of seizure, the latency, incidence of generalized asynchronized clonic movements which are superseded by flexion of limbs followed by extension and mortality in the case of PTZ-induced seizures and tonic hind-limb

extensor in Strychnine-induced seizure and Maximum electroshock test and hyperactivity, body tremors and fore limbs clonus followed by tonic extension of the hind limbs and generalized tonic-clonic seizure in the case of Picrotoxin induced seizure (Peter *et al.*, 1992). Any animal which do not convulse within thirty minutes after seizure induction was considered protected (Amole *et al*, 2009).

3.2 Methods

3.2.1 Fruit extraction

Aqueous fruit extraction of *Phoenix dactylifera* was carried out according to the method described by Al-Yahya (1986) and Mikki *et al.* (1988). All parts of the fruits in a suitable state of communition were placed in a closed vessel with the distilled water and were allowed to stand for a day with occasional stirring. The mixture was macerated and the marc pressed to remove retained solution. The liquids were combined and any precipitated solids were removed by decanting the clear fluid after a period of standing (or filtration). This was then placed in a water bath for evaporation to the jelly extract at 23^0C.

3.2.2 Phytochemical screening

The phytochemical screening of the aqueous fruit extract of *Phoenix dactylifera* was carried out in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The tests adopted were:

3.2.2.1 Molisch's test

This test was adopted for identifying carbohydrates in the aqueous extract. A few drops of Molisch's solution was added to 2 ml of aqueous solution of the extract, thereafter a

small volume of concentrated sulphuric acid was allowed to run down the side of the test tube to form a layer without shaking. The interface was observed for a purple colour as indicative of positive for carbohydrates (Mohammed *et al*., 2014).

3.2.2.2 Bontrager's test

This was adopted in identifying anthroquinones in the aqueous extract. One gram (1 g) of the aqueous plant extract was shaken with 10 ml of benzene and filtered. 5 ml of 10% ammonia was added to the filtrate. The mixture was shaken and the presence of pink, red or violet colour was indicative of anthroquinones (Brachmann *et al*., 2007).

3.2.2.3 Lieberman-Bucchard test

This test was adopted for identifying the presence of unsaturated steroid triterpenes in the extract. 0.5 g of the extract was dissolved in 10 ml anhydrous chloroform and filtered. The solution was divided into two equal portions for the following tests. The first portion of the solution above was mixed with 1 ml of acetic anhydride followed by the addition of 1 ml of concentrated sulphuric acid down the side of the test tube to form a layer underneath. The test was observed for green colouration as indicative of unsaturated steroid triterpenes (Mohammed *et al*., 2014).

3.2.2.4 Keller-Kialiani test

This was used for identifying cardiac glycoside. Two milliliters (2 ml) of the aqueous solution of the extract was added into three drops of strong solution of lead acetate. This was mixed thoroughly and filtered. The filtrate was shaken with 5 ml of chloroform in a separating funnel. The chloroform layer evaporated to dryness in a small evaporating dish. The residue was dissolved in a glacial acetic acid containing a

trace of ferric acid chloride; this was transferred to the surface of 2 ml concentrated sulphuric acid in a test tube. The upper layer and interface of the two layers were observed for bluish-green and reddish brown colouration respectively as indicative of the presence of cardiac glycosides (Mohammed *et al*., 2014).

3.2.2.5 Frothing test

This was adopted for identifying the presence of saponin glycoside in the extract. Three milliliters (3 ml) of the aqueous solution of the extract were mixed with 10 ml of distilled water in a test tube. The test tube was stoppered and shaken vigorously for about five minutes, it was allowed to stand for 30 minutes and observed for honeycomb froth, which will be indicative of saponins (Prashant *et al*., 2011).

3.2.2.6 Ferric chloride test

The presence of Tannins in the aqueous was identified using this test: Two milliliters (2 ml) of the aqueous solution of the extract were added to a few drops of 10% Ferric chloride solution (light yellow). The occurrence of blackish blue colour green or blue-green precipitate was taken as evidence for the presence of tannins (Evans, 2002).

3.2.2.7 Pew's test

This test was adopted to identify the presence of Flavonoids. Five milliliter (5 ml) of the aqueous extract was mixed with 0.1g of metallic and 8ml of concentrated sulphuric acid. The mixture was observed for red colour as indicative of flavonoids (Mohammed *et al*., 2014).

3.2.2.8 Dragendoff's test

This test was adopted to identify the presence of Alkaloids in the aqueous extract. One gram (1 g) of the aqueous was dissolved in 5 ml of 10% ammonia solution and extracted with 15 ml chloroform. The chloroform portion was evaporated to dryness and the resultant residue dissolved in 15 ml of dilute sulphuric acid. Dragendoff's reagent was added and turbidity and precipitate was observed as indicative presence of alkaloids (Prashant *et al*., 2011).

3.2.3 Proximate analysis

A handful of Dates not only make a nice yummy snack, but also benefit you with various nutrients present in it. Dates are low in saturated fats, cholesterol, Sodium and high in vitamins and important minerals. They are also popular for being high in dietary fibers. One and a half cup of dates contains almost 7 grams of dietary fibers. The proximate analysis was carried out to determine Moisture, Ash, Lipid (Fat), Protein and Carbohydrate content of the aqueous extract using the following methods:

3.2.3.1 Determination of moisture content

Aluminum dishes were washed and dried to a constant weight in an oven at 100^0C. They were later removed and cooled in a desiccator and weighed (W1). 2grams of the grounded (powder) sample was placed in the weighed moisture dish (W2). The dish containing the sample was kept in an oven for about 3hours, the sample were removed and cooled in the dessicator and weighed W3 (Hamza *et al.,* 2014). The % of moisture was calculated as:

$$W2 - W3 / W2 - W1 \times 100$$

3.2.3.2 Determination of ash content

Crucibles were cleansed and dried in the oven, after drying; they were cooled in the dessicator and weighed (W1). 2grams of the grounded (powder) sample was placed in the crucibles and weighed (W2). They were transferred into the Muffle Furnace for about 550^{o} C, then removed and cooled in the dessicator and weighed (W3) (Fouteye *et al.*, 2014).

The % of Ash was calculated as:

$$W3 - W1 / W2 - W1 \times 100$$

3.2.3.3 Determination of lipids (Fat)

A Dried 250ml clean boiling flask was placed in an oven, then transferred into dessicator and was allowed to cool. 2gram of the sample was weighed into labeled thimbles (filter paper). The boiling flask was filled with petroleum spirit or N-hexane. The soxhlet apparatus was assembled and was allowed refluxing for 8 hours. It was then removed and transferred to an oven to dry. Then it was transferred from the oven into a dessicator and allowed cooling then weighing (Al-Shahib and Marshall, 2003).

The % of Fat was calculated as:

$$\text{weight of fat} / \text{weight of sample} \times 100$$

3.2.3.4 Determination of Protein Digestion

2grams of the sample was weighed into a kjeldahl flask. Add 1tablet of catalyst (copper) was added to 25ml conc. Sulfuric Acid (H_2SO_4). The solution was heated in a fume cupboard until it assumes a green colour. The solution was allowed to cool and

then any black particle showing at the mouth and neck of the flask was washed down with distilled water. After cooling, the digest was transferred with several washings into 250ml with distilled water. Before use, there was steaming through the Markham distillation apparatus for about 15minutes. Under the condenser, a 100ml conical flask containing 10ml of Boric indicators was placed. 10ml of the digest was pipetted into the body of the apparatus via the small funnel aperture; then wash down with distilled water followed by 10ml of 40% NaOH solution. Steaming through for about 5-7 minutes to collect ammonium sulphate. The receiving flask was removed and washed down the tip of the condenser into the flask. The solution was titrated in the receiving flask using N/100 (0.01N) hydrochloric acid and the Nitrogen content was calculated and hence the Protein content of the sample (Hamza *et al.,* 2014).

3.2.3.5 Determination of carbohydrate (CHO)

By difference, in this method, carbohydrate content is obtained by calculations having estimated all other fractions by proximate analysis i.e. =100- (% of moisture + %Ash + %Protein + %Fat) (Sultana *et al.,* 2015).

3.2.4 Acute toxicity study in mice

The method used was described by Lorke (1983). All mice were administered the aqueous fruit extract per oral *(p.o*). The study was divided into two phases. In the first phase, nine (9) mice of either sex were randomly divided into groups of three mice each. Group 1 received 10 mg/kg extract while Groups 2 and 3 received 100 mg/kg and 1000 mg/kg respectively. The animals were observed for signs and symptoms of toxicity including mortality for twenty-four hours after treatment. In the second phase,

three mice of either sex were also randomly divided into three groups of one mouse each. The first mouse received extract at a dose of 1600 mg/kg while the second and third received the extract at doses of 2900 mg/kg and 5000 mg/kg respectively based on the outcome of the first phase. The mice were also observed for twenty four hours.

3.2.5 Pentylenetetrazole (PTZ)-induced seizure

This model was carried out according to the protocol prescribed by Peter *et al* (1992). The first group was administered Normal Saline (10 ml/kg), the second, third and fourth groups were administered graded doses of the aqueous fruit extract (250, 500 and 1000 mg/kg) orally *(p.o)* one hour before administration of PTZ (90 mg/kg) subcutaneously (Peter *et al.*, 1992). The fifth group received conventional Antiepileptic Drug, Sodium Valproate (*i.p*) thirty minutes before seizure induction. The animals were placed in different cages and observed for onset time of seizure and lethality up to thirty minutes after PTZ administration. The time taken before onset of myoclonic spasm of at least 5s duration and clonic convulsions was recorded (Peter *et al*., 1992).

3.2.6 Maximum electroshock (MES) test

The method described by Swinyard (1989) was employed in this study, fifty (50), one day old Ranger Cockerels were randomly divided into five groups each containing ten chicks (n =10). The first group received Normal saline (10 ml/kg) (*p.o)*. The second, third and fourth groups received 250, 500 and 1000 mg/kg (*p.o*). The fifth group was given Phenytoin (20 mg/kg) (*i.p*). One hour and thirty minutes after pretreatment in groups 1, 2, 3 4 and AED respectively, MES was administered to induce seizure using Ugo Basile electroconvulsive machine (Model 7801) connected to Claude Lyons stabilizer with corneal electrodes placed on the upper eyelids of the chicks (Toman *et*

al., 1946). The current, shock duration, frequency and pulse width were set and maintained at 90 mA, 0.80s, 200 pulses per second and 0.8 ms respectively (Swinyard, 1989). Tonic limb flexion, tonic limb extension, tonic hind limb extension, stupor and recovery or death was considered as seizure induced by MES. A substance is known to possess anticonvulsant property if it reduces or abolishes the extensor phase of MES convulsion (Sayyah, 2002).

3.2.7 4-Aminopyridine induced seizure

The protocol described by Yamaguchi and Rogawski (1992) was employed in this model. In mice, subcutaneously administered 4-AP (13.3 mg/kg) produces tonic-clonic convulsions and lethality (Yamaguchi and Rogawski, 1992). The first group was administered Normal Saline (10 ml/kg) (*p.o*). The second, third and fourth groups were administered graded doses of the extract (250, 500 and 1000 mg/kg) (*p.o*). The fifth group received Diazepam (20 mg/kg) (*i.p)*. The animals were observed for onset of tremor, tonic-clonic seizure and tonic hind limb extension and lethality thirty minutes after 4-Aminopyridine administration. The ability of the extract to abolish or delay this features was taken as an indication of an anticonvulsant (Yamaguchi and Rogawski, 1992).

3.2.8 Strychnine-induced seizure

This model of seizure was carried out employing the method prescribed by Anderson *et al* (1988). The first group was administered Normal Saline (10 ml/kg) (*p.o*). The second, third and fourth groups were administered graded doses of the extract (250, 500 and 1000 mg/kg) (*p.o*) and fifth group received Phenobarbitone (30 mg/kg) (*i.p)*. The animals were pretreated with the extract one hour in groups one, two, three and

four and thirty minutes in group five prior to administration of Strychnine (2 mg/kg) intramuscularly. The animals were observed for onset of tonic-clonic seizure and lethality thirty minutes after Strychnine administration. Mouse that did not experience seizure within thirty minutes was considered protected (Amole *et al.*, 2009).

3.2.9 Picrotoxin-induced seizure

This model of seizure was carried out employing the method prescribed by Swinyard (1989). The first group was administered Normal Saline (10 ml/kg) (*p.o*). The second, third and fourth groups were administered graded doses of the extract (250, 500 and 1000 mg/kg) (*p.o*). The fifth group received Diazepam (20 mg/kg) (*i.p)*. The animals were pretreated with the extract one hour in groups one, two, three and four and thirty minutes in group five prior to administration of picrotoxin (3.5 mg/kg) intraperitoneally. The animals were observed for onset of tonic-clonic seizure and lethality thirty minutes after picrotoxin administration. Mouse that did not experience seizure within thirty minutes was considered protected (Swinyard, 1989).

3.2.10 Isoniazid-induced seizure

This model of seizure was carried out employing the method prescribed by Coyer and South (1976). The first group was administered Normal Saline (10 ml/kg) (*p.o*). The second, third and fourth groups were administered graded doses of the extract (250, 500 and 1000 mg/kg) (*p.o*). The fifth group received Diazepam (20 mg/kg) (*i.p)*. The animals were pretreated with the extract one hour in groups one, two, three and four and thirty minutes in group five prior to administration of Isoniazid (300 mg/kg) subcutaneously. The animals were observed for onset of tonic-clonic seizure and

lethality thirty minutes after Isoniazid administration. Mouse that did not experience seizure within 30 minutes was considered protected (Coyer and South, 1976).

3.2.11 Statistical analysis

Data collected were statistically presented as mean ± SEM by statistical package for social sciences (SPSS), Version 20. One-way analysis of variance (ANOVA) followed by Dunnet's Post-hoc test. $P<0.05$ was considered significant.

CHAPTER FOUR

4.0 Results

4.1 Phytochemical Screening

The preliminary phytochemical screening revealed the presence of carbohydrates, unsaturated steroid tritepenes, flavonoids, cardiac glycoside and alkaloids in the aqueous extract (Figure 4.1).

4.2 Proximate Analysis

The proximate analysis of the aqueous fruit extract of *Phoenix dactylifera* revealed the presence of Moisture, Ash, Lipid, Protein and Carbohydrate in different percentage (Figure 4.2).

4.3 Acute Toxicity Study

At the end of the observation period, the extract did not produce any sign or symptom of toxicity or mortality. The LD_{50} is above 5000 mg/kg. From the LD_{50} determined, it was clear that the extract to be administered was decided to be less than 30% of the highest dose i.e 5000 mg/kg. The doses were at 20%, 10% and 5% i.e 1000 mg/kg, 500 mg/kg and 250 mg/kg.

4.4 Pentylenetetrazole-Induced Seizure

In control animals, onset of seizure was 200 ± 28.87 after PTZ injection and all animals died after tonic hind limb extension. Oral administration of aqueous fruit extract of *Phoenix dactylifera* at all the tested doses (250, 500 and 1000 mg/kg) neither show a

Figure 4.1: Phytochemicals of Aqueous Extract of *Phoenix dactylifera*

Phytochemical Constituent	Types of Test	Inference
Carbohydrate	Molisch's	+
Steroid	Lieberman-Bucchard's	+
Flavonoids	Pew's	+
Alkaloids	Dragendoff's	+
Tannins	Ferric chloride's	_
Saponin	Frothing's	_
Cardiac glycoside	Keller-Kialiani's	+
Anthraquinone	Bontrager's	_

(+ present; _ absent)

Figure 4.2: Proximate Analysis of *Phoenix dactylifera*

Nutrients	Amount (%)
Moisture	18.75
Ash	1.07
Lipid	30.18
Protein	1.74
Carbohydrate	49.97

delay in the onset of seizure and tonic hind limb extension nor provide any form of protection against mortality (Table 4.1).

4.5 Maximum Electroshock (MES) Test

In the MES test, though the extract did not abolish the extension of lower limbs but it showed a statistical significance ($p<0.05$) in the recovery time of the animals as compared with the control group (Table 4.2).

4.6 4-Aminopyridine-Induced Seizure

The oral administration of *Phoenix dactylifera* fruit extract in 4-Aminopyridine-induced seizure test showed no significance in both onset of hyperactivity and tonic hind limb extension (Table 4.3).

4.7 Strychnine-Induced Seizure

In this model, there was no protection shown by the extract against seizure but there was statistical significance ($p<0.05$) shown in the latency i.e the duration between the administration of strychnine and onset of seizure at doses 500 and 1000 mg/kg (Table 4.4).

4.8 Picrotoxin-Induced Seizure

The oral administration of aqueous of *Phoenix dactylifera* showed a significant ($p<0.05$) delay in the onset of forelimb clonus in Picrotoxin-induced seizure while there was no significance in the onset of generalized seizure when compared with the control group (Table 4.5).

Table 4.1: Effect of Aqueous Extract of *Phoenix dactylifera* Fruits Against PTZ (90mg/kg)-Induced Seizure

Group	QP (Seizure)	OS (Sec)	THLE (Sec)	QP (Mortality)
NS (10 ml/kg)	0/6	200 ± 28.8	260 ± 35.3	0/6
AEPD (250 mg/kg)	0/6	265 ± 34.9	264 ± 0.00	0/6
AEPD (500 mg/kg)	0/6	172 ± 2.4	214 ± 41.22	0/6
AEPD (1000 mg/kg)	0/6	191 ± 35.6	294 ± 49.00	0/6
SV (200 mg/kg)	6/6	Nil	Nil	6/6

NS = Normal saline, AEPD =Aqueous extract of *Phoenix dactylifera*, SV = Sodium valproate, QP = Quantal protection, OS = Onset of Seizure, THLE = Tonic hind limb extension. n = 6

Table 4.2: Effect of Aqueous Extract of *Phoenix dactylifera* Fruits Against Maximum Electroshock (MES) Test in Chicks

Group	QP (Seizure)	RT (Sec)
NS (10 ml/kg)	0/10	642 ± 158.7
AEPD (250 mg/kg)	0/10	82.4 ± 12.5**
AEPD (500 mg/kg)	0/10	75.4 ± 2.10**
AEPD (1000 mg/kg)	0/10	186.2 ± 16.5**
Phenytoin (20 mg/kg)	10/10	Nil

NS = Normal saline, AEPD =Aqueous extract of *Phoenix dactylifera*, QP = Quantal protection, RT= Recovery time, n = 6, ** $p < 0.05$

Table 4.3: Effect of Aqueous Extract of *Phoenix dactylifera* Fruits Against 4-Aminopyridine (13.3 mg/kg)-Induced Seizure in Mice

Group	QP (Seizure)	OT (Sec)	OH (Sec)	THLE (Sec)	QP (Mortality)
NS (10 ml/kg)	0/6	615 ± 89.1	648 ± 64.4	860 ± 106.1	0/6
AEPD (250 mg/kg)	0/6	593 ± 18.7	661 ± 27.9	945 ± 173.9	0/6
AEPD (500 mg/kg)	0/6	388 ± 30.7	675 ± 51.1	797 ± 86.2	0/6
AEPD (1000 mg/kg)	0/6	636 ± 61.8	745 ± 126.0	664 ± 80.3	4/6
DZP (20 mg/kg)	6/6	Nil	Nil	Nil	6/6

NS = Normal saline, AEPD = Aqueous extract of *Phoenix dactylifera*, OT = Onset of tremor, OH = Onset of hyperactivity, THLE = Tonic hind limb extension, DZP = Diazepam, QP = Quantal protection n = 6

4.9 Isoniazid-Induced Seizure

In this model of seizure, the aqueous extract of *Phoenix dactylifera* did not show significant anticonvulsant activity at the tested doses as shown in the table below (Table 4.6).

Table 4.4: Effect of Aqueous Extract of *Phoenix dactylifera* Fruits Against Strychnine (2 mg/kg)-Induced Seizure in Mice

Group	QP (Seizure)	L (Sec)	ST (Sec)	QP (Mortality)
NS (10 ml/kg)	0/6	175 ± 13.1	12.5 ± 1.70	0.0
AEPD (250 mg/kg)	0/6	212 ± 6.5	5.83 ± 1.01	0.0
AEPD (500 mg/kg)	0/6	248 ± 22.0**	28.8 ± 22.8	0.0
AEPD (1000 mg/kg)	0/6	291 ± 17.7**	108 ± 81.75	0.0
PBT (30 mg/kg)	6/6	Nil	Nil	6/6

NS = Normal saline, AEPD = Aqueous extract of *Phoenix dacylifera*, L = latency, ST= Seizure time, QP = Quantal protection, PBT = Phenobarbitone, n = 6, ** $p < 0.05$

Table 4.5: Effect of Aqueous Extract of *Phoenix dactylifera* Fruits Against Picrotoxin (3.5 mg/kg)-Induced Seizure in Mice

Group	QP (Seizure)	OFC (Sec)	OGS (Sec)	QP (Mortality)
NS (10 ml/kg)	0/6	690 ± 7.90	1169 ± 17.1	4/6
AEPD (250 mg/kg)	0/6	1099 ± 116.6	1192 ± 48.3	1/6
AEPD (500 mg/kg)	0/6	729 ± 79.5	1015 ± 93.2	5/6
AEPD (1000 mg/kg)	0/6	894 ± 125.2	1121 ± 123.3	3/6
DZP (20 mg/kg)	6/6	Nil	Nil	6/6

NS = Normal saline, AEPD = Aqueous extract of *Phoenix dactylifera*, QP = Quantal protection, OFC = Onset of forelimb clonus, OGS = Onset of generalized seizure

n = 6

Table 4.6: Effect of Aqueous Extract of *Phoenix dactylifera* Fruits Against Isoniazid (300 mg/kg)-Induced Seizure in Mice

Group	QP (Seizure)	OTCS (Sec)	THLE (Sec)	QP (Mortality)
NS (10 ml/kg)	0/6	1583 ± 65.6	2258 ± 62.7	0/6
AEPD (250 mg/kg)	0/6	1955 ± 92.2	2349 ± 196.4	0/6
AEPD (500 mg/kg)	0/6	1582 ± 184.6	2056 ± 74.2	0/6
AEPD (1000 mg/kg)	0/6	1728 ± 103.9	2210 ± 77.2	0/6
DZP (20 mg/kg)	6/6	Nil	Nil	6/6

NS = Normal saline, AEPD = Aqueous extract of *Phoenix dactylifera*, QP = Quantal protection, OTCS = Onset of tonic-clonic seizure, THLE = Tonic hind limb extension, DZP = Diazepam, n = 6

CHAPTER FIVE

5.0 Discussion

This present study tries to evaluate the anticonvulsant of date which has been used in ages for the treatment of epilepsy. The preliminary phytochemical screening revealed the presence of carbohydrates, unsaturated steroid triterpenes, flavonoids, cardiac glycoside and alkaloids in the aqueous extract. These phytochemical constituents have been reported to be associated with different pharmacological activities of plants (Al-Farsi and Lee, 2008). Triterpenes and steroids, among other phytochemicals have been reported to possess anticonvulsant activity (Shibata, 2001; Wahab *et al.*, 2009).

The proximate analysis revealed the presence of moisture, ash, lipid, protein and carbohydrate in different measures. These are responsible for the high nutrient content of the fruit. The acute toxicity study carried out revealed the extract is practically non-toxic and it's safe for consumption probably due to the presence of the nutrients and vitamins which are abound in *Phoenix dactylifera* (Hamza *et al.*, 2014).

The most frequently used chemoconvulsant for the screening of potential anticonvulsant agents is PTZ. The mechanism by which PTZ produces its action is believed to be through antagonism of the GABA receptor complex (Amole *et al.*, 2009). Also, drugs that reduce T-type Ca^{2+} currents, such as ethosuximide can prevent seizures induced by PTZ (Karunakar *et al.,* 2009). Drugs protecting against tonic – clonic seizures induced by PTZ are considered to be useful to control myoclonic and absence seizures in humans (Karunakar *et al.*, 2009). Since the *Phoenix dactylifera* extract did not offer protection against seizure induced by PTZ, it is probable that it does not interact with any of the mechanisms by which PTZ produces seizure i.e

GABAergic neurotransmission, enhancement of T-type Ca^{2+} current or opening of glutamatergic neurotransmission mediated by NMDA receptor. This is not in agreement with Kasture *et al*. (2000) which reported that triterpene isolated from *R. cordifolia* inhibit seizure induced by PTZ.

The Maximal electroshock test is the most widely used animal model in antiepileptic drug discovery, because seizure induction is simple and the predictive value for detecting clinically effective antiepileptic is high. This model is seen as the most validated procedure for preclinical screening of drugs and it is predicted effectiveness against generalized seizures of the tonic-clonic (*grand mal*) type and partial seizures. It permits the evaluation of the ability of a substance to prevent seizure spread through neural tissues (Castel-Branco *et al.,* 2009). The pharmacology of acute maximal electroshock test does not differ from the pharmacology of generalized tonic-clonic seizures in genetic models with chronic epilepsy (Duraisami *et al.,* 2009). It has often been stated that antiepileptic drugs that block MES-induced tonic extension act by blocking seizure spread. Moreover, MES-induced tonic extension can be prevented either by drugs that inhibit voltage-dependent Na^{+}channels, such as phenytoin, valproate, felbamate and lamotrigine or by drugs that block glutamatergic excitation mediated by the N-methyl-D aspartate (NMDA) receptor, such as felbamate (Yudkoff *et al.*, 2006). Electroshock causes the inhibition of GABA release and this in turn, may inhibit GABA synthesis (Sermet *et al.,* 1998). Thus, the delay observed in the onset of seizure and decrease in the recovery time exhibited by the extract showed that it might have probably blocked the seizure spread by inhibiting Na^{+}channels and glutamatergic excitation through NMDA receptor. This might have possible due to the presence of

flavonoids and triterpene which have been reported by Kasture *et al*. (2000) to inhibit seizure.

The drug 4-AP is known to block voltage-activated potassium channels expressed in a variety of cell types including neurons. It causes epileptiform activity in *in vitro* preparations and is a potent convulsant in animals and man. Potassium channels play a significant role in controlling all aspect of neuronal excitability (Wickenden, 2002). Sodium channel blockers, such as phenytoin which prevent seizure spread effectively antagonize seizures induced by K^+ channel blocker such as 4-AP while those with specific actions on other cellular targets may be weak or inactive, presumably because they are unable to attenuate the spread of intense (non-NMDA receptor mediated) excitation evoked by 4-AP. The oral administration of the extract neither offer protection nor increase seizure latency induced by subcutaneous injection of 4-AP and also did not exhibit action on both the hyperactivity and tonic hind limb extension induced by 4-AP administration. The inability of the extract to produce significant activities against 4-AP induced seizure suggests it might likely not be interacting with K^+ channel in producing its anticonvulsant activities.

Strychnine has been demonstrated to have a well-defined mechanism of convulsant action reported to be by directly antagonizing the inhibitory spinal cord and brainstem reflexes of glycine and thus increasing spinal reflexes (Amole *et al.*, 2009). Strychnine acts primarily as an antagonist of the glycine receptor, which is a ligand-gated chloride channel in neurons in the spinal cord and neurons. Strychnine binds to the receptor, preventing the inhibitory effects of glycine on the postsynaptic neurons (Sharma, 2008). This study showed an increase in the latency of hind limb tonic extensor jerks at

doses 500 and 1000 mg/kg suggesting the extract might have inhibited the antagonistic effects of strychnine on the glycine receptor.

Picrotoxin is known to be GABA antagonist exerting its effect by binding to the picrotoxin binding site which is closely related to the chloride ionophore (which is a ligand-gated ion channel concerned chiefly with the passing of chloride ions across the cell membrane) in the $GABA_A$ receptor complex (Thirupathy and Saravanan, 2009). Therefore, picrotoxin prevents Cl^- channel permeability and thus promotes an inhibitory influence on the target neuron (Martin and Olsen, 2000). Picrotoxin reduces conductance through the channel by reducing not only the opening frequency but also the mean open time. In this study, the aqueous extract of *Phoenix dactylifera* did not offer any anticonvulsant activity against picrotoxin-induced seizure.

From the result obtained from the present study, it showed that the extract does not offer any anticonvulsant activity against Isoniazid-induced seizure at all the tested doses. This may probably due to the inability of the extract to increase the concentration of pyridoxine which is responsible for the seizure induced by INH i.e the extract does not interact with the synthetic pathway of GABA. This is evident in the non-significance of the onset of tonic clonic seizure and tonic hind limb extension of the treated groups compared with the control group.

The data presented in this study provided scientific evidence that the phytochemical obtained from the aqueous extract of *P. dactylifera* fruit may contain phytochemicals that are relevant to the management of convulsive disorder.

CHAPTER SIX

6.0 Summary, Conclusion and Recommendations

6.1 Summary

The aqueous fruit extract of *Phoenix dactylifera* contain some psychoactive constituents which can be used in ameliorating generalized seizures of the tonic clonic (*grand mal*) type. This is evident in the result obtained from the study.

6.2 Conclusion

The aqueous fruit extract of *Phoenix dactylifera* possesses a moderate anticonvulsant activity against Maximum Electroshock and strychnine induced seizure tests which may be beneficial in *Grand mal* epilepsy and is mediated via the augmentation of inhibitory neurotransmitters, possibly glycinergic system.

6.3 Recommendations

From the present study, it is recommended that:

i. The aqueous extract of the fruit should be evaluated for its effects on epileptogenesis.
ii. Further works should be carried out in order to isolate pure compounds responsible for the observed pharmacological effect.

6.4 Contributions to Knowledge

i. The aqueous extract of *Phoenix dactylifera* significantly ($p<0.05$) reduced the recovery time in the MEST at all the doses tested (250-1000 mg/kg).

ii. The extract at the doses 500 and 1000 mg/kg delayed the latency to seizure in the strychnine induced seizure test suggesting the involvement of glycinergic system.

REFERENCES

Abdul, A. and Allaith, A. (2008). Antioxidant activity of Bahraini date palm (*Phoenix dactylifera*.) fruit of various cultivars. *International Journal of Food Science and Technology*,43:1033–1040.

Abdullah, Y. and Al-Taher, B. (2008). Possible anti-diarrheal effect of the Date spathe aqueous extract in rats. *Scientific Journal of King Faisal University (Basic and Applied Science)*, 9:1429-1435.

Adeyemi, O. O., Akindele, A. J., Yemitan, O., K., Aigbe, F. R. and Fagbo, F. I. (2010). Anticonvulsant, anxiolytic and sedative activities of the aqueous root extract of *Securidaca longepedunculata frensen*. *Journal of Ethnopharmacology*, 130(2):191-195.

Al-Kuran, O., Al-Mehaisen, L., Bawadi, H., Beitawi, S. and Amarin, Z. (2011). The effect of late pregnancy consumption of date fruit on labour and delivery. *Journal of Obstetrics and Gynaecology,* 31 (1): 29–31.

Allaith, F. and Abdul, A. A. (2007). *In vitro* evaluation of antioxidant activity of different extract of *Phoenix dactylifera* fruits as functional foods. *Deutsche Lebensmittel Rundschau*, 101:305-308.

Al-Farsi, M. A. and Lee, C. Y. (2008). Nutritional and functional properties of dates: a review. *Critical Reviews in Food Science and Nutrition*, 48: 877–887.

Ali, M. and Khamis, A. S. (2004). Mineral ion content of the seeds of six cultivars of Bahraini date palm (*Phoenix dactylifera). Journal of Agricultural and Food Chemistry*, 52: 6522–6525.

Al-Qarawi, A., A., Abdel-Rahman, H., Ali, B., H., Mousa, H. M. and El-Mougy, S. A. (2004). Protective effect of extracts from Dates on carbon tetrachloride-induced hepatotoxicity in rats. *International Journal Applied Resources and Veterinary Medicine*, 2:176-180.

Al-Qarawi, A. A., Abdel-Rahman, H., Ali, B. H., Mousa, H. M. and El-Mougy, S. A (2005). The ameliorative effect of dates (*Phoenix dactylifera*) on ethanol-induced gastriculcer in rats. *Journal of Ethnopharmacology,* 98:313–317.

Al-Qarawi, A. A., Abdel-Rahman, H., Ali, B. H., Mousa, H. M. and El-Mougy, S. A. (2008). Nephroprotective action of *Phoenix dactylifera* in gentamicin-induced nephrotoxicity. *Pharmaceutical Biology,* 4:227-230.

Al-Shahib, W. and Marshall, R. J. (2003). Fatty acid contents of the seeds from 14 varieties of date palm (*Phoenix dactylifera*), *International Journal of food Science and Technology,* 38:709-712.

Al-Shahib, W. and Marshall, R. J. (2003). The fruit of the Date palm: its possible use as the best food for the future? *International Journal of food Science and Nutrition,* 54:247-259.

Al-Yahya, M. A. (1986). Phytochemical and biological studies on Saudi medicinal plants II.The major component of the spathe oil of *Phoenix dactylifera. Fitoterapia,* 57 (4): 592-595.

Amabeoku, G. J., Green, I. and Kabatende, J. (2007). Anticonvulsant activity of *Cotyledon orbiculeta* leaf extract in mice. *Journal of Ethnopharmacology,* 112(1):101-107.

Amabeoku, G. (2008). Anticonvulsant activity of *Nylandtia spinosa* aqueous and methanol leaf extract in mice. *Human Experimental Toxicology*, 112(1):101-107.

Amole, O. O., Yemitan, O. K. and Oshokiyo, K. A. (2009). *African Journal of Pharmacy and Pharmacology,* 3 (6):319-322.

Anderson, M. C., Chung, E. and Van Woert., M. H. (1988). Strychnine seizure potentiation by azaspirodecanedione anxiolytics in rats. *European Journal of Pharmacology,* 155:279-283.

Asadi-Shekaari, M., Panahi, M., Dabiri, S. H., Zahed, S. K. and Pour, T.P. (2008). Neuroprotective effects of aqueous date fruit extract on focal cerebral ischemia in rats. *Pakistan Journal of Medical Sciences,* 24(5):661–665.

Baliga, M. S., Baliga, B. R. V., Kandathil, S. M., Bhat, H. P. and Vayalil, P. K. (2011). A review of the chemistry and pharmacology of the date fruits. *Food Research International Journal*, 44(7):1812–22.

Barhmanpour, S., Talaei, T., Vojdan, Z., Panjehshahin, M. R., Zareei, S. and Ghaeminia, M. (2006). Effect of *Phoenix dactylifera* on sperm parameters and reproductive system of adult male rats. *International Journal of Medical Sciences*, 31:4.

Barh, D. and Mazumdar, B. C. (2008). Comparative nutritive values of palm saps before and after their partial fermentation and effective use of wild Date sap in treatment of anemia. *Research Journal of Medicine and Medical Science*, 3: 173-176.

Brachmann, A. O., Joyce, S. A.; Jenke-Kodama, H.; Schwar, G.; Clarke, D. J and Bode, H. B. (2007). "A type II polyketide synthase is responsible for anthroquinone biosynthesis in photorhabdus luminescens". *Chembiochem: a European Journal of Chemical Biology*, 8(14): 1721-8.

Castel-Branco, M. M., Alves, G. L., Figueiredo, I. V., Falcao, A. C. and Caramona, M. M. (2009). Maximal electroshock seizure (MES) model in the preclinical assessment of potential new antiepileptic drugs. *Methods and Findings in Experimental and Clinical Pharmacology*, 31(2): 101-106.

Coyer, J. R. and South M. J. (1976). Isoniazid-induced seizure in mice. *Seizure*. 15 (2): 22-6.

Dada, T. O. and Odeku, E. L. (2010). Epilepsy in the Nigerian patient. A review of 234 cases. *West Africa Medical Journal*, 15: 153-163.

Duraisami, R., Srinivasan, D. and Ramaswamy, S. (2009). *Journal of Bangladesh Pharmacological Society*, 4: 51-54.

Elleuch, M., Besbes, S., Roiseux, O., Blecker, C., Deroanne, C. and Drira, N. E. (2008). Date flesh: chemical composition and characteristics of the dietary fibre. *Food Chemistry*, 111:67–82.

Emilo, P (2014). Adverse effects of antiepileptic drugs. *Farmacovigilanza*, 80 (1): 1.

Faqir, M. A., Sardar, I. B., Ahmad, H., El-Ghora, M. I. K., Muhammad, N., Shahzad, H. and Muhammad, S. A. (2012). Phytochemical characteristics of Date Palm (*Phoenix dactylifera*) fruit extracts. *Pakistan Journal of Food Science*, 22(3): 117-127.

Fouteye, M., Mohammed, L., Abdoulaye, S., Zeinel, A. O., Bouna, M. V. O., Mohammed, A., Taleb-khyr, O., Djeh, A. O., Mohammed, S. and Boukhary, O. (2014). Assessment of physico-chemical diversity in fruit of Mauritanian date palm (*Phoenix dactylifera). African Journal of Agricultural Research*, Vol. 9(28): 2167-76.

Gates, J. R. (2000). Side effect, profile and behavioral consequences of antiepileptic medications. *Epilepsy behaviour*, 3:153-159.

Glauser, T. A., Cnaan, A., Shinar, S., Hirtz, D. G., Duglos, D. and Mansur, D. (2010). Ethosuximide, valproic acid and lamotrigine in childhood absence epilepsy. *England Journal of Medicine*, 362: 790-799.

Hamza, A. M., Agho, Collins, S. G., Ado, S. G., Ikuenobe, C. E., Ataga, C. D. and Odewale, J. O. (2014). Proximate compositions evaluation and variability among cultivars of Date palm (*Phoenix dactylifera)* in Nigeria. *International Journal of Plant and Soil Science*, Volume 3, Issue 3.

Hela, S. I., Megahed, H. S., Salem, S. M. and Younness, E. R. (2013). Monotherapy versus polytherapy in epileptic adolescents. *Macedonian Journal of Medical Science*, 6(2): 174-177.

Hessen, E., Lossius, M. I. and Gjerstad, L. (2009). Antiepileptic monotherapy significantly impairs normative scores on common tests of executive functions. *Acta Neurology* 119: 194-198.

Hong, Y. J., Tomas-Barberan, F. A. A., Kader, A. and Mitchell, A. E. (2006).The flavonoids glycosides and procyanidin composition of Deglet Nourdates (*Phoenix dactylifera*). *Journal of Agriculture and Food Chemistry*, 54:2405–2411.

Hosseinzadeh, H. and Parvardeh, S. (2004). Anticonvulsant effects of thymoquinonethe major constituent of *Nigella sativa* seeds in mice. *Phytomedicine*, 11(1):56-64.

Igoli, J. O., Ogaji, O. G., Tor-Anyiin, T. A. and Igoli, N. P. (2005). Traditional medicine practice amongst the Igede people of Nigeria Part II. *African Journal of Traditional, Complementary and Alternative Medicines,* 2(2): 134-152.

Jackson, M. J. and Turkington, D. (2006). Depression and anxiety in epilepsy. *Journal of Neurological and Neurosurgical Psychiatry*, 76:145-147.

Javanmardi, J., Stusho, O., Locke, E. and Vivanco, J. M. (2003). Antioxidant activity and total phenolic content of Iranian Ocimumaccessions. *Food Chemistry*, 83: 547-550.

Karunakar, H., Shalin, P., Thakker, A., Joshi, B., Shastry, C. S. and Chandrashekhar, K. S. (2009). *Tropical Journal of Pharmaceutical Resources*, 8(2): 117-125.

Kasture, V. S., Chopde, C. T. and Deshmukh, V. K (2000). Anticonvulsive activity of *Albizzia lebbeck, Hibiscus rosa sinesis* and *Butea monosperma* in experimental animals. *Journal of Ethnopharmacology*,71: 65-75.

Kenoyer, J. M. and Heuston, K. B. (2005). *The Ancient South Asian World*. The World in Ancient Times.Oxford University Press. ISBN 978-0-19-522243-2. Retrieved 30 July, 2013.

Khoshnood-Mansoorkhani, M. J. and Moein, M. R. (2010). Anticonvulsant activity of *Teucrium polium* against seizure induced by PTZ and MES in mice. *Iranian Journal of Pharmaceutical Research*, 9(4):395-401.

Krentz, A. J. and Bailey, C. J. (2005). Oral antidiabetic agents: current role in type 2 diabetes mellitus. *Drugs*, 65: 385-411.

Long, L. (2003). Levetiracetam monotherapy during pregnancy- a case series. *Epilepsy Behaviour*, 4(4): 447-448.

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

Lukasiuk, K. and Pitkanen, A. (2009). Seizure-induced gene expression. In: Encyclopedia of basic epilepsy research. Oxford: Academic Press : 1302-1309.

Magaji, M. G., Yaro, A. H., Musa, A. M., Anuka, J. A., Abdu-Aguye, I. and Hussaini, I. M. (2012). Central depressant activity of butanol fraction of *Scurinega virosa* root bark in mice. *Journal of Ethnopharmacology*, 141:128-133.

Maiha, B. B., Magaji, M. G., Yaro, A. H., Ahmad, S. I. and Magaji, R. A. (2009). Anticonvulsant studies on *Cochlospermum tinctorium* and *Paullinia pinnata*

extracts in laboratory animals. *Nigerian Journal of Pharmaceutical Sciences*, 8(1): 102-108.

Martin, D. L. and Olsen, R. W. (2000). GABA in the Nervous System. Lippincot Williams and Wilkins, Philadelphia.Pp 205-220.

Mansouri, A., Embarek, G., Kokkalou, E. and Kefalas, P. (2005). Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (*Phoenix dactylifera*). *Food Chemistry*, 89:411–416.

Mehrzadi, S., Sadr, S., Hosseinzadeh, A., Gholamine, B. Shahbazi, A., Fallahhusseini, H. and Ghaznavi, H. (2015). Anticonvulsant activity of the ethanolic of *Punica granatum* seed. *Neurological Research*, 37(6):470-475.

Mikki, M. S., Al-Taisan, S. M. and Abdul Azziz, A. A. (1988). Isolation and identification of the chemical constituents of the spathe of the Date palm. Al-Hassa Regional Agricultural Research Center, Hofuf, Saudi Arabia (unpublished work).

Miller, C. J., Dunn, E. V. and Hashim, I. B. (2003). The glycaemic index of dates and date/yoghurt mixed meals. Are dates 'the candy that grows on trees'? *European Journal of Clinical Nutrition*, 57:427–430.

Mohamed, D. A. and Al-Okbi, S. Y. (2004). *In vivo* evaluation of antioxidant and anti-inflammatory activity of different extracts of Dates in adjuvant arthritis. *Polish Journal of food and nutritional sciences*, 13:397-402.

Mohammed, S. A.; Sanni, S.; Ismail, A. M.; Kyari, A. S.; Abdullahi, S. and Amina, I. (2014). Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of *Acacia nilotica* (Thorn mimosa). *Veterinary Research Forum,* 5(2): 95-100.

Muazu, J. and Kaita, A. H. (2008). A review of traditional plants used in the treatment of epilepsy amongst the Hausa/Fulani tribes of northern Nigeria. *African Journal of Traditional, Complementary and Alternative Medicines,* 5(4): 387-390.

Musa, M. A., Abdullahi, I. M., Muhammad, J. K. and Magaji, M. G. (2014). Phytochemical screening and anticonvulsant studies on ethyl acetate fraction of *Globimetula braunii* on laboratory animals. *Asian Pacific Journal of Tropical Biomedicine,* 4(4): 285–289.

Nasser, Z., Majid, A. and Morteza, J. (2011). Date Fruit Extract Is a Neuroprotective Agent in Diabetic Peripheral Neuropathy in Streptozotocin-Induced Diabetic Rats: A Multimodal Analysis. *Oxidative Medicine and Cellular Longevity*, 2011:976948.

Ornoy, A. (2009). Valproic acid in pregnancy: how much are we endangering the embryo and fetus?. *Reproductive Toxicology,* 28(1): 1-10.

Osuntokun, B. O., Bademosi, O. and Familusi, J. B. (2005). Electroencephalographic correlates of epilepsy in Nigerian children. *Developmental Medicine and Child Neurology*, 16(5): 659-663.

Peter, P. D. D., Rudi, D., Bart, M. and Yin-Quan, P. (1992). Chemical models of epilepsy with some reference to their applicability in the development of anticonvulsants. *Epilepsy Research,* 12: 87-110.

Prashant, T.; Bimlesh, K. and Mandeep, K (2011). Phytochemical screening and extraction: A review. *Internationale Pharmaceuticca Sciencia*, 1(1):98-106.

Ragaa, H. M., Amal, M. E.,Mohamad, G. A., Zekry, K. Z., Hussien, A. A., Amany, A. E., Magdy, A., Sabry, S. and Mahmoud, E. (2014). Neuro-activity Effects

of Phoenix dactylifera fruit and Pits Extractin Chronic Stressed Rat Models. *Asia Pacific Journal of Life Sciences*, 4:2.

Raza, M., Alghasham, A. A. M., Alorainy, M. S. and El-Hadiyah, T. M. (2008).“Potentiation of valproate-induced anticonvulsant response by *Nigella sativa* seed constituents: the role of GABA receptors,” *International Journal of Health Sciences*, 2:15–25. Advances in Neurology vol 79 Pp 551-560.

Rezvani, M. E., Roohbakhsh, A., Mosaddegh, M. H., Esmailidehaj, M., Khaloobagheri, F. and Esmaeli, H. (2011). Anticonvulsant and depressant effects of aqueous extracts of *Carum copticum* seeds in male rats. *Epilepsy Behaviour*, 22(2):220-225.

Rogawski, M. A. and Loscher, W. (2004). The neurobiology of antiepileptic drugs. *Nature Reviews Neuroscience*, 5 (7):553-564.

Sabah, A. A., Jassim, B. and Mazen, A. N. (2007). *In vitro* evaluation of the Antiviral activity of an extract of Date on a Pseudomonas Phage. *Food Chemistry*, 15:1-6.

Salah, A. and Al-Maiman, M. (2005). Effect of *Phoenix dactylifera* seed fibers on plasma lipids in rats. *Journal of King Saudi University*, 17: 117-123.

Sayyah, P. E. (2002). Animal Models of seizure and epilepsy in the development of antiepileptic drugs. *Epilepsy*, 23: 345-356.

Sermet, E., Gregoire, M. C., Galy, G., Lavenne, F., Pierre, C., Veyre, L., Lebras, D.,Cinotti, L., Comar, D., Dalery, J. and Bobilier, P. (1998). Paradoxical metabolic response of the human brain to a single electroconvulsive shock. *Neuroscience Letter*; 254: 41-44.

Sharma, R. K. (2008). Concise textbook of forensic medicine and toxicology. 4th Edition. Elsevier. Pp 1-23.

Shibata, S. (2001). Chemistry and cancer preventing activities of gingsponins and some related triterpenoid compounds. *Journal of Korean Medical Sciences,* 16 (supplement):S28-37.

Silvano, S. T., Joseph, V. and Paul, A. E. (2016). Anticonvulsant and anxiolytic activity of leaf of *Melanthera scandens* in a rat model. *African Journal of Pharmacology and Pharmacy*, 222.

Stafford, G., Pedersen, M., Jager, A. and van Staden, T. (2008). South african medicinal plants used to treat Mental illnesses. *African Journal of Traditional, Complementary and Alternative Medicine,* 32(3):12-14.

Sultana, P., Dilruba, E., Afzal, S., Mrityunjoy, B., Subed C. D. S., Golam, M. S. J., Amirul, I. M., Narayan, R. and Mohammed, S. S. (2015). Nutritional analysis of date fruits (*Phoenix dactylifera)* in perspective of Bangladesh, *American Journal of Life Sciences*, Volume 3(4): 274-78.

Swinyard, E. A. (1989). Laboratory evaluation of antiepileptic drugs: Review of laboratory methods. Epilepsia, 10:107–119.

Toman, J. E. P., Swinyard, E. A. and Goodman, L. S. (1946). Properties of maximal seizures and their alteration by anticonvulsant drugs and other agents. *Journal of Neurophysiology*, 69: 45-54.

Thirupathy, K. P. and Saravanan, A. (2009). *African Journal of Pharmacy and Pharmacology*, 3: 63-65.

Vayalil, P. K. (2002). Antioxidant and antimutagenic properties of aqueous extract of date fruit. *Journal of Agricultural and Food Chemistry*; 50: 610–617.

von Stulpnagel, C., Kluger, G., Leiz, S. and Holthausen, H. (2010). Levetiracetam as add-on therapy in different subgroups of “benign” idiopathic focal epilepsies in childhood. *Epilepsy behaviour,* 17: 193-198.

Vyawahare, N. S., Pujari, R. R., Rajendran, R., Khsirsagar, A. D., Ingawale, D. K., and Patil, M. N. (2009). Neurobehavioral effects of Phoenix dactylifera in mice *Journal of Young Pharmacists,* 1(3):225-232.

Wahab, A., Haq, R. U., Ahmed, A., Khan, R. A. and Raza, M. (2009). "Anticonvulsant activities of nutmeg oil of *Myristica fragrans*," *Phytotherapy Research*, 23 (2) 153–158.

Walid, A. and Marshall, J. R. (2003)."The fruit of the date palm: its possible use as the best food for the future?"*International Journal of Food Sciences and Nutrition*

Wan, I. W. I. and Mohammed, N. F. M. R. (2013). Evaluation on the Benefits of Date Palm (*Phoenix dactylifera*) to the Brain. *Alternative and Integrative Medicine,* 2:4.

Wesnes, K. A., Edgar, C., Dean, A. D. P. and Wroe, S. J. (2009). The cognitive and psychomotor effects of remacemide and cabamazepine in newly diagnosed epilepsy. *Epilepsy behaviour*, 14: 522-528.

Wickenden, A. D. (2002). Potassium channels as antiepileptic drug target. *Neuropharmacology*, 43: 1055-1060.

Yamaguchi, S. and Rogawski, M. A. (1992)."Effects of anticonvulsant drugs on 4-aminopyridine-induced seizures in mice". *Epilepsy Research*, 11 (1): 9–16.

Yudkoff, M., Daikhin, Y., Nissim, I., Horyn, O., Luhvry., B and Lazarow, A. (2006). Ketosis and brain handling of glutamate, glutamine and GABA. *Neurochemical International Journal,* 48: 650-656.

Zolfagharian, F., Razavi, B. M. and Hosseinzadeh, H. (2016). Anticonvulsant effect of *Satureja hortensis* aerial parts extract in mice. *Avicenna Journal of Phytomedicine*, 6(3): 305-312.