Research Article

Evaluation of the anticonvulsant activity of aqueous leaf extract of *Emilia praetermissa* Milne-Redh (Asteraceae) in rats and mice

Ogochukwu N. Nwaefulu [a,*], Raymond I. Ozolua [b], Ibrahim O. Otokiti [b], and Ifeanyi Ebido [a]

[a] *Department of Pharmacology & Toxicology, Prof Dora Akunyili College of Pharmacy, Igbinedion University, Okada, Edo State, Nigeria*
[b] *Department of Pharmacology & Toxicology, Faculty of Pharmacy, University of Benin, Benin*

_____

* **Corresponding author:** Department of Pharmacology & Toxicology, Prof Dora Akunyili College of Pharmacy, Igbinedion University, P.M.B 0006, Okada, Edo State, Nigeria. **Tel**: +234-805-2460208. **Email**: ogom4christ@ymail.com

Background: *Emilia praetermissa* has been used for the treatment of convulsive disorders in children by herbalists in Eastern Nigeria. It is also used for the treatment of ulcers, fever, splenomegaly, filarial infections, bacterial infections and ringworms.

Objective: This objective was to investigate the inhibitory property of the aqueous leaf extract of *E. praetermissa* (AEP) against electrically and chemically induced convulsions in rats and mice.

Methodology: Groups of rats were administered 5, 10 and 20 mg/kg aqueous leaf extract of *E. praetermissa* and after one hour they were subjected to maximal electroshock. Groups of mice treated with same doses of the extract were subjected to chemically-induced convulsion using pentylentetrazol or strychnine. Phenobarbitone (30 mg/kg) and/or diazepam (0.5 mg/kg) were used as standards. Comparisons were made by use of Student's *t*-test. All data were analyzed using GraphPad Instat software (USA). *P* < 0.05 indicated statistically significant difference.

Results: The doses of aqueous leaf extract of *E. praetermissa* significantly (*P*< 0.05) reduced the extensor seizure latency in maximal electroshock model, and onset of tonic convulsion in the chemical models. Aqueous leaf extract of *E. praetermissa* (20mg/kg; *P*< 0.007) was significantly superior to diazepam (0.5 mg/kg; *P*< 0.04) in reducing the onset of strychnine-induced seizure.

Conclusion: These results suggest that the ethnomedicinal application of *E. praetermissa* in Eastern Nigeria has scientific basis that requires further investigation.

Key words: *Emilia praetermissa,* aqueous extract, anticonvulsant activity.

1. Introduction

Epilepsy is the most common non-infectious neurological disease in developing countries (Senanayake and Roman, 1993). It is estimated that the condition affects approximately 50 million people worldwide, with 40 million of them living in developing countries (Leonardi and Ustun, 2002). The highest incidence occurs in children under the age of five and in the elderly (Theodore et al, 2006). The condition continues to await drugs with improved effectiveness as the available ones fail to provide adequate control of seizures in about one-third of patients and do not prevent progressive epileptogenic changes (Jeub et al, 2002). Furthermore, undesirable side effects from the drugs used clinically often render treatment difficult thereby suggesting the need for new types of anticonvulsants drugs which may be of plant origin (Sayyah et al, 2002).

Medicinal plants have continued to play important roles in effective delivery of health care in developed and less developed countries alike. It has been estimated that about 25% of all prescribed medicines today are substances derived from plants (Gamaniel, 2000). About 80% of the world inhabitants rely mainly on traditional medicines for their primary health care (WHO, 2000).

Emilia praetermissa Milne-Redh (Asteraceae) was first found in Sierra Leone and Nigeria and later in other West African countries, including Cameroun, Cote d' Ivoire, Ghana, Guinea and Liberia (Hepper, 1963).

E. praetermissa is used as vegetables and as fodders for rodents. It is also used for the treatment of ulcers, fever, splenomegaly, filarial infection, and ringworms. Other uses include the treatment of various bacterial infections such as leprosy, syphilis and gonorrhea. The leaf extract have also been used as laxative (Jeffrey, 1997).

Several studies have been carried out on medicinal plants but there seems to be no scientific information on the anticonvulsant activity of *E. praetermissa.* The expressed fresh leaf extract is mixed with the blood of wall geckos (*Tarentola mauritanica*) and used in Eastern Nigeria for the treatment of convulsive disorders in children. We therefore designed this study to investigate the effects of the aqueous leaf extract on animal models of convulsion.

2. Materials and Methods

2.1 Plant Material and Extraction

The plant was collected from Ifite-Oraifite, Anambra State, Nigeria in the month of September 2011. It was authenticated by the Forest Research Institute of Nigeria, Ibadan, Nigeria, where a herbarium sample with voucher number **FHI-108836** has been deposited. Subsequent collections for the study were from the same site.

The fresh leaves (340 g) were rinsed by washing with tap water to remove debris. The leaves were later rinsed in distilled water and allowed to drain. They were cut into small pieces, ground with a pestle in a porcelain mortar and macerated with 1000 ml of distilled water for 24 h. It was then filtered with a white clean cloth. The filtrate was centrifuged at 1000 rpm for five minutes to remove suspended particulate matter. The supernatant fraction then represented the aqueous extract of *E. praetermissa.* Ten milliliters (10 ml) aliquots of the extract were measured into separate beakers of known weight and evaporated to dryness. Each 10 ml portion yielded 93.43 mg of the extract. This quantity was then reconstituted in water and administered to the animals.

2.2 Drugs and chemicals

Solutions of pentylenetetrazol 70 mg/kg (SIGMA, USA), diazepam 0.5 mg/kg (Roche, Switzerland) and penobarbitone sodium 30 mg/kg (BDH Chemicals, England) were prepared fresh in distilled water. The injection solution of strychnine 3 mg/kg (SIGMA, USA) was prepared by dissolving in dimethyl sulphoxide (DMSO). All other chemicals were of analytical grade and were manufactured by reputable companies.

2.3 Experimental animals and groups

Experiments were performed using adult male albino rats (120 – 180 g) and mice of both sexes (30 – 40 g). The animals were bred locally in the animal house of the Department of Pharmacology & Toxicology, University of Benin, Benin City, Nigeria. They were housed in standard cages (males separate from females in the case of the mice). They had free access to pellets (Bendel Feeds and Flour Mill Ltd, Ewu, Nigeria) and tap water. Animals were exposed to natural room temperature and lighting conditions and were handled according to standard protocols for the use of laboratory animals (National Institute of Health USA: Public Health Service Policy on Humane Care and Use of Laboratory Animals, 2002).

They were randomly assigned to five groups of 10 rats per group for the maximal electroshock (MES) model, and two sets of 6 groups (10 mice per group) each for the pentylenetetrazol and strychnine-induced convulsions.

2.4 MES seizure model

Electro-convulsive shock inducing Hind Limb Tonic Extension (HLTE) in 99% of the animals was previously described. (Sayyah et al, 2002; Yazdi et al, 2011) The electrical stimulus (50 mA; 50 Hz; one second duration) was applied through ear-clip electrodes using an electroshock apparatus (ICE MOD 849, Industria Costruzioni Elettromeccaniche- Milan, Italy). Three groups of rats were treated intraperitoneally with AEP at doses of 5, 10 and 20 mg/kg respectively. The remaining two groups were treated intraperitoneally with 30 mg/kg phenobarbitone and 2 ml/kg distilled water respectively. One hour after treatment, the animals were subjected to electroshock as described. Extensor seizure latency was measured using a stop watch and the number of animals protected was also noted.

2.5 Chemically-induced seizure model

The first set of six groups of mice was given intraperitoneal injection of 5, 10 and 20 mg/kg AEP; 30 mg/kg phenobarbitone; 0.5 mg/kg diazepam; and 2 ml/kg distilled water respectively. One hour later, seizure was induced by intraperitoneal administration of 70 mg/kg of pentylentetrazol (PTZ). For the second set of six groups, each was given doses of AEP, phenobarbitone, diazepam and distilled water as in the first. One hour later, 3 mg/kg strychnine was administered intraperitoneally. Onset and duration of convulsion were recorded the first 30 min after chemical induction of seizure

2.6 Statistics analysis

Data are presented as mean ± SEM (standard error of the mean) and "*n*" represents the number of animals used for a particular experiment. Comparisons were made by use of Student's *t*-test. All data were analyzed using GraphPad Instat software (USA). $P < 0.05$ indicated statistically significant difference.

3. Results

3.1 Effect of AEP on MES –induced seizure

In the MES experiment, AEP dose-dependently prolonged the average extensor seizure latency significantly when compared to control. The animals were significantly protected at 5 mg/kg ($P < 0.03$), 10 mg/kg ($P < 0.02$) and 20 mg/kg ($P < 0.005$). Phenobarbitone (30 mg/kg) completely protected the mice against MES-induced seizures. It has 100% protection (**Table 1**).

3.2 Effect of AEP on Chemically-Induced Convulsion

In the PTZ group; AEP prolonged the onset of tonic convulsions at all doses, although it was only significant at 10 mg/kg ($P < 0.04$) and 20 mg/kg ($P < 0.03$). It also reduced the duration of the tonic convulsion and delayed the time it took for the mouse to die after the administration of PTZ (**Table 2**). Phenobarbitone (30 mg/kg) completely protected the mice against PTZ-induced seizures. Diazepam (5mg/kg) also significantly prolonged the onset of tonic convulsion; this is comparable with the effect of AEP (10mg/kg).

In the strychnine group, the onset of tonic convulsion was prolonged in all doses but very significantly at 20 mg/kg ($P < 0.004$) (**Table 3**). This was significantly different from the latency observed with diazepam in which the animals died with the onset of the tonic seizure. The time it took for the mice to die after the administration of strychnine was significantly prolonged at 5 mg/kg ($P < 0.04$) and 20 mg/kg ($P < 0.004$). The delay in death time observed with 20 mg/kg was significantly ($P < 0.04$) different from that observed with diazepam.

Table 1: Effects of aqueous leaf extract of *E. praetermissa* (AEP) on MES-induced seizure in rats.

Animal Group	Extensor Seizure Latency (Sec)	Number of Animals Protected/Used	% Protection
Control(2 ml/kg H_2O)	3.24 ± 0.02	0/10	0
Phenobarb (30 mg/kg)	0.00 ± 0.00	10/10	100
AEP (5 mg/kg)	5.79 ± 1.29*	5/10	50
AEP (10 mg/kg)	6.66 ± 1.55**	4/10	40
AEP (20 mg/kg)	8.31 ± 1.74***	6/10	60

* $P < 0.03$, **$P < 0.02$, ***$P < 0.005$ compared to control. AEP = aqueous extract of *E. praetermissa*; DZP = diazepam; phenobarb = phenobarbitone. n = 10 per group

Table 2: Effect of AEP on pentylentetrazol-induced seizures in mice

Animal Group	Onset of Tonic Convulsion (Min)	Duration of Tonic Convulsion (Min)	Death Time (Min)
Control (2 ml/kg H_2O)	1.85 ± 0.62	0.14 ± 0.20	4.73 ± 0.62
DZP (0.5 mg/kg)	3.61 ± 0.47*	0.13 ± 0.02	6.29 ± 1.07
Phenobarb (30 g/kg)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
AEP (5mg/kg)	1.61 ± 0.38	0.10 ± 0.05	3.66 ± 1.14
AEP (10 mg/kg)	3.23 ± 1.13*	0.10 ± 0.01	6.76 ± 2.73
AEP (20 mg/kg)	2.96 ± 0.92**	0.11 ± 0.01	5.68 ± 1.56

* $P < 0.04$, **$P < 0.03$ compared to control. AEP = aqueous extract of *E. praetermissa*; DZP = diazepam; phenobarb = phenobarbitone. n = 10 per group

Table 3: Effect of AEP on strychnine-induced seizure in mice

Animal Group	Onset of Tonic Convulsion (Min)	Duration of Tonic Convulsion (Min)	Death Time (Min)
Control (2 ml/kg H_2O)	3.15 ± 0.88	0.00 ± 0.00	3.15 ± 0.88
DZP (0.5 mg/kg)	7.27 ± 1.14*	0.00 ± 0.00	7.27 ± 1.14
Phenobarb (30 mg/kg)	7.34 ± 2.52	0.07 ± 0.00	9.00 ± 2.23
AEP (5 mg/kg)	6.32 ± 1.40	0.10 ± 0.02	6.58 ± 1.49[a]
AEP (10 mg/kg)	6.30 ± 1.63	0.05 ± 0.01	6.34 ± 1.63
AEP (20 mg/kg)	12.69 ± 2.53**	0.04 ± 0.01	12.81 ± 2.53[b]

*$P < 0.04$, compared to AEP; **$P < 0.007$, [a]$P < 0.007$, [b]$P < 0.004$ compared to control. AEP = aqueous extract of *E. praetermissa*; DZP = diazepam; phenobarb = phenobarbitone. n = 10 per group

4. Discussion

The preliminary phytochemical screening of the aqueous leaf extract of *Emilia praetermissa* has been reported to show the presence of tannins, flavonoids, steroids, cardiac glycosides, carbohydrate, reducing sugar and terpenoids and absence of alkaloids and saponins (Anaka et al, 2013).

AEP contains a wide array of secondary plant metabolites including flavonoids. It has been shown that flavonoids are an important class of natural compounds that have demonstrated CNS activities such as affinity for GABA$_A$ receptors and anticonvulsant effects (Miliauskas et al, 2004; Huen et al, 2003). For example anticonvulsant activity has been reported for apigenin a glucoside flavonoid of *T. polium* (Abdollahi et al, 2003; Kawashty et al, 1999) Triterpenes have also been reported to possess anticonvulsant activity in some experimental seizure models like PTZ and MES (Khoshnood-Mansoorkhani et al, 2010). Other secondary plant metabolites that have demonstrated anticonvulsant activities include monoterpenes for example pinene, (Librowski et al, 2000; Sayyah et al, 2005) eugenol and methyleugenol (Sayyah and Mandgary, 2003).

Modulation of glutamatergic and GABAergic transmission is a mechanism indicated for anticonvulsant action of the monoterpenes like linalool and eugenol (Szbadics and Erdelyi, 2000).Therefore, it seems that the anti-seizure profile of AEP may be related in part to its flavonoid, steroid and terpenoid content.

The most popular and widely used animal seizure models are the traditional MES and PTZ tests. The ability of a plant extract to prevent seizures or delay/prolong the latency or onset of the hind limb tonic extensions was considered as an indication of anticonvulsant activity (Ojewole, 2009). The MES test is considered to be a predictor of likely therapeutic efficacy against generalized tonic-clonic seizures (Khoshnood-Mansoorkhani et al, 2010; Sayyah et al, 2005). By contrast, the PTZ test represents a valid model for human generalized myoclonic seizures and also generalized seizures of the petit mal (absence) type (Sayyah and Mandgary, 2003; Ngo et al, 2001).

MES-induced seizure can be prevented either by drugs such as phenytoin, valproic acid, felbamate and lamotrigine that inhibit voltage-dependent Na$^+$ channels or by drugs that block glutamatergic receptor such as felbamate (Khoshnood-Mansoorkhani et al, 2010). On the other hand, drugs that reduce T-type Ca^{2+} currents, such as ethosuximide can prevent seizures induced by PTZ (Khoshnood-Mansoorkhani et al, 2010). Drugs that enhance GABA$_A$ receptor mediated inhibitory neurotransmission such as benzodiazepines and phenobarbitone and perhaps valproic acid and felbamate can prevent this type of seizure (Sayyah et al, 2002; Khoshnood-Mansoorkhani et al, 2010). Extensor seizure latency is the time interval between the application of the stimulus and the appearance of the extensor spasm (Crossland, 1980). Substances which abolish the tonic phase of the convulsion (induced by MES) may be effective against grand mal seizures. Drugs which abolish the tonic phase will, in smaller doses, lengthen the extensor seizure latency (Crossland, 1980).

The results from the present study indicate that AEP possesses anticonvulsant activity against both MES and PTZ-induced seizures.

Two mechanisms have been proposed for the mode of PTZ-induced convulsion. It is proposed that PTZ induces convulsion by either inhibiting gamma amino butyric acid (GABA) pathway in CNS (Corda, et al, 1990) or by increasing the central noradrenergic activity Jayaraman, et al, 2010). The effectiveness of the extract in this model therefore suggests that it possibly enhances GABA-ergic transmission or attenuates noradrenergic pathways transmission in the CNS. It is also possible that AEP enhances the central action of glycine since it was effective in antagonizing strychnine induced seizures. Strychnine produced its convulsant effect by blocking the receptors for glycine (Amabeoku and Kinyua, 2010).

5. Conclusion

The results of this study suggest that AEP possesses anticonvulsant effects in rats and mice. Although the exact mechanisms are not clear, it seems that central GABAergic and glycinergic neurotransmissions are enhanced. While this report lends some credence to the use of the plant in Eastern Nigerian ethnomedicine, further studies are necessary.

Conflict of Interest Declaration

The authors declare no conflict of interest.

References

Abdollahi M, Karimpour H and Monsef-Esfehani H (2003). Antinociceptive effects of *Teucrium polium* L. total extract and essential oil in mouse writhing test. *Pharmacol. Res.* **48**: 31-5.

Amabeoku GJ and Kinyua CG (2010). Evaluation of the anticonvulsant activity of *Zanthoxylum capense* (Thunb.) Harv. (Rutaceae) in mice. *Int. J. Pharmacol.* **6**: 844-53.

Anaka ON, Owolabi OJ and Emenike CF (2013). Anti-hyperlipidemic effect of aqueous leaf extract of Emilia praetermissa milne-redh (Asteraceae) in rats. *Int. J. Biosci.* **3**: 68-77.

Corda MG, Giorgi O, Longoni B, Orlandi M and Biggio G (1990). Decrease in the function of the gaminobutyric acid-coupled chloridechannel produced by the repeated administration of pentylenetetrazol to rats. *J. Neurochem*. **55**: 1221-61.

Crossland J (1980). Epilepsy and the pharmacology of convulsions and of anticonvulsant drugs. In: Lewis's Pharmacology. 5th Edn. Churchill Livingstone, Edinburgh London Melbourne and New York. Pp 531.

Derese B, Desalegne E, Adem F and Agalu A (2012). Traditional medicine practices in Northeast Ethiopia. *Int. J. Trad. Nat. Med.* **1**: 64-74.

Gamaniel KS (2000). Toxicity from medicinal plants and their products. Nig J *Nat. Prod. Med.* **4**: 4-5.

Hepper FN (1963). *Emilia.* In: Flora of West Tropical Africa, Crown Agents for Overseas Governments and Administrations 2nd ed. J. Hutchinso J. and Dalziel J.M. (eds). London, UK **2**: Pp 244 - 245.

Huen M, Leung J, Ng W, Lui W, Chan M, Tze-Fei Wong J and Xue H (2003). 5, 7- Dihydroxy-6-methoxyflavone, a benzodiazepine site ligand isolated from *Scutellaria baicalensis* Georgi, with selective antagonistic properties. *J. Biochem. Pharmacol.* **66**: 125-32.

Jayaraman R, Anitha T, Joshi VD (2010). Analgesic and anticonvulsant effects of *Acorus calamus* roots in mice. *Intl. J. Pharm. Tech. Res.* **2**: 552-5.

Jeffrey C (1997). What is *Emilia coccinea* (Sims) G. Don (Compositae)? A revision of the large headed *Emilia* species of Africa. *Kew Bull.* **52**: 205 – 12.

Jeub M, Beck H, Siep E, Rüschenschmidt C, Speckmann EJ, Ebert U, Potschka H, Freichel C, Reissmüller E, Löscher W (2002). Effect of phenytoin on sodium and calcium currents in hippocampal CA1 neurons of phenytoin-resistant kindled rats. *Neuropharmacol.* **42**:107-116.

Kawashty S, Gamal El-Din E and Saleh N (1999). The flavonoid chemosystematics of two *Teucrium* species from southern Sinai, Egypt. *J. Biochem. Sys. Ecol.* **27**: 657-60.

Khoshnood-Mansoorkhani MJ, Moein MR and Oveisi N (2010). Anticonvulsant Activity of *Teucrium polium* Against Seizure Induced by PTZ and MES in Mice. *Iran J. Pharma. Res.* **9**: 395–401.

Leonardi M, Ustun T (2002). The global burden of epilepsy. *Epilepsia.* **43**: 21-25.

Librowski T, Czarnecki R, Mendyk A and Jastrzebska M (2000). Influence of new monoterpene homologues of GABA on the central nervous system activity in mice. *Polish J. Pharmacol.* **52**: 317-21*.*

Lisowski S (1997). Le genre *Emilia* (Cass) Cass. (Asteraceae) dans la flore de Gunee (*Afrique occidentale*). *Bulletin du Jardian Botanique National de Belgique.* **66**: 201-06.

Miliauskas G, PRV and Van Beek T (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts*. J. Food Chem.* **85**: 231-37.

National Institutes of Health (2002). Public health service policy on humane care and use of laboratory animals. Office of the Laboratory Animal Welfare. USA Pp 1-19.

Ngo Bum E, Schmutz M, Meyer C, Rakotonirina A, Bopelet M, Portet C, Jeker A, Rakotonirina S, Olpe H and Herling P (2001). Anticonvulsant properties of the methanolic extract of *Cyprus articulatus* (Cyperaceae). *J. Ethnopharmacol.* **76**: 145-50.

Ojewole JAO (2009). Anticonvulsant Activity of *Hypoxis hemerocallidea* Fisch. & C. A. Mey. (Hypoxidaceae) Corm ('African Potato') aqueous extract in mice. *Phytother. Res.* **22**: 91-6.

Sayyah M and Mandgary A (2003). Anticonvulsant Effect of *Ferula gummosa* root extract against experimental seizures. *Iran Biomed. J.* **7**: 139-43.

Sayyah M, Mandgary A and Kamalinejad M (2002). Evaluation of the anticonvulsant activity of the seed acetone extract of *Ferula gummosa* Boiss. against seizures induced by pentylenetetrazol and electroconvulsive shock in mice. *J. Ethnopharmacol.* **82**: 105-09.

Sayyah M, Moaied S and Kamalinejad M. Anticonvulsant activity of *Heracleum persicum* seed. *J. Ethnopharmacol.* **98**: 209-11.

Senanayake N, Roman GC (1993). Epidemiology of epilepsy in developing countries. *Bull. World Health Organ.* **71**: 247-258.

Szbadics J and Erdelyi L (2000). Pre-and postsynaptic effects of eugenol and related compounds on *Helix pomatia* L. neurons. *Acta Biol. Hung.* **51**: 265-73.

Theodore WH, Spencer SS, Wiebe S, Langfitt JT, Ali A, Shafer PO, Berg AT, Vickrey BG (2006). Epilepsy in North America: a report prepared under the auspices of the global campaign against epilepsy, the International Bureau for Epilepsy, the International League Against Epilepsy, and the World Health Organization. *Epilepsia.* **47**: 1700-1722.

World Health Organization (2000). General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine, Geneva, Switzerland. Pp 1-80.

Yazdi A, Soroush S, Sayyah M and Ezzati MH (2011). Evaluation of the Anticonvulsant Activity of the Leaves of *Glycyrrhiza glabra* var. *glandulifera* Grown in Iran, as a Possible Renewable Source for Anticonvulsant Compounds. *Iran J. Pharma. Res.* **10**: 75-82.