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Anticonvulsant and Sedative Activities of Extracts of *Erythrophleum Ivorense* Stem Bark in Mice

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Abstract
Erythrophleum ivorense is used traditionally for many ailments such as convulsion, swellings, pain and emesis. Therefore, this study aimed to investigate the anticonvulsant and sedative activities of extracts of *Erythrophleum ivorense* stem bark in mice. Anticonvulsant effect of the extracts was assessed using picrotoxin, pentylenetetrazole and strychnine-induced convulsion and sedative effect was evaluated using pentobarbitone-induced hypnotic method. The results of this study showed that, crude methanolic extract and ethyl acetate extract significantly ($p < 0.05$) delayed the onset, shortened the duration and offered protection against pentylenetetrazole-induced convulsion. The extracts also antagonized picrotoxin-induced convulsion profoundly but did not antagonize strychnine-induced convulsion. Crude methanolic extract and ethyl acetate extract significantly ($p < 0.05$) prolonged the time of sodium pentobarbital-induced hypnosis. The median lethal dose (LD_{50}) of the crude methanolic extract was found to be 87 mg/kg. In conclusion, crude methanolic extract and ethyl acetate extract of *Erythrophleum ivorense* possessed anticonvulsant and CNS depressant properties.

Keywords: *Erythrophleum ivorense*, Anticonvulsant, sedative, extracts, toxicity

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INTRODUCTION

Epilepsy has become one of the serious brain disorders that affect millions of people at some point during their life time. It is characterized by recurrent seizure which is a manifestation of paroxysmal and disordered neuronal discharges in the brain [1]. These synchronized discharges of brain cells lead to alterations in sensory, motor and other activities. Seizures could be partial when localized part of the brain is involved or general when both sides of the brain are involved in the synchronous discharge [2]. Epilepsy is usually caused by brain damage that results from trauma, infections, stroke or developmental abnormality [3]. The physical, social and psychological effects of seizures on epileptic patients are enormous and hence remedies are conscientiously sought to alleviate the suffering of these individuals. The search for anticonvulsant drugs has resulted in the availability of many synthetic drugs. Examples of antiepileptic drugs (AED) that are presently in clinical use include phenytoin, carbamazepine , ethosuximide, phenobarbitone and clonazepam. These drugs interact with neurotransmitters that are involved in inhibitory and excitatory transmissions in the brain [4]. However, these drugs are associated with high incidence of adverse effects and the high cost of treatment is usually unaffordable especially for patients in countries with poor economy. Therefore there is growing interest in alternative approach for the treatment of seizures and the use of medicinal plants has gained popularity around the world. *Hypericum perforatum, Piper methysticum, Actaea racemosa* and *Erythrophleum ivorense* are among plants that have been used to treat seizures [5]. In spite of the claims that they are effective anticonvulsants, many of these plants have not been subjected to scientific investigations in order to validate such claims.

The plant *Erythrophleum ivorense* belongs to the family *Fabaceae*. It is called "Epo-obo" among Yoruba people of South Western, Nigeria. It is also called by several other names in West Africa countries such as 'forest ordeal tree', 'red water tree' and 'sasswood tree'. The stem bark of *Erythrophleum ivorense* is traditionally used in the treatment of convulsive disorder, emesis, pain, swelling, smallpox and as anthelminthic and laxative [6]. This study was designed to investigate anticonvulsant and sedative properties of extracts of *Erythrophleum ivorense* stem bark.

MATERIALS AND METHODS

Experimental animals

Healthy Swiss albino mice of either sex weighing 20-25g were obtained from the animal house of the College of Medicine, University of Ibadan, Nigeria. They were housed in standard cages under normal laboratory conditions of temperature (25 ± 5°C) and a 12/12 h light/dark cycle. The animals were fed balanced animal feed and clean tap water *ad libitum*. The study was approved by the Animal Ethic Committee of University of Ibadan, Nigeria and the 'Principle of Laboratory Animal Care' [7] was followed.

Collection of plant materials

Fresh uncrushed stem bark of *Erythrophleum ivorense* was collected from Iwo Local Government area of Osun State, South West, Nigeria. It was authenticated in the herbarium of Department of Botany, Obafemi Awolowo University Ile-Ife, Nigeria where voucher specimen was deposited with voucher number 16878.

Preparation of plant extract

Erythrophleum ivorense stem bark was air- dried and reduced to powdery form using electric blending machine. The powdered *Erythrophleum ivorense* stem bark was extracted in 75% methanol. The crude methanol extract (CME) was fractionated using ethyl acetate, dichloromethane and n-hexane to yield 55.2% ethyl acetate fraction (EAF), 31.4% dichloromethane fraction (DCMF), and 13.4% n-hexane fraction (HF). The crude methanol extract and fractions were prepared by dissolving in 2% dimethylsuphoxide (DMSO).

Acute toxicity of crude methanol extract

Male Swiss mice were divided into seven groups of 10 animals each. The extract was administered intraperitoneally to the animals in groups 2 – 6 at single doses of 10, 20, 40, 80, 160 mg/kg body weight respectively. The control group (group 1) received equal volume of the vehicle (2% DMSO). The animals were observed for toxic symptoms within 24 hr after the administration of the extract. Behavioral parameters and mortality were monitored for the first 2 hr and thereafter for 24 hr. Lethal dose in 50 % of the total population (LD_{50}) was determined using the method of Lorke [8].

Phytochemical analysis

The preliminary phytochemical screening of the extracts (CME, EAF, DCMF and HF) of *Erythrophleum ivorense* stem bark was carried out using standard procedures [9, 10].

Pentobarbital-induced sleeping time

The method used by Bourin was adapted [11]. Briefly, the animals were divided into 13 groups of 5 mice each. Three doses (5, 10 and 20 mg/kg body weight) of each of the extracts were tested using a group of animals for each dose. One group of animals was pretreated with 2 ml of normal saline. All pretreatments were done intraperitoneally 30 min before administration of sodium Pentobarbitone (40 mg/kg). Time interval between loss and regain of righting reflex was taken as index of hypnosis. Duration of sleep was considered completed when mice did not accept the decubitodorsal position for three consecutive trials.

Anticonvulsant activity

The method described by Elisha *et al* [12] was adapted for the study. Mice were randomly divided into experimental groups (n = 5). Three doses (5, 10 and 20 mg/kg body weight) of each of the extracts were tested using a group of animals for each dose. One group of animals was pretreated with 2 ml of normal saline. Thirty minutes after these pretreatments, the convulsant drugs, picrotoxin (10mg/kg i.p), pentylenetetrazole (85 mg/kg i.p) or strychnine (3 mg/kg i.p) was administered. Convulsion was evaluated when mouse's body muscle was contracting and relaxing rapidly and repeatedly. Anticonvulsant activity is expressed when onset of action of convulsion is prolonged or duration of convulsion is shortened.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Dunnest's multiple comparison test. Data obtained from the study were expressed as mean ± SEM. P-values less than 0.05 were considered significant.

RESULTS

Acute Toxicity Test

Acute toxicity study showed that the crude methanol extract is fairly toxic. Some of the signs of toxicity observed were reduced motor activity, ataxia, scratching and increased respiratory rate and death. The value of LD_{50} obtained was 87 mg/kg body weight.

Phytochemical screening

The results show that the HF of *E. ivorense* is devoid of the main compound families as indicated in Table 1.

Secondary metabolites	Extracts			
	CME	EAF	DCMF	HF
Alkaloids	++	++	++	--
Saponins	++	++	++	--
Tannins	++	++	--	--
Flavonoids	++	++	++	--
Cardiac glycosides	++	++	--	--

Table 1: Phytochemical constituents of extracts of *Erythrophleum ivorense* stem back

DCMF contained alkaloids, saponins, flavonoids while EAF contained alkaloids, saponins, tannins, flavonoids and cardiac glycosides. Likewise CME showed similar constituents to EAF.

Pentobarbital-induced sleeping time

The extracts were found to exhibit hypnotic property. As shown in Table 2, CME and EAF significantly ($p<0.05$) prolonged the sleeping time induced by sodium pentobarbitone in a dose related manner. The effects produced by DCMF and HF were not significant.

Treatments	Doses (mg/kg)	Duration of sleep (min)
Control	0	135.25 ± 2.04
CME	5	281.06 ± 4.02*
	10	295.38 ± 3.96*
	20	325.02 ± 4.21*
EAF	5	293.40 ± 5.05*
	10	307.21 ± 3.01*
	20	341.60 ± 4.45*
DCMF	5	165.60 ± 5.09
	10	158.10 ± 3.31
	20	146.07 ± 2.11
HF	5	187.21 ± 6.07
	10	163.08 ± 4.14
	20	132.20 ± 4.05

Table 2: Effect of extracts of *Erythrophleum ivorense* on Pentobarbital-induced sleeping time in mice

Each value represents the mean ± SEM (n = 5); *p < 0.05 compared with control group

Anticonvulsant activities

Crude methanolic (CME) and ethyl acetate fraction (EAF) significantly ($p < 0.05$) prolonged the onset of seizures and shortened the duration of action in picrotoxin-induced seizure, while Dichloromethane fraction (DCMF) and n-hexane fraction (HF) did not significantly ($p > 0.05$) delay onset of convulsion or offer any protection against induced convulsion in the animals (Table 3). Similarly, CME and EAF at the highest dose administered (20 mg/kg b.w) significantly ($p<0.05$) prolonged onset of seizure and shortened duration of action in pentylentetrazole-induced convulsion (Table 4). As shown in table 5, the extract at the tested doses did not modify the action of Strychnine in mice. They did not significantly ($p > 0.05$) prolonged the onset of seizures and also failed to shorten the duration of convulsion in strychnine-treated mice.

Treatments	Doses (mg/kg)	Onset of convulsion (min)	Duration of convulsion (min)
Control	0	5.45 ± 0.07	4.62 ± 0.08
CME	5	10.02 ± 0.06*	3.41 ± 0.04
	10	12.84 ± 0.24*	2.84 ± 0.06*
	20	14.40 ± 0.35*	2.46 ± 0.06*
EAF	5	8.41 ± 0.07*	4.23 ± 0.05
	10	10.44 ± 0.28*	3.42 ± 0.04
	20	13.20 ± 0.30*	2.67 ± 0.21*
DCMF	5	5.22 ± 0.11	3.40 ± 0.04
	10	5.43 ± 0.10	3.83 ± 0.08
	20	5.01 ± 0.11	3.62 ± 0.07
HF	5	5.26 ± 0.11	4.20 ± 0.05
	10	4.82 ± 0.07	4.41 ± 0.06
	20	5.07 ± 0.11	3.85 ± 0.08

Table 3: Effects of extracts of *Erythrophleum ivorense* on picrotoxin-induced convulsion in mice.

Each value represents the mean ± SEM (n = 5); *p<0.05 compared with control group

Treatments	Doses (mg/kg)	Onset of convulsion (min)	Duration of convulsion (min)
Control	0	1.82 ± 0.12	2.67 ± 0.08
CME	5	1.60 ± 0.09	2.85 ± 0.07
	10	1.86 ± 0.11	3.09 ± 0.00
	20	15.22 ± 0.21*	1.81 ± 0.12*
EAF	5	1.43 ± 0.05	2.43 ± 0.06
	10	1.27 ± 0.01	2.05 ± 0.00
	20	14.64 ± 0.45*	1.44 ± 0.05*
DCMF	5	1.66 ± 0.09	3.26 ± 0.10
	10	1.62 ± 0.09	2.80 ± 0.07
	20	1.80 ± 0.11	3.03 ± 0.00
HF	5	1.23 ± 0.07	2.44 ± 0.06
	10	1.62 ± 0.04	2.60 ± 0.08
	20	1.28 ± 0.05	2.63 ± 0.08

Table 4: Effects of extracts of *Erythrophleum ivorense* on pentylenetetrazole-induced convulsion in mice

Each value represents the mean ± SEM (n = 5); *p < 0.05 compared with control group

DISCUSSIONS

There are several traditional claims regarding the usefulness of *Erythrophleum ivorense* in the treatment of pain, inflammation and convulsion. However, this plant has not been subjected to any systematic pharmacological screening so far. Hence, it was considered that investigations on these medicinal properties may give scientific authentication to the traditional claims.

To assess the acute toxicity of the plant, the crude methanolic extract (CME) was selected as it gave the positive test for most of the constituents during phytochemical screening and because of its high extractive value.

Treatments	Dose (mg/kg)	Onset of convulsion (min)	Duration of convulsion (min)
Control	0	2.63 ± 0.18	2.44 ± 0.22
CME	5	2.67 ± 0.11	2.29 ± 0.20
	10	2.78 ± 0.24	2.22 ± 0.20
	20	2.43 ± 0.22	2.25 ± 0.20
EAF	5	2.81 ± 0.25	3.20 ± 0.21
	10	3.20 ± 0.21	2.85 ± 0.25
	20	3.26 ± 0.21	3.42 ± 0.23
DCMF	5	3.08 ± 0.06	3.48 ± 0.23
	10	3.24 ± 0.21	3.45 ± 0.23
	20	3.27 ± 0.21	2.65 ± 0.18
HF	5	3.40 ± 0.24	2.83 ± 0.25
	10	2.83 ± 0.25	3.28 ± 0.21
	20	2.61 ± 0.18	3.46 ± 0.23

Table 5: Effects of extracts of *Erythrophleum ivorense* on strychnine-induced convulsion in mice

Value represents the mean ± SEM (n = 5). One-way ANOVA revealed no significant (p > 0.05) difference among treated groups

The median lethal dose (LD_{50}) of the extract was found to be 87 mg/kg and this indicates that the extract is fairly toxic.

The crude methanolic extract and ethyl acetate fraction (EAF) of the plant were found to exhibit sedative effects, as shown by their ability to prolong pentobarbitone-induced sleeping time. It is well known that drugs with sedative properties prolonged the time of sleep produced by barbiturates [13]. Studies have shown that the potentiation of barbiturate hypnosis is an index for CNS depression [14]. It may therefore be suggested that the ability of the extract to prolong barbiturate-induced sleeping time indicates that it possesses CNS depressant property. Picrotoxin, a GABA-A receptor antagonist, produces seizures by blocking the chloride-ion channels linked to GABA-A receptors, thus preventing the entry of chloride ions into the neurons. This leads to decreased GABAergic transmission and activity in the brain. Thus, convulsions arising from picrotoxin are due to decreased GABA-A receptor-mediated inhibition which tips the balance in favor of glutamate-mediated excitatory transmission [15]. The ability of CME and EAF to attenuate seizures induced by picrotoxin may possibly be due to an interaction with GABA-A receptors and / or GABAergic transmission. It is known that some anticonvulsant drugs enhance GABAergic neurotransmission by increasing chloride ion flux through the chloride channels of GABA-A receptors [16]. Therefore it is possible that CME and EAF antagonize picrotoxin-induced seizure by opening the chloride channel associated with GABA-A receptors. It is also possible to achieve these effects by suppressing glutamate-mediated excitation [17]. Pentylenetetrazole-induced convulsion represents a valid model for human generalized and absence seizure [18]. Pentylenetetrazole has been used experimentally to study seizure phenomenon and to identify pharmaceuticals that may control seizure susceptibility. The exact mechanism of epileptogenic action of pentylenetetrazole at the neuronal level is still unclear, but it has been generally reported to produce seizures by inhibiting gamma-aminobutyric acid (GABA) activity [19]. Enhancement of GABAergic neurotransmission has been shown to inhibit or attenuate seizure, while inhibition of GABAergic neurotransmission or activity is known to promote and facilitate seizure [20]. Anticonvulsant agents such as diazepam and phenobarbitone inhibit pentylene-tetrazole-induced seizure by enhancing the action of GABA-A receptor, thus facilitating the GABA-A receptor- mediated opening of chloride-ion channels [21]. Thus inhibition of pentylenetetrazole-induced seizures by CME and EAF suggest that both produce this effect by enhancing GABAergic neurotransmission.

Also, drugs that promote an increase in onset and shortening of period of convulsion in picrotoxin- and pentylenetetrazole-induced convulsion is suggestive of their anticonvulsant activity [22]. CME and EAF prolonged onset of convulsion, while the average duration of convulsion was markedly reduced. Although the parameters used for evaluation of anticonvulsant activity in the present study are not conclusive, it gives a preliminary indication about the anticonvulsant effect of the extracts. CME and EAF also depressed the central nervous system. It is, therefore, thought that the anticonvulsant property of the extract and fraction may be linked, at least in part, to its ability to depress the central nervous system. The pharmacological effects of CME and EAF observed in this study are likely due to the phytochemical constituents such as flavonoids, tannins or alkaloids [23].

In conclusion, extracts of *E. ivorense* possesses sedative and anticonvulsant effects. The results of this experimental animal study lend pharmacological credence to the folkloric and ethnomedicinal uses of the plant in the management of convulsive disorder. There is a need for more precise studies to isolate the active compounds and elucidate the mechanism of action responsible for the central nervous system effects of *E. ivorense.*

5. References

[1] Vyawahare NS, Khandelwal AR, Batra VR, Nikam AP. Herbal anticonvulsants. Journal of Herbal Medicine and Toxicology 2007; 1(1): 9-14

[2] Kolawole OT, Akiibinu MO, Ayankunle AA. Anticonvulsant and depressant activity of methanol leaf extract of *Croton zambesicus*. International Journal of Tropical Disease and Health 2012; 2 (1): 33-41

[3] Sharma S, Sankhyan N, Ramesh K, Gulati S. Child Neurology: Epilepsy of infancy with migrating focal seizures. Neurology 2011; 77(4): 21-24.

[4] Clinckers R, Smolders I, Meurs A, Ebinger G, Michotte Y. Hippocampal dopamine and serotonin elevations as pharmacodynamics markers for the anticonvulsant efficacy of oxcarbazepine and 10, 11-dihydro-10-hydroxy carbamazepine. Neuroscience Letters 2005; 390: 4853.

[5] Schachter SC. Botanicals and herbs: a traditional approach to treating epilepsy. Neurotherapeutics 2009; 6: 415-420

[6] Oliver-Bever B. Medicinal Plants in Tropical West Africa. Cambridge University Press, New York. 1986; pp. 82-83

[7] NIH. Guide for the use of laboratory animals. DHHS, PHS, NIH Publication No. 85- 23, 1985 Revised.

[8] Lorke D. A new approach to acute toxicity testing. Archives of Toxicol. 1983; 54: 275-287

[9] Harbone J.B. Phytochemical Methods. 2nd ed. Chapman and Hall, London 1984; pp. 279

[10] Trease GE, Evans WC. Pharmacognosy. 15th ed. Saunders Publishers, London, 2002; pp. 42-44, 221-293.

[11] Bouri M, Poisson L, Larousse C. Piracetam interaction with neuroleptics in psychopharmacological tests. Neuropharmacol. 1986; 19: 93-96.

[12] Elisha EE, Maliki SJ, Abraham BK. Effect of *Jasmium officinale* flower on the central nervous system of mouse. International Journal of Crude Drug Research 1988; 26: 221-227

[13] Nyeem MAB, Alam MA, Awal MA, Mostofa M, Uddin SJ, Islam N, Rouf R. CNS depressant effect of the crude ethanolic extract of the flowering tops of Rosa damascene. Iranian Journal of pharmacology and Therapeutics 2006; 5(2): 171-174

[14] Dehar N, Walia R, Ratol S. Potentiation of thiopentone sodium induced hypnosis by Berberis Aristata in rodents. Asian J Pharm Clin Res. 2012; 5(1): 131-133

[15] Smith M, Wilcox KS, White HS. Discovery of antiepileptic drugs. Neurotherapeutics 2007; 4: 12-17

[16] Amoateng P, Woode E, Kombian SB. Anticonvulsant and related neuropharmacological effects of the whole plant extract of *Synedrella nodiflora* (L.) Gaertn (Asteraceae). Pharm Bioallied Sci. 2012; 4(2): 140-148

[17] Czapinski P, Blaszczyk B, Czuczwar SJ. Mechanism of action of antiepileptic drugs. Current Topics in Medicinal Chemistry 2005; 5: 3-143

[18] Vijayalakshmi A, Ravichandiran V, Anbu J, Velraj M, Jayakumari S. Anticonvulsant and neurotoxicity profile of the rhizome of *Smilax china* Linn. in mice. Indian J Pharmacol. 2011; 43(1): 27-30.

[19] Rocha L. Subchronic treatment with antiepileptic drugs modifies pentylenetetrazol-induced seizures in mice: Its correlation with benzodiazepine receptor binding. Neuropsychiatr Dis Treat. 2008; 4(3): 619-625

[20] Shin EJ, Bach JH, Nguyen TTL, Jung BD, Oh KW, Kim MJ, Jang CG, Ali SF, Ko SK, Yang CH, Kim HC. *Gastrodia Elata* Bl attenuates cocaine-induced conditioned place preference and convulsion, but not behavioral sensitization in mice: importance of $GABA_A$ receptors. Curr Neuropharmacol. 2011; 9(1): 26-29

[21] Praveen KU, Naga PK, Murali KB, Swarnalatha M. Evaluation of antiepileptic activity of methanolic extract of *Brassica nigra* seeds in mice. International Journal of Pharmaceutical Innovations 2013; 3(2): 73-84

[22] Salih MAM, Mustafa AA. Substance in broad beans (*Vicia faba*) is protective against experimentally induced convulsions in mice. Epilepsy Behav. 2008; 12:25-29

[23] Owolabi OJ, Amaechina FC, Okoro M. Effect of ethanol leaf extract of *Newbouldia laevis* on blood glucose levels in diabetic rats. Tropical Journal of Pharmaceutical Research 2011;10 (3): 249- 254.