

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 (3 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. HF, EAF, BF and AF significantly ($P < .05$) prolonged 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.

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

11 1.0 Introduction

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

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

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

36 *Milicia excelsa* (welw.) C.C. Berg belongs to the family Moraceae popularly known as Iroko tree or
 37 African teak. It is a large deciduous tree 30 to 50 m high, occurring naturally in humid forests of West
 38 Africa [18]. Its latex, leaf, stem bark, root, fruit, and ashes are used in African traditional medicine to
 39 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]. Preliminary investigations from our laboratory showed that the median lethal dose (LD₅₀) of the ethanol leaf extract, n-hexane (HF), ethylacetate (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°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 [28]. 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), Pentylentetrazol (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 [29]. 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.). 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 [30, 31]. 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.). 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 [29]. Mice were divided into five different groups containing 6 mice per group (n=6). Group 1 received normal saline (10 mL/kg, p.o.)

for 60 minutes prior to SCN (4 mg/kg, i.p.) injection, groups 2- 4 were pre-treated with the extract at the doses of 250, 500, 1000 mg/kg, p.o. for 60 minutes prior to SCN (4 mg/kg, i.p.) injection and group 5 was pre-treated with diazepam (1 mg/kg, i.p.) as 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 of the most active fraction (AF), another set of mice were pretreated with flumazenil (GABA_A receptor antagonist, 3.0 mg/kg, i.p.) [32], cyproheptadine (5-HT receptor antagonist, 4 mg/kg i.p.) [33], and L-NNA (Nitric oxide synthase inhibitor, 10 mg/kg, i.p.) [32], 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 \pm 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 ($P < 0.05$) delayed by EME at all the doses used (250, 500 and 1000 mg/kg, p.o.), onset of tonic convulsion was significantly ($P < 0.05$) delayed at 250 and 500 mg/kg, p.o. while the time of death was also significantly ($P < 0.05$) 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 and AF at 1000 mg/kg significantly ($P < 0.05$) prolonged the onset of clonic and tonic convulsions and death latency while at 500 mg/kg, they both significantly ($P < 0.05$) delayed the death latency when compared to the vehicle treated control group. EAF at 250 mg/kg and AF at 500 mg/kg significantly ($P < 0.05$)

prolonged the onset of clonic convulsion and tonic convulsions respectively when compared to the vehicle treated control group. Diazepam, a reference anticonvulsant drug at 1 mg/kg, i.p. significantly ($P < 0.05$) prolonged the onset of clonic, tonic convulsions and death latency with 100% protection. EME offered 33.3% and 50% protection at 250 and 500 mg/kg, p.o respectively while AF offered 0, 50 and 83.33% protection at 250, 500 and 1000 mg/kg respectively. HF and BF had no effect 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.) significantly ($P < 0.05$) delayed the onset of clonic and tonic convulsions and death latency. AF significantly ($P < 0.05$) prolonged the onset of tonic convulsion and death latency but had no significant ($P < 0.05$) 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. HF and BF had no effect no significant effects on clonic, tonic convulsion and death latency. Diazepam, a reference anticonvulsant drug at 1 mg/kg, i.p. significantly ($P < 0.05$) prolonged the onset of clonic, tonic and death latency and offered 100% protection. 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, EAF and AF at all the doses used (250, 500 and 1000 mg/kg, p.o.) significantly delayed the onset of clonic convulsion while they have no significant effect on the onset of tonic convulsion and death latency. However, phenobarbitone (30 mg/kg, i.p.), a standard anticonvulsant drug significantly prolonged the onset of clonic, tonic and death latency with 33.3% protection. 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 ($P < 0.05$) 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 (**P* < 0.05) reversed the anticonvulsant effect of AF when compared to AF treated mice alone. 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 ± 2125*	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 ethanolic 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 ethanolic leaf extract, n-hexane, ethylacetate, 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 ethanolic 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) + DPZ (1)	401.7 ± 57.2 ^B	1004.5 ± 246.5 ^B	1018.0 ± 246.8 ^B		0
CYPRO (4) + AF (1000)	582.2 ± 49.1	1037.0 ± 100.3* [#]	1054.7 ± 99.4* [#]	0/6	0
CYPRO (4) + DPZ (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

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 (3 mg/kg, i.p.), L-NNA; L-N^G-Nitroarginine (10 mg/kg, i.p.).

Values are Mean \pm SEM, ANOVA; one way analysis of variance followed by Student-Newman Keuls Test, $n=6$, $*P < 0.05$, $^{##}P < 0.05$, and $^{\beta}P < 0.05$ 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).

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 [34]. 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 [35] or by blocking the effect of GABA at central GABA_A receptors, which have been associated with epilepsy [36]. Many plants are known for their anticonvulsant activities [37, 38, 39, 40]. For example, the anticonvulsant effect of *Passiflora incarnata* has been reported in mice [41]. Also, the anticonvulsant effect of *Annona squamosa* has also been demonstrated following the administration of picrotoxin in mice [42]. Since, EME, EAF, and AF suppressed picrotoxin-induced convulsion, it can, therefore, be inferred that the extract and these fractions have anticonvulsant effects.

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 [33]. PTZ induced seizure is analogous to petit mal type of seizures and human generalized seizures [43]. Agents positive on the PTZ test are considered useful in humans [44]. A number of reports have shown agents having anticonvulsant effects against PTZ-induced convulsion [45, 46, 47]. For instance, the anticonvulsant effect of flutamide in PTZ-induced convulsion has been evaluated [48]. Likewise, the anticonvulsant effect of *Securinega virosa* following the administration of PTZ in mice has been reported [49]. Since EME and AF offered varying degrees of anticonvulsant in mice, it can, therefore, be suggested that they have anticonvulsant effect against PTZ-induced convulsion in mice.

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 [50]. Several reports have demonstrated that some plants showed anticonvulsant effects in strychnine-induced convulsion [51, 52, 2]. For example, it has been reported that the extract of *Crinum glaucum* prolonged the onset of convulsion in mice in SCN-induced convulsion model [32] while the extract of *Asystasia gangetica* offered some anticonvulsant protection in strychnine induced convulsion in mice [53]. It can, therefore, be suggested that EME, EAF, and AF have anticonvulsant effects in this model. Of all the fractions, the AF produced a consistent anticonvulsant effect in picrotoxin-, pentylenetetrazole-, and strychnine-induced convulsions in this study.

Since AF produced consistent anticonvulsant effects in all the convulsion models used, the mechanisms of anticonvulsant effects of this fraction were therefore investigated. 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 [32]. An earlier report has indicated that pretreatment of *Crinum glaucum* extract in mice with flumazenil abolished the anticonvulsant effect of this extract, the reversal of which was attributed to the blockade of GABA_A- benzodiazepine receptor transmission [32]. Similarly, the reversal of the anticonvulsant effect of *Passiflora incarnata* extract by pretreatment with flumazenil in mice has also been ascribed to the antagonism of GABA_A- benzodiazepine receptor neurotransmission [41]. 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 [54] while the 5-HT receptor antagonist clozapine, resulted in a decrease in extracellular GABA level [55] 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, Paul and Jayakumar, 2000 reported NO to be a modulator of GABA in the brain either by increasing GABA concentration or decreasing GABA transaminase (GABA-T) activity [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 > HF > BF 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"

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