Original Research Article

Mechanism of Anticonvulsant Effects of Ethanol Leaf Extract and Fractions of *Milicia Excelsa* (Moraceae) in Mice.

ABSTRACT

Aims: This study investigated the anticonvulsant potential of ethanol leaf extract and fractions of *Milicia excelsa* (Moraceae).

Study Design: This study used experimental animal models predictive of human convulsion in mice

Place and Duration of Study: Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria, between January 2014 to February 2015.

Methodology: The anticonvulsant effect of ethanol leaf extract (EME), n-hexane (HF), ethyl acetate (EAF), n-butanol (BF) and aqueous (AF) fractions of the extract was evaluated using picrotoxin-, pentylenetetrazole-, and strychnine-induced convulsion models. The neural mechanism of anticonvulsant effect of the most active fraction (AF) was also investigated using flumazenil (3 mg/kg, i.p.), cyproheptadine (4 mg/kg, i.p.) and L-N^G-Nitroarginine (10 mg/kg, i.p.) in picrotoxin-induced convulsion model.

Results: EME and AF significantly (P < .05) delayed the onset of clonic and tonic convulsions and death latency with varying degree of protection in picrotoxin-, pentylenetetrazole-induced convulsion models. EME, EAF and AF significantly (P < .05) pronlonged the onset of clonic convulsion in strychnine-induced convulsion in mice. Flumazenil, cyproheptadine and L-N^G-Nitroarginine abolished the anticonvulsant effect of AF suggesting the involvement of GABAergic, serotonergic and nitergic pathways.

Conclusion: This study concludes that *Milicia excelsa* leaf contains biologically active anticonvulsant principles, thus lending pharmacological credence to the suggested traditional use. Further study may be undertaken to isolate and elucidate the chemical structure of the biologically active ingredient(s) responsible for the observed anticonvulsant

effect.

Keywords: *Milicia excelsa*, anticonvulsant, nitergic, serotonergic, GABAergic

1.0 Introduction

Epilepsy (often interchangeably called seizure disorder) is one of the common and serious neurological disorders [1], characterized by spontaneous and recurrent seizure [2], resulting from sudden and excessive discharge by some cerebral neurons in the brain [3]. It is estimated to be affecting 50 million people worldwide [4], with 40 % being women [5]. Abnormal cellular discharge may be associated with a variety of causative factors such as- trauma, oxygen deprivation, tumors, infection and metabolic derangements producing long lasting plastic changes in the brain affecting neurotransmitters release and transport, the properties of receptors and channels, regulation of gene expression, synaptic reorganization and astrocyte activity [5].

All the currently available antiepileptic drugs (AEDs) are synthetic drugs, [1] and in spite of their availability, almost one-third of epileptic patients appear to be refractory to all pharmacological interventions [6, 7]. Besides the inability of these drugs to effectively and efficiently control seizure, their adverse effects remained to be fully circumvented [8]. They have debilitating adverse effects on cognition and behaviour [9]) which are commonly and consistently observed with barbiturates, benzodiazepines, and topiramate [10, 11]. Hence, the search for antiepileptic agents with better selective activity and lower toxicity should be a continuous endeavour [12].

Medicinal plants have been used in the treatment of different human ailments in different parts of the world [13]. Natural products from folkloric medicines have contributed in no small measures to the discovery of modern drugs and can serve as an alternative source for the discovery of AEDs with novel structures and better safety and efficacy profiles [14]. To this end, elaborate studies should be geared towards botanicals claimed in traditional medicines to be beneficial against serious disorders such as epilepsy [15, 16]. This can indeed be a good beginning in search for safer and more effective remedies [17]. Numerous plants claimed to be useful in traditional medicine for the treatment of epilepsy have been demonstrated to be potent in models of epileptic research and several such plants remain to be scientifically evaluated and validated [17].

Milicia excelsa (welw.) C.C. Berg belongs to the family Moraceae popularly known as Iroko tree or African teak. It is a large deciduous tree 30 to 50 m high, occurring naturally in humid forests of West Africa [18]. Its latex, leaf, stem bark, root, fruit, and ashes are used in African traditional medicine to

prepare ethnomedicines for the treatment of malaria [19], anaemia [20], lactation failure [21], mental illnesses [22, 23, 24], sexual dysfunction [25], rheumatism [26] and convulsion [27].

Lupeol acetate, ursolic acid, triacontyl (E)-ferulate, 2-(3,5-dihydroxyphenyl)benzofuran-5,6-diol) and a benzylic diglycoside identified as 3,4-dimethoxybenzyl beta-D-xylopyranosyl (1 --> 2)-beta-D-glucopyranoside have been isolated from the leaf of *Milicia excelsa* [28].

Preliminary investigations from our laboratory showed that the median lethal dose (LD_{50}) of the ethanol leaf extract, n-hexane (HF), ethyl acetate (EAF), n-butanol (BF) and aqueous (AF) fractions were greater than 5000 mg/kg via oral route in mice (Personal communication).

The objective of this study was to investigate the anticonvulsant potential of the ethanol leaf extract, HF, EAF, BF, and AF using mice models. To the best of our knowledge; the anticonvulsant potential of the leaf has not been reported upon comprehensive literature search.

2.0 Materials and Methods

2.1 Plant identification and authentication

Milicia excelsa leaves were collected within the campus of the Obafemi Awolowo University (OAU). It was identified and authenticated by Mr. G. A. Ademoriyo of the Herbarium Unit, Department of Botany, Faculty of Sciences, OAU, Ile-Ife and herbarium number Ife-17482 was obtained.

2.2 Preparation of Plant Materials

The leaves of *Milicia excelsa* were air dried at room temperature. The dried leaves were pulverized and 1.0 kg of the powder was extracted with 3 liters of seventy percent (70%) ethanol for 72 h. The marc was re-extracted once and the combined extract was concentrated *in vacuo* at a temperature of 40^{o}C to yield 70 g (7.0%) crude extract and coded (EME) (Personal communication). Sixty gram (60g) of the crude extract was successively partitioned into n-hexane, ethylacetate, n-butanol and aqueous fractions. The fractions were again concentrated *in vacuo* to give n-hexane, ethyl-acetate, n- butanol, and aqueous fractions (Personal communication). EME and the fractions were freshly prepared by dissolution in 2% Tween 20 in normal saline on each day of the experiment.

2.3 Laboratory animal

Male and female adult albino mice weighing 18–25 g were obtained from the Animal House, Department of Pharmacology, Faculty of Pharmacy, OAU, Ile-Ife. The animals were acclimatized for one week before the commencement of the experiments. They were housed in cages lined with wood

beddings and maintained at room temperature, under natural light/darkness cycle. They were fed standard animal pellets and water *ad libitum*. Experimental protocols were carried out strictly according to the National Institute of Health [29]. The experiments were performed between 9.00 am and 3.00 pm on each day of the experiment.

2.4 Drugs

Diazepam (DZP) (Roche, Basel, Switzerland); Picrotoxin (PTX), Pentylenetetrazol (PTZ), Strychnine (SCN), L-N^G-Nitroarginine (L-NNA), Cyproheptadine (CYPRO), Tween 20 (Sigma Chemicals Co, St. Louis, Missouri, U.S.A.), Flumazenil (Hikma Farmaceutical, Portugal, S.A.), Phenobarbitone (May and Baker, Lagos, Nigeria) and normal saline (Unique Pharmaceutical Limited, Lagos, Nigeria). EME and its fractions were dissolved with 2% Tween 20 and made up to the required volume with normal saline. The drugs, EME and fractions were freshly prepared on each day of the experiments.

2.5 Pharmacological experiments

2.5.1 PTZ induced convulsion

Tonic-clonic convulsion was induced by PTZ (85 mg/kg, i.p.) as previously described [30]. Mice were divided into five different groups containing 6 mice per group (n=6). Group I mice (negative control) were orally ingested 2% Tween 20 in normal saline (10 mL/kg). Groups II-IV (treatment groups) were orally ingested with EME at the doses of 250, 500 and 1000 mg/kg. Sixty minutes after respective treatments, Groups I-IV received PTZ (85 mg/kg, i.p.). The procedures were repeated for HF, EAF, BF and AF at the doses 250, 500 and 1000 mg/kg respectively. Group V mice (positive control group) received diazepam (1 mg/kg, i.p.) 30 minutes prior to PTZ (85 mg/kg, i.p.) injection. Each mouse was observed for the onset of clonic, tonic convulsion and death latency in seconds immediately following PTZ injection. Animals that survived beyond 30 minutes were considered protected.

2.5.2 PTX- induced convulsion

Tonic-clonic convulsion was induced by PTX (10 mg/kg, i.p.) as adapted from previous [31, 32]. Mice were divided into five different groups containing 6 mice per group (n=6). Group I mice (negative control) were orally ingested 2% Tween 20 in normal saline (10 mL/kg). Groups II-IV (treatment groups) were orally ingested with EME at the doses of 250, 500 and 1000 mg/kg. Sixty minutes after respective treatments, Groups I-IV received PTX (10 mg/kg, i.p.). The procedures were repeated for HF, EAF, BF and AF at the doses 250, 500 and 1000 mg/kg respectively. Group V mice (positive control group) received diazepam (1 mg/kg, i.p.) 30 minutes prior to PTX (10 mg/kg, i.p.) injection.

Each mouse was observed for the onset of clonic, tonic convulsion and death latency in seconds immediately following PTX injection. Animals that survived beyond 30 minutes were considered protected.

2.5.3 SCN- induced convulsion

SCN (4 mg/kg, i.p.) was used to induce tonic-clonic convulsions [30]. Mice were divided into five different groups containing 6 mice per group (n=6). Group I received normal saline (10 mL/kg, p.o.) for 60 minutes prior to SCN (4 mg/kg, i.p.) injection, groups II-IV were pre-treated with EME at the doses of 250, 500, and 1000 mg/kg, p.o. for 60 minutes prior to SCN (4 mg/kg, i.p.) injection. The procedures were repeated for HF, EAF, BF and AF at the doses of 250, 500 and 1000 mg/kg respectively. Group V was pre-treated with Phenobarbitone (30 mg/kg, i.p.), a standard drug for 30 minutes prior to SCN (4 mg/kg, i.p.) injection. Each animal was observed for tonic-clonic convulsion. Animals that survived beyond 30 minutes were regarded as protected. The onset of convulsion and time of the death of each mouse was recorded in seconds.

2.5.4 Mechanism of anticonvulsant effect

In order to delineate the mechanism of anticonvulsant action, AF was used, and considered as the most active fraction, because it gave the highest percentage protection of 83.3 and 100 at the highest dose of 1000 mg/kg, p.o in PTZ-, and PTX-induced convulsion models respectively. To this effect, another set of mice were pretreated with flumazenil ($GABA_A$ receptor antagonist, 3.0 mg/kg, i.p.) [33], cyproheptadine (5-HT receptor antagonist, 4 mg/kg i.p) [34], and L-NNA (Nitric oxide synthase inhibitor, 10 mg/kg, i.p.) [33], for 15 minutes prior to oral administration of AF (1000 mg/kg, p.o.). One hour later, the mice were given PTX (10 mg/kg, i.p.). The onset of clonic, tonic convulsion and death latency were recorded for each mouse. Animals that survived beyond 30 minutes were considered protected.

2.6 Statistical Analysis

Results are expressed as mean ± S.E.M. The significance of different between groups were analysed using one way analysis of variance (ANOVA), followed by post hoc analysis using the Student-Newman-keuls test. GraphPad InStat® Biostatistics software (GraphPad Software, Inc., La Jolla, USA) was used and the level of significance for all tests was set at $*P < 0.05$.

3.0 Results

3.1 Effects of HF, EME, EAF, BF and AF of *Milicia excelsa* on PTZ-induced convulsion model in mice.

The onset of clonic convulsion was significantly [$F_{(4, 25)} = 5254.9$, $P < 0.0001$] delayed by EME at all the doses used (250, 500 and 1000 mg/kg, p.o.), onset of tonic convulsion was significantly [$F_{(4, 25)} = 14.548$, $P < 0.001$] delayed at 250 and 500 mg/kg, p.o. while the time of death was also significantly [$F_{(4, 25)} = 24.513$, $p< 0.0001$] prolonged at 250 and 500 mg/kg, p.o. compared to the vehicle treated control group. The extract at 1000 mg/kg, p.o. did not have any significant effect on the onset of clonic, tonic and the time of death. EAF at 500 mg/kg, 1000 mg/kg and DZP (1 mg/kg) significantly [$F_{(4, 25)} = 4099.4$; $P < 0.0001$)] and [$F_{(4, 25)} = 213.29$; $P < 0.0001$)] prolonged the onset of clonic and tonic convulsions respectively while at 500, 1000 mg/kg and DZP (1 mg/kg) significantly [$F_{(4, 25)} = 184.93$; $P < 0.0001$)] delayed the death latency when compared to the vehicle treated control group. AF at 1000 mg/kg, and DZP (1 mg/kg) significantly [$F_{(4, 25)} = 21.230$; $P < 0.0001$)] and AF at 500, 1000 mg/kg and DZP (1 mg/kg) significantly [$F_{(4, 25)} = 20.168$; $P < 0.0001$)] prolonged the onset of clonic and tonic convulsions respectively while at 500, 1000 mg/kg and DZP (1 mg/kg) significantly [$F_{(4, 25)} = 18.845$; $P < 0.0001$)] delayed the death latency when compared to the vehicle treated control group. EME offered 33.3% and 50% protection at 250 and 500 mg/kg, p.o respectively. AF at 500 and 1000 mg/kg offered 50 and 83.3% protection respectively while EAF offered no protection at all the doses used in this study. HF and BF had no significant effects on clonic, tonic convulsion and death latency. The result is presented in Table 1.

3.2 Effects of EME, HF, EAF, BF and AF of *Milicia excelsa* on PTX induced convulsion model in mice.

The result obtained showed that EME at all the doses used (250, 500 and 1000 mg/kg, p.o.) and diazepam (1 mg/kg) significantly [$F_{(4, 25)} = 10.288$; $P < 0.0001$)], [$F_{(4, 25)} = 7.838$; $P = 0.0003$)] and [$F_{(4, 25)} = 6.541$; $P = 0.001$)] delayed the onset of clonic and tonic convulsions and death latency. AF significantly [$F_{(4, 25)} = 6.739$; $P = 0.0008$)], [$F_{(4, 25)} = 7.516$; $P = 0.0004$)] and diazepam (1 mg/kg) prolonged the onset of tonic convulsion and death latency but had no significant effect on the onset of clonic convulsion. EME offered 100, 50 and 33.3% protection at 250, 500 and 1000 mg/kg respectively. EAF offered 33.3% protection at 1000 mg/kg and 16.3% protection at 250 and 500 mg/kg respectively. AF offered 50, 66.7 and 100% protection at 250, 500 and 1000 mg/kg

respectively. EAF, HF and BF had no effect no significant effects on clonic, tonic convulsion and death latency. The result is presented in Table 2.

3.3 Effects of EME, HF, EAF, BF and AF of *Milicia excelsa* on SCN-induced convulsion model in mice.

EME, and AF at all the doses used (250, 500 and 1000 mg/kg, p.o.) significantly [$F_{(4, 25)} = 29.060$; $P < 0.0001$)] and [$F_{(4, 25)} = 6.411$; $P = 0.0011$)] delayed the onset of clonic convulsion while they have no significant effect on the onset of tonic convulsion and death latency. EAF at the doses of 500 and 1000 mg/kg, significantly [$F_{(4, 25)} = 4.389$; $P = 0.008$)] prolonged the onset of clonic convulsion. EME, EAF and AF offered 0% protection at all the doses used in this study. HF and BF had no significant effects on clonic, tonic convulsion and death latency. Phenobarbitone, a reference anticonvulsant drug at 30 mg/kg, i.p. significantly prolonged the onset of clonic, tonic convulsion, death latency and offered 33.3% protection. The result is presented in Table 3.

3.4 Effect of pretreatment with flumazenil, cyproheptadine, and L-NG-Nitroarginine before AF in PTX-induced convulsion in mice.

The result presented in Table 4 showed the pretreatment of animals with flumazenil (3 mg/kg, i.p, a $GABA_A$ receptor antagonist), cyproheptadine (3 mg/kg, i.p, a 5-HT receptor antagonis) and L-NNA (Nitric oxide synthase inhibitor, 10 mg/kg, i.p.), before treatment with AF (1000 mg/kg, p.o.) administration significantly [$F_{(5, 30)} = 8.498$, $p< 0.001$], [$F_{(5, 30)} = 14.464$, $p< 0.001$] and [$F_{(5, 30)} = 12.011$, $p< 0.001$] reversed the onset of tonic convulsion; significantly [$F_{(5, 30)} = 12.539$, $p< 0.001$], [$F_{(5, 30)} = 17.211$, $p< 0.001$] and [$F_{(5, 30)} = 13.084$, $p< 0.001$] reversed death latency of AF when compared to AF treated mice. Thus, reversed the anticonvulsant effect of AF. The results are presented in Table 4.

Table 1: The anticonvulsant effects of EME, EAF, and AF in PTZ-induced convulsion model in mice.

Treatments (mg/kg) + PTZ	Onset of clonic convulsion (secs)	Onset of tonic convulsion (secs)	Death latency (secs)	Quantal protection	% Protection
VEH	58.0 ± 4.4	254.2 ± 40.0	314.2 ± 43.6	0/6	0
EME (250)	167.8 ± 6.4*	1145.0 ± 260.7*	1425.7 ± 198.2*	2/6	33.3
EME (500)	127.8 ± 10.3*	1197.8 ± 238.9*	1496.8 ± 202.5*	3/6	50
EME (1000)	102.2 ± 19.4*	426.7 ± 93.0	451.0 ± 96.0	0/6	0
EAF (250)	53.0 ± 2.2	307.5 ± 22.2	377.5 ± 35.7	0/6	0
EAF (500)	148.5 ± 26.0*	148.5 ± 26.0	171.2 ± 28.5*	0/6	0
EAF (1000)	79.0 ± 4.4	523.5 ± 90.0*	535.8 ± 88.6*	0/6	0
AF (250)	118.5 ± 9.1	430.8 ± 92.4	505.2 ± 110.3	0/6	0
AF (500)	107.0 ± 4.3	1277.7 ± 249.9*	1305.5 ± 237.2*	3/6	50
AF (1000)	969.7 ± 371.4*	1585.2 ± 214.8*	1587.5 ± 212.5*	5/6	83.3

DZP (1)	1800.0± 0.0*	1800.0± 0.0*	1800.0± 0.0*		100

VEH; vehicle 2% Tween 20 in Normal saline (10 mL/kg, p.o), EME, EAF, and AF represent ethanol leaf extract, ethyl acetate, and aqueous fractions of *Milicia excelsa* respectively, DZP; diazepam. PTZ; pentylenetetrazole. Values are Mean ± SEM, ANOVA; one way analysis of variance followed by Student-Newman Keuls Test, n=6, *P < 0.05 compared to the vehicle treated control.

Table 2: The anticonvulsant effects of EME, EAF, and AF in PTX- induced convulsion model in mice.

Treatments (mg/kg) + PTX	Onset of clonic convulsion (secs)	Onset of tonic convulsion (secs)	Death latency(secs)	Quantal protection	% Protection
VEH	414.2 ± 33.1	833.8 ± 203.4	972.5 ± 161.3	0/6	0
EME (250)	663.7 ± 42.3*	1786.6 ± 13.2*	1800.0 ± 0.0*	6/6	100
EME (500)	563.0 ± 43.0*	1434.3 ± 166.0*	1532.3 ± 179.1*	3/6	50
EME (1000)	508.2 ± 47.6*	1307.2 ± 163.0*	1538.8 ± 170.8*	2/6	33.3
EAF (250)	422.0 ± 15.2	1203.8 ± 151.5	1277.2 ± 131.5	1/6	16.7
EAF (500)	466.3 ± 21.6	1230.3 ± 145.8	1254.5 ± 148.5	1/6	16.7
EAF (1000)	411.5 ± 13.5	1104.7 ± 56.8	1406.7 ± 127.2	2/6	33.3
AF (250)	462.7 ± 38.4	1456.5 ± 163.0*	1451.7 ± 159.0*	3/6	50
AF (500)	437.5 ± 16.4	1453.3 ± 161.0*	1547.5 ± 159.7*	4/6	66.7
AF (1000)	486.5 ± 11.2	1695.0 ± 66.5*	1800.0 ± 0.0*	6/6	100
DZP (1)	843.3 ± 77.9*	1763.0 ± 37.0*	1800.0 ± 0.0*	6/6	100

VEH; vehicle 2% Tween 20 in Normal saline (10 mL/kg, p.o), EME, EAF and AF represent ethanol leaf extract, n-hexane, ethyl acetate, n-butanol and aqueous fractions of *Milicia excelsa* PTX; picrotoxin, DZP; diazepam, Values are Mean ± SEM, ANOVA; one way analysis of variance followed by Student-Newman Keuls Test, n=6, *P < 0.05 compared to the vehicle treated control group.

Table 3: The anticonvulsant effects of EME, EAF, and AF in SCN-induced convulsion model in mice.

Treatments (mg/kg) + SCN	Onset of clonic convulsion (secs)	Onset of tonic convulsion (secs)	Death latency(secs)	Quantal protection	% Protection
VEH	136.0 ± 7.7	141.0 ± 8.6	148.7 ± 10.8	0/6	0
EME (250)	169.5 ± 8.1*	181.8 ± 6.8	212.3 ± 15.1	0/6	0
EME (500)	215.2 ± 13.6*	228.5 ± 13.6	238.0 ± 12.7	0/6	0
EME (1000)	186.2 ± 13.2*	189.2 ± 14.1	203.3 ± 16.3	0/6	0
EAF (250)	305.3 ± 49.1*	305.3 ± 49.1	322.5 ± 51.3	0/6	0
EAF (500)	258.7 ± 30.5*	297.3 ± 40.8	321.5 ± 44.6	0/6	0
EAF (1000)	265.3 ± 41.9*	299.0 ± 40.5	314.2 ± 41.3	0/6	0
AF (250)	240.7 ± 49.2*	280.2 ± 43.2	297.2 ± 41.7	0/6	0
AF (500)	250.0 ± 8.1*	275.7 ± 12.1	289.7 ± 9.7	0/6	0
AF (1000)	263.7 ± 13.8*	313.7 ± 11.3	343.0 ± 17.6	0/6	0
PBT (30)	298.7 ± 13.0*	1007.7 ± 274.9*	1042.7 ± 261.0*	2/6	33.3

VEH; vehicle 2% Tween 20 in Normal saline (10 mL/kg, p.o), EME, EAF and AF represent ethanol leaf extract, ethylacetate, and aqueous fractions of *Milicia excelsa* SCN; strychnine, PBT; phenobarbitone, Values are Mean ± SEM, ANOVA; one way analysis of variance followed by Student-Newman Keuls Test, n=6, *P < 0.05 compared to vehicle treated control group.

Table-4

Treatments (mg/kg) + PTX	Onset of clonic convulsion (secs)	Onset of tonic convulsion (secs)	Death latency(secs)	Quantal protection	% Protection
VEH	414.2 ± 33.3	833.8 ± 198.2	882.5 ± 188.3	0/6	0
AF (1000)	486.5 ± 11.2	1695.0 ± 66.5*	1800.0 ± 0.0*	6/6	100
DPZ (1)	843.3 ± 77.9*	1763.0 ± 37.0*	1800.0 ± 0.0*	6/6	100
FLU (3)	307.2 ± 17.6	753.7 ± 43.0	767.8 ± 42.7	0/6	0
CYPRO (4)	511.2 ± 9.9	1241.0 ± 126.3	1263.2 ± 126.3	0/6	0
L-NNA (10)	386.7 ± 35.2	793.7 ± 170.1	966.7 ± 138.2	0/6	0
FLU (3) + AF (1000)	454.0 ± 95.9	862.8 ± 195.7*#	930.2 ± 91.5*#	0/6	0
FLU (3) + DZP (1)	401.7 ± 57.2β	1004.5 ± 246.5β	1018.0 ± 246.8β		0
CYPRO (4) + AF (1000)	582.2 ± 49.1	1037.0± 100.3*#	1054.7 ± 99.4*#	0/6	0
CYPRO (4) + DZP (1)	846.5 ± 70.2	1800.0 ± 0.0	1800.0 ± 0.0	6/6	100
L-NNA (10) + AF (1000)	442.0 ± 38.5	1152.2 ± 191.3#	1171.7 ± 189.6#	0/6	0
L-NNA (10) + DZP (1)	1581.3 ± 138.6α	1800.0 ± 0.0	1800.0 ± 0.0	6/6	100

VEH; vehicle; 2% Tween 20 in Normal saline (10 mL/kg, p.o), AF; Aqueous fraction of ethanolic leaf extract of *M. excelsa*, DZP; diazepam (1 mg/kg, i.p.), FLU; flumazenil (3 mg/kg, i.p.), PTX; picrotoxin (10 mg/kg, i.p.), CYPRO; cyproheptadine (4 mg/kg, i.p.), L-NNA; L-N^G-Nitroarginine (10 mg/kg, i.p.). Values are Mean ± SEM, ANOVA; one way analysis of variance followed by Student-Newman Keuls Test, n=6, *$P < 0.05$, $^{\#}P < 0.05$, and $^{\beta,\alpha}P < 0.05$ compared to vehicle, AF and DZP group respectively.

4.0 DISCUSSION

The findings of this present work provide scientific evidence for the anticonvulsant activities of ethanolic leaf extract of *Milicia excelsa* (EME), its ethyl acetate fraction (EAF) and aqueous fraction (AF) in mouse model of convulsion as well as the neural mechanism of the anticonvulsant effect in the most active fraction (AF).

From the LD_{50} determined from our preliminary investigations, 1/20, 1/10 and $1/5^{th}$ of the LD_{50} ($LD_{50} \geq 5000$ mg/kg) which corresponded to 250, 500 and 1000 mg/kg were selected for EME and its fractions and considered as low, medium and high doses respectively [35] for the anticonvulsant investigations.

EME, EAF, and AF suppressed picrotoxin-induced convulsion at varying degrees, indicating that they may have anticonvulsant activities that may be acting via the enhancement of chloride currents through picrotoxin–sensitive chloride channels [36]. Picrotoxin is a CNS stimulant, which interacts with GABA receptor complex and blocks the chloride ionophore; hence, it elicits its convulsant effects by

blocking the presynaptic inhibition mediated by GABA [37] or by blocking the effect of GABA at central $GABA_A$ receptors, which have been associated with epilepsy [38]. This finding is in conformity with many medicinal plants known for their anticonvulsant activities in PTX-induced convulsion models [39], [40].

EME and AF offered a varying degree of protection in PTZ-induced convulsion model, suggesting that they might contain biologically active principle(s) with anticonvulsant effect acting via GABA-benzodiazepine receptor neurotransmission since GABA is central to the anticonvulsant effect in PTZ induced convulsion [34]. PTZ induced seizure is analogous to petit mal type of seizures and human generalized seizures [41]. Agents positive on the PTZ test are considered useful in humans [42]. This finding is in line with many medicinal agents known for their anticonvulsant activities in PTZ-induced convulsion models [43], [44].

The prolongation of the onset of clonic, tonic convulsion and the time of death produced by EME, EAF, and AF suggest that they may have anticonvulsant effects on the strychnine-sensitive channels through the glycine receptor. Strychnine induces convulsions by antagonizing competitively the postsynaptic inhibitory effects of glycine in the spinal cord [45]. This finding is in conformity with many medicinal plants known for their anticonvulsant activities in SCN-induced convulsion models [2] [33] [46], [47] [48].

Since AF produced consistent anticonvulsant effects in PTX-, and PTZ-induced convulsion models, and these chemoconvulsants act via GABA receptor neurotransmission, the mechanisms of anticonvulsant effects of AF was therefore investigated in PTX-induced convulsion model using antagonism of GABAergic, serotonergic (5-HT) and nitric oxide inhibition. Earlier report has implicated GABA antagonism, 5-HT antagonism and NOS inhibition in anticonvulsant effects of a medicinal plant [33]. We therefore explored these mechanism to suggest if AF was acting via any of these mechanism, and to suggest also, if there exist any probable functional interaction between 5-HT, NO and GABA in the anticonvulsant effect of AF as suggested in other studies [33] [34].

The finding showed that AF might be acting via $GABA_A$- benzodiazepine receptor neurotransmission in its anticonvulsant effects since pretreatment with flumazenil ($GABA_A$-benzodiazepine receptor antagonist) abolished the anticonvulsant effect of AF. Pretreatment of rodents with flumazenil before administration of test substances have been reported to reverse the anticonvulsant effects of some medicinal plant substances [33] [34]. Hence, the reversal of the anticonvulsant effect of AF may also

be mediated via the GABAergic mechanism. Pretreatment of AF with cyproheptadine abolished the anticonvulsant effect of this fraction. This result indicates that this fraction may exert its anticonvulsant effect through the 5-HT receptor. Since enhanced GABAergic transmission is central to preventing picrotoxin-induced seizure, it is therefore likely that the anticonvulsant activity of this fraction may involve interaction between serotonergic and GABAergic transmission. For instance, the previous report has demonstrated that administration of 5-HT receptor agonist, 1-(2, 5-dimethoxy-4-iodophenyl-2- amino propane (DOI) resulted in significant increase in extracellular GABA levels [49] while the 5-HT receptor antagonist clozapine, resulted in a decrease in extracellular GABA level [50] in the brain. It can, therefore, be inferred that AF appears to either promote GABA synthesis and/or release through 5-HT receptor activation. Pretreatment of AF with L-NNA (a nitric oxide synthase inhibitor) reversed the anticonvulsant effect of this fraction. This suggests that there may be a functional interaction between nitric oxide and GABA in the brain since enhanced GABAergic transmission is central to preventing picrotoxin-induced convulsion. For instance, NO has been reported to be a modulator of GABA in the brain either by increasing GABA concentration or decreasing GABA transaminase (GABA-T) activity [51].

One of the isolated compounds from the leaf of *Milicia Excelsa* is ursolic acid [28]. It is a pentacyclic triterpenoid carboxylic acid which is found in many medicinal plants [52]. Previous studies have shown that ursolic acid possessed anticonvulsant effects [52] [53]. Since *Milicia excelsa* leaf contained ursolic acid, it could, therefore, be suggested that ursolic acid either in additive or synergy with other phytocompounds in the leaf could be responsible for the observed anticonvulsant effect of *Milicia excelsa* leaf in this study.

How the AF transversed the blood brain barrier (BBB) to exert the observed anticonvulsant effect could not be established in this study. It can probably be suggested that the phytocompounds in AF could transverse the BBB by active transport since hydrophilic drugs are substrates for drug transporters of the BBB [54]. Moreso, previous studies have demonstrated the anticonvulsant effects of AF of medicinal plants [55], [56].

5.0 Conclusion

In conclusion, the results of this study indicated that *M. excelsa* leaf extract and fractions showed varying degree of anticonvulsant effects. The magnitude of activity of the fractions was of the order AF > EAF while the anticonvulsant effects may be mediated via GABAergic, serotonergic and nitergic

pathways. The findings of this study therefore lend pharmacological credence to the suggested ethnomedicinal uses of the leaf in treating mental illnesses.

CONSENT

It is not applicable.

ETHICAL APPROVAL

“All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee”

REFERENCES

1. Hema B, Bhupendra S, Mohamed Saleem TS, Gauthaman K. Anticonvulsant Effect of *Drosera burmannii* Vahl. Int J Appl Res Nat Prod, 2009; 2(3): 1-4.
2. Hamad MN, Sulaiman AA, Numan IT, Abdul Razak SA. Study of the anticonvulsant effect of ethyl acetate fraction of *Matricaria recutita* extract in mice. Int J Pharm Pharm Sci, 2014; 6:224-7.
3. Suresh K, Reecha M, Gundeep B, Anupam J, Anupam S. Plants and Plant Products with Potential Anticonvulsant Activity – A Review. Pharmacogn Commun, 2012; 2(1): 3-99.
4. Fisher R, van Emde Boas W, Blume W, Elger C, Genton P, Lee P et al. Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE), Epilepsia, 2005; 46: 470-472.
5. Scheuer ML, Pedley TA. The evaluation and treatment of seizures. New Engl J Med, 1990; 323: 1468–74.
6. Bialer M. New antiepileptic drugs that are second generation to existing antiepileptic drugs, Expert Opin Investig Drugs, 15(6): 637-47.
7. Perucca E, French J, Bialer M. Development of new antiepileptic drugs: Challenges, incentives, and recent advances, Lancet Neurol, 2007; 6(9): 793-804.
8. Gates JR. Side Effect Profiles and Behavioral Consequences of Antiepileptic Medications, Epilepsy and Behav, 2000; 3: 153-159.
9. Duncan JS. The promise of new antiepileptic drugs, Br J Clin Pharmacol, 2002; 53(2):123–131.

10. Vermeulen J, Aldenkamp AP. Cognitive side-effects of chronic antiepileptic drug treatment: a review of 25 years of research. Epilepsy Res, 1995; 22(2): 65–95.
11. Bromley RL, Leeman BA, Baker GA, Meador KJ. Cognitive and neurodevelopmental effects of antiepileptic drugs. Epilepsy & Behav, 2011; 22(1): 9–16. 90.
12. Al-Taher AY. Anticonvulsant effects of 3, 4-Dimethoxy toluene, the major constituent of *Phoenix dactylifera L* Spathe in mice. Scientific Journal of King Faisal University (Basic and Applied Sciences), 2008; 9(2): 115 – 122.
13. Idris ML, Nkafamiya II, Akinterinwa A, Japari JI. Preliminary Studies on Some Medicinal Plants in Girei, Adamawa State of Nigeria. Br J Pharm Res, 6(3): 203-213
14. Raza M, Shaheen F, Choudhary MI, Rahman AU, Sombati S, Suria A et al. Anticonvulsant effect of FS-1 subfraction isolated from roots of *Delphinim Denudatum* on hippocampal pyramidal neurons, Phytother Res, 2003; 17(1): 38-43.
15. Samrén EB, Duijn CM, van Koch S, Hiilesmaa VK, Klepel H, Bardy AH et al. Maternal use of antiepileptic drugs and the risk of major congenital malformations: a joint European prospective study of human teratogenesis associated with maternal epilepsy. Epilepsia, 1997; 38(9): 981-90.
16. Kumar S, Sharma G, Sharma A, Gorge M, Joseph L. Anticonvulsant effect of chloroform extract of *phyllostachys bambusoides*, Int J Pharm Pharm Sci, 2011; 3(5): 25-27.
17. Shaheen F, Choudhary MI, Sombati S, Rafiq A, Suria A, Rahman A et al. Anticonvulsant activities of ethanolic extract and aqueous fraction isolated from *Delphinium denudatum.* J Ethnopharmacol, 2001; 78:1: 73–78.
18. Agyeman VK, Ofori DA, Cobbinah JR, Wagner MR. Influence of *Phytolyma lata* (Homoptera psyllidae) on seed growth of *Milicia excelsa*. Ghana J Forestry, 2009; 25: 29-39.
19. Kone WM, Koffi AG, Bomisso EL, Tra Bia FH. Ethnomedical Study and Iron Content of Some Medicinal Herbs Used in Traditional Medicine in Cote D'Ivoire for the Treatment of Anaemia. Afr J Tradit Complement Altern Med, 2012; 9(1): 81 – 87.
20. Titanji VPK, Zofou D, Ngemenya MN. The antimalarial potential of medicinal plants used for the treatment of malaria in Cameroonian folk medicine. Afr J Tradit Complement Altern Med, 2008; 5(3): 302 – 321.

21. Betti JL. An ethnobotanical study of medicinal plants among the Baka Pygmies in the Dja Biosphere Reserve, Cameroon. African Study Monographs, 2004; 25(1): 1-27.
22. Ofori DA. *Milicia excelsa* (Welw.) C. C. Berg. In: Louppe, D., Oteng-Amoako, A. A., and Brink, M. (Editors). Prota, 2007; 7(1): Timbers/Bois d'oeuvre 1. [CD-Rom]. PROTA, Wageningen, Netherlands.
23. Sonibare MA, Soladoye MO, Subuloye TO. Ethnobotanical Survey of Anti Psychotic Plants in Lagos and Ogun States of Nigeria. Eur. J. Sci. Res, 2008; 19(4): 634 - 644.
24. Ibrahim JA, Muazzam I, Jegede IA, Kunle OF, Okogun JI. Ethno-medicinal plants and methods used by Gwandara tribe of Sabo Wuse in Niger State, Nigeria, to treat mental illness. Afr. J. Tradit. Complement. Altern. Med, 2007; 4(2): 211–8.
25. Betti JL, Yongo OD, Mbomio DO, Iponga DM, Ngoye A. An Ethnobotanical and Floristical Study of Medicinal Plants Among the Baka Pygmies in the Periphery of the Ipassa-Biosphere Reserve, Gabon. Eur J Med Plants, 2013; 3(2): 174 – 205.
26. Ndah NJ, Egbe AE, Bechem E, Asaha S, Yengo T, Chia EL et al. Ethnobotanical study of commonly used medicinal plants of the Takamanda Rainforest South West, Cameroon. Afr. J. Plant Sci, 2013; 7(1): 21-34.
27. Wahab OM. Ethnomedicinal Antiepileptic Plants Used in Parts of Oyo and Osun States, Nigeria. Bot Res Int, 2015; 8 (4): 77-81.
28. Ouete JL, Sandjo LP, Kapche DW, Yeboah SO, Mapitse R, Abegaz BM, Opatz T, Ngadjui BT. Excelsoside: a new benzylic diglycoside from the leaves of Milicia excelsa. Z Naturforsch C. 2014; 69(7-8): 271-5.
29. National Institute of Health (NIH-1985), Guide for the Care and Use of Laboratory Animals, National Research Council. National Academy Press, Washington, DC, 1996.
30. Swinyard EA, Woodhead JH, White HS, Franklin MR. Experimental selection, qualification and evaluation of anticonvulsants. In: Levy R, Mattson R, Meldrum BS, Penry JK**,** Dreifuss FE. (Eds), Antiepileptic Drugs, 3rd ed. Raven press New York pp 1989; 85 - 102.
31. Vellucci SV, Webster RA. Antagonism of caffeine-induced seizures in mice by Ro15-1788. Eur J Pharmacol, 1984; 97(3-4): 289-293.

32. Ogbonnia SO, Jager AK, van Staden T, Coker HAB. Aniconvulsant activity of *Schumanniophyton Magnificum* root extracts in mice, West Afr J Pharmacol Drug Res, 2003; 19(1-2): 33-36.

33. Ishola IO, Olayemi SO, Idowu AR. Anticonvulsant, Anxiolytic and Hypnotic effects of Aqueous Bulb Extract of *Crinum glaucum* A. Chev (Amaryllidaceae), Role of GABAergic and Nitrergic Systems, Pak J Biol Sci, 2013; 16: 701-710.

34. Kolawole OT, Akiibinu MO, Ayankunle AA. Anticonvulsant and Depressant Activity of Methanol Leaf Extract of *Croton zambesicus.* Int J Trop Dis Health, 2012; 2(1): 33-41.

35. Ali A, Rao NV, Shalam MD, Gouda TS, Kumar SM. Anticonvulsive effect of seed extract of *Caesalpinia bonducella* (Roxb.). Iran J Pharmacol Ther. 2009; 8: 51–55.

36. Smith IS, Thopson SJK, Sargent JB, Heal JD. BTS 72664 – a novel CNS drug with potential anticonvulsant, neuroprotective and antimigraine properties. CNS Drug Rev, 2001; 7: 146-171.

37. Tolstukhina TI, Flerov MA. ATPase activity in neurone and neurogia during convulsion induced by picrotoxin. Vopr Med Khim, 1999; 45(2): 145- 149.

38. Nicol RA. Introduction to the pharmacology of the central nervous system (CNS). In: Katzung BG (editor). Basic and Clinical Pharmacology. 9th ed. New York, McGraw-Hill, 2007; pp. 489-507.

39. Chauhan AK, Dobhal MP, Joshi B. A review of medicinal plants showing anticonvulsant activity, J Ethnopharmacol, 1988; 22: 11-23.

40. Nsour WN, Lau CBS, Wong ICK. Review on phytotherapy in epilepsy, Seizure 2000; 9: 96-107.

41. Nassiri-Asl M, Shariati-Rad S, Zamansoltani F. Anticonvulsant effects of aerial parts of *Passiflora incarnata* extract in mice: involvement of benzodiazepine and opioid receptors. BMC Complement Altern Med, 2007; 7: 26.

42. Porwal M, Sharma K, Paras K, Malik P. Anticonvulsant effect of *Annona squamosa* leaves in mice. Pharmacologyonline, 2011; 2: 44 - 52.

43. Mcdonald RL, Kelly KM. Antiepileptic drug mechanisms of action. Epilepsia, 1995; 36: 2-12.

44. Hegde K, Thakker SP, Joshi AB, Shastry CS, Chandrashekhar KS., Anticonvulsant Activity of *Carissa carandas* Linn. Root Extract in Experimental Mice. Trop J Pharm Res, 2009; 8(2): 117 – 125.
45. Ahmadiani A, Mandgary A, Sayyah M. Anticonvulsant Effect of Flutamide on Seizures Induced by Pentylenetetrazole: Involvement of Benzodiazepine Receptors, Epilepsia, 2003; 44(5): 629–635.
46. Magaji MG, Yakubu Y, Magaji RA, Musa AM, Yaro AH, Hussaini IM. Psychopharmacological potentials of methanol leaf extract of *Securinega virosa* (roxb. ex willd) baill. in mice. Pak J Pharm Sci, 2013; 16: 1 - 5.
47. Bigler ED. Comparison of effects of bicuculline, strychnine, and picrotoxin with those of pentylenetetrazol on photically evoked after discharges. Epilepsia, 1977; 18: 465 - 470.
48. Hemalatha S, Wahi AK, Singh PN, Chansouria JPN. Anticonvulsant and free radical scavenging activity of Hybanthus enneaspermus: a preliminary screening. Indian J Tradit Know, 2003; 2(4): 383 – 388.
49. Jawaid T., Argal S., Singh S., Botanicals and herbs: A traditional approach in treatment of epilepsy. J Pharm Res, 2011; 4: 1138 - 1140.
50. Adeyemi OO, Aigbe FR, Olofinjana OE. Investigation of the Anticonvulsant, Sedative and Anxiolytic Activities of the Aqueous Leaf and Stem Extract of *Asystasia Gangetica* (Linn.). *University of Lagos Journal of Basic Medical Sciences,* 2014; 2(1): 21 – 26.
51. Abi-Saab WM, Bubser M, Roth RH, Deutch AY. 5-HT_2 receptor regulation of extracellular GABA levels in the prefrontal cortex. *Neuropsychopharmacology*, 1999; 20(1): 92- 96.
52. Nieoczym D, Socała K, Wlaź P. Assessment of the Anticonvulsant Potency of Ursolic Acid in Seizure Threshold Tests in Mice. Neurochem Res, 2018; 43(5): 995–1002.
53. Taviano MF, Miceli N, Monforte MT, Tzakou O, Galati EM. Ursolic acid plays a role in *Nepeta sibthorpii* Bentham CNS depressing effects. Phytother Res, 2007; 21:382–385
54. Jouyban A and Soltani S: Blood–Brain Barrier Permeation. In: Toxicity and Drug Testing. Acree B (ed.). 2012; pp. 3-24.
55. Ya'u J, Yaro AH, Malami S, Musa MA, Abubakar A, Yahaya SM. Anticonvulsant activity of aqueous fraction of Carissa edulis root bark. Pharm Biol, 2009; 53(9): 1329-1338

418 56. Abubakar K, Adebisi IM, Ugwah-Oguejiofor JC, Idris GO, Idris B, and Mshelia HE.
419 Phytochemical Screening and Anticonvulsant Activity of the Residual Aqueous Fraction of
420 *Tapinanthus globiferus* Growing on *Ficus glums*. Herb Med, 2016; 2(2): 1-6.