EVALUATION OF ANTICONVULSANT, SEDATIVE AND ANXIOLYTIC ACTIONS OF *JATROPHA CURCAS*AND *ALLIUM SATIVUM* IN MICE.

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DEDICATION

This dissertation is dedicated to the Almighty God who made all things possible, my husband and our children Jesutofunmi and Oluwabukunmi.

CERTIFICATION

We hereby certify that this work titled, '' Evaluation of Anticonvulsant, Sedative and Anxiolytic actions of *Jatropha curcas* and *Allium sativum* in mice'' was carried out in the Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria by OSASAN, Josephine Yetunde under our supervision:

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TABLE OF CONTENTS

CHAPTER TWO

CHAPTER FOUR

LIST OF TABLES

Table

Pages

LIST OF FIGURES

Figure

Pages

LIST OF ABBREVIATION

1. SE - Status epilepticus
2. PTZ – Pentylenetetrazole
3. PTX - Picrotoxin
4. STR – Strychnine
5. PILO –Pilocarpine
6. MES – Maximal electroshock
7. ILAE - International League Against Epilepsy
8. GABA – Gamma amino butyric acid
9. EEG – Electroencephalogram
10. JME- Juvenile myoclonic epilepsy
11. MRI – Magnetic resonance imaging
12. MRS – Magnetic resonance spectroscopy
13. fMRI – Functional magnet resonance imaging
14. PET – Position emission tomography
15. MEG – Magnetoencaphalography
16. EMU – Epilepsy monitoring unit
17. AEDS - Antiepileptic drugs
18. FDA - Federal Drug Administration
19. CNS – Central nervous system
20. REM - rapid-eye-movement
21. NREM - non- rapid-eye-movement

22. EMG - electromyogram
23. EOG - electro-oculogram
24. SCN - suprachiasmatic nuclei
25. ICSD - International Classification of Sleep Disorders
26. OSAS - Obstructive sleep apnoea syndrome
27. PLMS - periodic limb movements in sleep
28. RBD - REM behaviour disorder
29. RLS - Restless legs syndrome
30. DLBD - Diffuse Lewy- Body disease with dementia
31. FASPS - Familial advanced sleep-phase syndrome
32. PTSD - Post-traumatic stress disorder
33. GAD - Generalized anxiety disorder
34. OCD - Obsessive-compulsive disorder
35. CBT - Cognitive-behavioural Therapy
36. OFT – open field test
37. EPM – elevated plus maze
38. HB – Hole board
39. DMSO – Dimethylsultoxide
40. L.D. – Lethal dose
41. ANOVA – Analysis of Variance
42. SEM – Standard error of mean
43. HLTE - hind limb tonic extension
44. I.P. - Intraperitoneal

45. P.O. - Per oral

ABSTRACT

This study investigated the anticonvulsant, anxiolytic and hypnotic effects of the methanolic extracts of *Jatropha curcas* Linn (Euphorbiaceae) leaf and *Allium sativum* Linn (Liliaceae) bulb in mice. It also determined the most active fraction of the two extracts. This was with a view to providing scientific information for their ethnomedicinal claims.

The cold methanolic extracts were obtained by maceration. The LD_{50} of the extracts were determined using Lorke's method. Doses of (*J. curcas:* 500, 1000, 1500 mg/kg, per oral (p.o.) and 50, 100, 150 mg/kg, intraperitoneal (i.p.) and *A. sativum* 500, 1000, 2000 mg/kg, p.o., and 200, 400, 800 mg/kg, i.p.) were screened for anxiolytic, hypnotic and anticonvulsant activities. The anxiolytic effect was evaluated in Open Field Test (OFT), Elevated-Plus Maze (EPM) and Holeboard (HB), the hypnotic effect of the extracts was determined in pentobarbital - induced sleep model where the sleep latency and total sleeping time were assessed, while the anticonvulsant test was carried out using strychnine (STR), pentylenetetrazole (PTZ), picrotoxin (PTX), pilocarpine (PILO) and maximum electroshock (MES) - induced convulsion models. *J. curcas* extract with most potent anticonvulsant effect was separated into n-hexane, ethylacetate and butanol fractions. Each fraction (n-hexane 10, 15, 20 mg/kg, i.p., ethylacetate 10, 20 40 mg/kg, i.p. and butanol 100, 200 300 mg/kg, i.p.). Each fraction was evaluated for anxiolytic, hypnotic and anticonvulsant effects (PTZ, PTX and PILO - the models with highest anticonvulsant protection from the earlier models). The mechanism of action of the ethylacetate fraction that showed the highest anticonvulsant activity to pentylenetetrazole - induced convulsion was evaluated using appropriate antagonist, flumazenil. The results were analysed using one-way ANOVA followed by the Student- Newman keul-test.

The oral LD_{50} of *J. curcas* and *A. sativum* were 3808 and ≥ 5000 mg/kg, respectively, and interperitoneally 346 and 2154 mg/kg, respectively in mice. The LD_{50} of n-hexane, ethylacetate and butanol fractions of *J. curcas* also gave 49, 89 and 775 mg/kg, i.p. respectively in mice. The effect of the extracts in the OFT significantly ($p<0.05$) decreased locomotor, rearing and grooming activities and in HB there was a significant ($p<0.05$) decrease in number of head dips suggesting that the extracts possessed central nervous system depressant activity. The extracts also reduced sleep latency and prolonged sleeping time. The results showed that the extract of *J. curcas* gave a better anticonvulsant protection than *A. sativum* in PTX and PTZ – induced convulsion and significantly ($p < 0.05$) prolonged onset of clonic tonic seizures and death latency. In the PILO-induced convulsion the duration of seizure was significantly ($p < 0.05$) reduced and death latency was significantly prolonged. The three fractions of *J. curcas* that were evaluated showed varying degrees of anxiolytic, hypnotic and anticonvulsant effects. The ethylacetate fraction was found to be the most potent compared to the n-hexane and butanol fractions. The mechanism of action of the ethylacetate fraction is due to its interaction with GABA – benzodiazepine receptors.

The study concluded that *J. curcas* and *A. sativum* possessed anxiolytic, hypnotic and anticonvulsant activities. The anticonvulsant activity of *J. curcas* was higher than that of *A. sativum,* thus providing scientific evidence in support of the traditional use of the plant in the management of epilepsy.

CHAPTER ONE

1.0 INTRODUCTION

In traditional medicine, various parts of plants such as the leaf, stem, bulb and root have been used for medicinal purposes over the centuries. These parts have been claimed to possess various clinical activities such as anti-ulcer (Devi *et al*., 2008), anti-inflammatory, laxative, analgesic, anticancer, antibacterial, wound healing, anti-stress (Baliga *et al*., 2013), sedative (Lopes *et al*., 2011), antipyretic (Velazquez *et al*., 2009), antimicrobial (Liolios *et al*., 2010), anxiolytic (Ambawade *et al*., 2001), anti-diarrhoeal (Aniagu *et al*., 2005), antidiabetic, antioxidant (Nain *et al*., 2012). In various parts of the world, many medicinal plants based on folk medicine or traditional uses have been investigated. Herbal products from medicinal plants have contributed immensely to the discovery of modern drugs and can also be used as an alternative source of therapy such as antiepileptic drugs because of better safety and efficacy compared to the problems associated with orthodox antiepileptic drugs as seen in the various side effects, dose-related problems, chronic toxicity, teratogenic effects (Mathur *et al*., 2010; Aldenkamp, 2006), and the fact that some patients are never seizure free despite the use of these modern antiepileptic drugs (AEDS) (Raza *et al*., 2003).

Convulsion or seizure is one of the most common neurological problems in our society today. The frequency is on the increase and this can no longer be overlooked. Studies have shown that the overall incidence of epilepsy in developed societies has been found to be around 50 cases per 100,000 persons per year and on a very high side in old age (Poole *et al*., 2000; Ropper and Brown, 2005). Out of these number of patients, only about two-third, have their seizures well controlled with currently available antiepileptic drugs while in the remaining one-third patients

seizures are refractory to treatment suggesting that seizures are not well controlled by drugs (Bialer, 2006., Perucca *et al*., 2007). For instance, neonatal seizures which were once treated effectively with phenobarbital and phenytoin suddenly became refractory in some patients, which brought about the use of other antiepileptic drugs such as levetiracetam and topiramate (Tulloch *et al*., 2012). Also studies have shown that refractory status epilepticus occurs when status epilepticus (SE) fails to respond to appropriate typical antiepileptic drugs used in management of epilepsy (Synowiec *et al*., 2013). Therefore, there is need for more research to help develop new strategies and medicinal agents for treating patients who become refractory to the medications and therapeutic targets currently used (Boyd *et al*., 2012). The use of these available antiepileptic drugs produce many undesirable adverse effects in patients, which include dose related neurotoxic effects such as drowsiness, fatigue, dizziness, mental dullness, blurry vision, incoordination, idiosyncratic adverse effects like serious rash (Stevens-Johnson Syndrome, toxic epidermal necrolysis), hematologic changes, weight gain, severe hypertrophy of gums, hepatotoxicity, bone density loss and congenital malformations for instance teratogenesis in women of reproductive age (St. Louis, 2009). Looking at these numerous side effects, there is need for the discovery and developments of new antiepileptic drugs with improve seizure control and minimal side effects.

Therefore, considering the problems associated with modern antiepileptic drugs, herbal products may be of advantage over the orthodox drugs in the management of seizure. Hence, there is need for more research into medicinal plants with possible anticonvulsant activity based on traditional use. Thus, in line with this, the following plants *Jatropha curcas* and *Allium sativum* were screened for anticonvulsant effect using behavioural models for anticonvulsant such as

pentylenetetrazole (PTZ), strychnine (STR),picrotoxin (PTX), pilocarpine (PILO) and maximal electroshock (MES).

1.1. LITERATURE REVIEW

1.1.1. Seizures

1.1.1.1 Definition of seizures

Seizures are central nervous system disorder characterized by sudden, excessive and abnormal electrical discharge from the brain cells resulting in a variety of events or symptoms such as feelings or loss of consciousness, alteration in awareness, abnormal movements, atypical or odd behavior, distorted perceptions that are of limited duration but recur if untreated (Harvey and Champe, 2009; Porter and Meldrum, 2009). The International League Against Epilepsy (ILAE) defines epilepsy as "a condition characterized by two or more recurrent epileptic seizures over a period longer than 24 hours, unprovoked by any immediate identified cause" (Sander, 1997). A modification in the definition was suggested later, the new definition is said to require the occurrence of at least one seizure plus a clear predisposing factor. This modification could have practical consequences because the prevalence of epilepsy can be overestimated (Fisher *et al*., 2005).

1.1.1.2 Epidemiology of Seizures

Epilepsy is a very common disorder affecting as much as 50 million people worldwide and about eighty percent of them are living in developing countries, of which 90% do not have access to and hence do not receive appropriate treatment (Scott *et al*., 2001). The prevalence of epilepsy in

the developed countries ranges from 4 to 10 cases per 1,000. Studies in the developing and tropical countries have reported a higher prevalence rate of epilepsy, ranging from 14 to 57 cases per 1,000 persons (Burneo *et al*., 2005; Carpio and Hauser, 2009). The prevalence of epilepsy out of every 1000 population is 10-13 in Africa, 14-18 in Latin America, 5-8 in Nigeria and the prevalence is higher in males than females, 11 out of 16 in reviewing record data and 16 out of 29 in door to door evaluation studies (Banerjee *et al*., 2009).