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

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Authors' contributions

This work was carried out in collaboration between all authors. Author LAA managed the experimental process, performed the statistical analysis of the study, managed the literature searches and wrote the first and final version of the manuscript. Author MAA designed the study, managed the experimental process and managed the analyses of the study. Author EMO designed the study, managed the experimental process and managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

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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 and February 2015.
Methodology: The anticonvulsant effect of ethanol leaf extract (EME), n-hexane (HF), ethyl

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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 prolonged death latency with varying degrees of protection in picrotoxin-, and 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.

1. 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 neuronal 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. These may affect 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]. These make the search for antiepileptic agents with better selective activity and lower toxicity 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 in the literature.

2. 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 (60 g) 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 acclimatised 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 (FLU) (Hikma Farmaceutical, Portugal, S.A.), Phenobarbitone (PBT) (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 randomized 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 randomized 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 randomized 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 PTZ (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 Dunnett (compare all vs vehicle) while the results of the mechanism of anticonvulsant effects were analysed using one way analysis of variance (ANOVA), followed by the Student- Newman-keuls test post hoc analysis. 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.

The significance of difference between groups was 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. 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$_{(10, 55)}$ = 24.299, P < 0.0001] delayed by AF at 1000 mg/kg and the onset of tonic convulsion was significantly [F$_{(10, 55)}$ = 13.774, P < 0.001] delayed by EME at 250 mg/kg, and by AF at 500 and 1000 mg/kg and by DZP (1 mg/kg) when compared to the vehicle treated control group. EME at 250 and 500 mg/kg, AF at 500 and 1000 mg/kg and DZP at 1 mg/kg significantly [F$_{(10, 55)}$ = 19.021, P < 0.001] prolonged the death latency when compared to the vehicle treated control group. EME offered 33.3% and 50% protection at 250 and 500 mg/kg, respectively. AF at 500 and 1000 mg/kg offered 50 and 83.3% protection respectively. Although, EAF increased the onset of clonic, tonic convulsions and death latency at 1000 mg/kg, but not significant from the vehicle treated control group. 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

EME at 250 mg/kg, and DZP (1 mg/kg) significantly [F$_{(4, 25)}$ = 10.288; P < 0.0001)], [F$_{(4, 25)}$ = 7.838; P = 0.0003)] and [F$_{(10, 55)}$ = 12.078; P < 0.001)] delayed the onset of clonic convulsion. EME at 250 and 500 mg/kg, AF at the doses of

250, 500 and 500 mg/kg and DZP (1 mg/kg) significantly [$F_{(10, 55)}$ = 4.733; $P < 0.001$)] prolonged the onset of clonic convulsion while the death latency was significantly [$F_{(4, 25)}$ = 3.823; $P = 0.0006$)] elongated by EME at 250, 500 and 1000 mg/kg and AF at 500 and 1000 mg/kg when compared to the vehicle treated control group. 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

EAF at all the doses used (250, 500 and 1000 mg/kg), AF at 500 and 1000 mg/kg and PBT (30 mg/kg) significantly [$F_{(10, 55)}$ = 3.571; $P = 0.0001$)] elongated the onset of clonic convulsion when compared to the vehicle treated control group. However, EME, EAF and AF at all the doses used did not show any significant effect on the onset of tonic convulsion and death latency, but PBT (30 mg/kg) significantly [$F_{(10, 55)}$ = 7.265; $P < 0.0001$)] and [$F_{(10, 55)}$ = 8.434; $P < 0.0001$)] prolonged the onset of tonic and death latency respectively. PBT (30 mg/kg) 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 onset of tonic convulsion and death latency of AF when compared to AF treated mice. Pretreatment with flumazenil before DZP significantly ($P < 0.05$) reversed the onset of clonic, tonic convulsions and death latency of DZP group when compared to DZP treated mice alone. However, pretreatment of DZP group with cyproheptadine showed no significant effect on the onset of clonic, tonic convulsions and death latency when compared to DZP group alone. Pretreatment of DZP group with L-NNA significantly ($P < 0.05$) elongated the onset of clonic convulsion without any significant effect on the onset of tonic convulsion and death latency when compared to DZP group alone.

4. 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

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)	75.2 ± 4.3	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 ± 241.8*	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 Dunnett (compare all vs vehicle) post hoc 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 ± 198.2	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 Dunnett (compare all vs vehicle) post hoc 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 ± 14.7	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)	289.7 ± 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 Dunnett (compare all vs vehicle) post hoc test, n=6, *P < 0.05 compared to vehicle-treated control group.*

(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 1/5th of the LD$_{50}$ (LD$_{50}$ ≥ 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 anti-convulsant 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].

Table 4. Effect of pretreatment with flumazenil, cyproheptadine, and L-NG-Nitroarginine before AF in PTX-induced convulsion 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 ± 33.3	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
DZP (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 ± 57.2[β]	1004.5 ± 246.5[β]	1018.0 ± 246.8[β]		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 ± 138.6[α]	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 ^{β,α}P < 0.05 compared to vehicle, AF and DZP group respectively

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 antagonising 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 mechanisms 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]. More so, previous studies have demonstrated that anticonvulsant effects of some medicinal plants may be due to their aqueous fractions [55,56].

5. CONCLUSION

In conclusion, the results of this study indicated that *M. excelsa* leaf extract and fractions showed a 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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