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Anticonvulsant Effects of a Leaf Extract of *Ficus exasperata* Vahl (Moraceae) in Mice

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Abstract: *Ficus exasperata* Vahl (Moraceae) is a terrestrial afro-tropical tree used as an antiepileptic remedy in African traditional medicine. This study investigated the anticonvulsant effect of a hydroalcoholic leaf extract of *F. exasperata* against seizures induced by pentylenetetrazole, picrotoxin or maximal electroshock in mice. The extract (30-300 mg kg⁻¹, p.o.) significantly delayed the onset and decreased the duration of pentylenetetrazole- and picrotoxin-induced convulsions. It also caused significant reduction in the duration of maximal electroshock-induced tonic hind limb extension of mice. In addition, at some anticonvulsant doses, the extract produced motor impairment on the rotarod. The present findings strongly indicate that the leaf extract of *F. exasperata* has anticonvulsant properties in mice and may explain its use as an antiepileptic agent in African traditional medicine.

Key words: Seizures, picrotoxin, pentylenetetrazole, maximal electroshock, rotarod

INTRODUCTION

Ficus exasperata Vahl (Moraceae), is a terrestrial afro-tropical tree that grows up to about 20 m tall and prefers evergreen and secondary forest habitats (Berg, 1989; Berg and Wiebes, 1992; Lansky and Paavilainen, 2010). The leaf extract has been used in African traditional medicine to treat epilepsy, high blood pressure, rheumatism, arthritis, intestinal pains and colics, bleeding and wounds (Irvine, 1961; Mshana *et al.*, 2000). Phytochemical characterisation of the leaves of *F. exasperata* showed the presence of alkaloids, flavonoids, saponins and tannins (Ayinde *et al.*, 2007; Ijeh and Ukwemi, 2007; Woode *et al.*, 2009). There are some reports regarding the biological activities of the plant. An aqueous leaf extract produced a dose-related reduction in mean arterial blood pressure (Ayinde *et al.*, 2007) as well as significant anti-ulcerogenic effect in aspirin-induced ulcerogenesis (Akah *et al.*, 1998). The ethanolic leaf extract has also been shown to have anti-nociceptive, anti-pyretic, anti-inflammatory, anti-arthritic and antioxidant properties (Woode *et al.*, 2009; Abotsi *et al.*, 2010).

In this study, we investigated the anticonvulsant potential of a hydroalcoholic extract of the leaves of *Ficus exasperata* Vahl in the pentylenetetrazole-, picrotoxin- and maximal electroshock-induced seizure tests in mice. Pentylenetetrazole-, picrotoxin- and maximal electroshock-induced seizures are well accepted models of epilepsy (Giardina, 2001; Akula *et al.*, 2007). Pentylenetetrazole-

and picrotoxin-induced seizure tests help to identify anticonvulsant agents that are effective against myoclonic/generalized absence epilepsy while the maximal electroshock-induced seizure test predicts drugs effective against generalized tonic-clonic epilepsy (Giardina, 2001; Castel-Branco *et al.*, 2009). The effect of the extract on motor coordination was also evaluated with the rotarod test.

MATERIALS AND METHODS

Collection of plant material and extraction: Leaves of *Ficus exasperata* were collected from the campus of Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana in June 2007. Leaves were authenticated in the Department of Pharmacognosy of the university where a voucher specimen (No.FP/08/023) has been kept. The leaves were shade-dried and pulverized in a hammer-mill. One kilogram of the powdered material was extracted with 70% v/v of ethanol in a Soxhlet apparatus for 24 h. The hydroalcoholic extract was then evaporated to a greenish syrupy mass under reduced pressure in a rotary evaporator, air-dried and kept in a desiccator. Yield obtained was 10% w/w. This is referred to subsequently as the extract or FEE.

Animals: Male ICR mice (35±5 g) were purchased from the Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon and housed in the animal house of the Department of Pharmacology,

KNUST, Kumasi. Animals were housed in groups of five in stainless steel cages ($34 \times 47 \times 18 \text{ cm}^3$) with soft wood shavings as bedding, fed with normal pellet diet (Ghana Agro Food Company Limited (GAFCO), Tema), given water *ad libitum* and maintained under laboratory conditions (temperature $25 \pm 2^\circ\text{C}$, relative humidity 60-70% and 12 h light-dark cycle). To acclimatize the animals to the test conditions, they were brought to the laboratory a week before the experiments. All procedures and techniques used in these studies were in accordance with accepted principles for laboratory animal use and care (EU directive of 1986: 86/609/EEC) and were approved by the Departmental Ethics Committee.

Drugs and chemicals: The following drugs and chemicals were used: diazepam, Pentylentetrazole (PTZ), picrotoxin (Sigma-Aldrich Inc., St. Louis, MO., USA); carbamazepine (Tegretol®, Novartis, Basel, Switzerland).

Pentylentetrazole-induced seizure test: PTZ (60 mg kg^{-1} , s.c.) was used to induce clonic convulsions (Oliveira *et al.*, 2001; Bhattacharya and Sanyal, 1978). Mice were divided into six groups ($n = 6$) and received FEE ($30\text{-}1000 \text{ mg kg}^{-1}$, p.o.), diazepam (1.0 mg kg^{-1} , i.p.) or vehicle (normal saline; 10 mL kg^{-1} , i.p.) 30 min (i.p.) or 1 h (p.o.) before the injection of PTZ (60 mg kg^{-1} , s.c.), respectively. The animals were placed individually in an observation chamber and videotaped for 30 min. The videos were later scored to determine the latency and duration of clonic convulsions for each mouse.

Picrotoxin-induced seizure test: The test was conducted similar to that described by Leewamich *et al.* (1996) with modifications. Mice were divided into seven groups ($n = 6$) and pre-treated with saline (10 mL kg^{-1} , i.p.), FEE ($30\text{-}300 \text{ mg kg}^{-1}$ p.o.) or diazepam ($0.1\text{-}1 \text{ mg kg}^{-1}$, i.p.) 30 min (i.p.) or 1 h (p.o.) before the administration of picrotoxin (3 mg kg^{-1} , i.p.). Animals were immediately placed individually in an observation chamber and videotaped for 30 min. The videos were later scored to determine the latency, frequency and duration of picrotoxin-induced clonic convulsions.

Maximal Electroshock Seizure Test (MEST): The test was performed as described by Schmutz *et al.* (1990) with some modifications. Mice were divided into seven groups ($n = 10$) and received FEE ($30\text{-}300 \text{ mg kg}^{-1}$, p.o.), vehicle (10 mL kg^{-1} , p.o.) or carbamazepine ($3\text{-}30 \text{ mg kg}^{-1}$, p.o.). One hour after the treatments, tonic convulsions of the hind extremities of mice were induced by passing alternating current (50 Hz, 60 mA and 0.2 sec) from an Electroconvulsive Therapy (ECT) apparatus (Model 7800,

Ugo Basile, Camerio, Italy) via ear electrodes. The current used was predetermined before experimentation and was the maximal current that caused hind limb extension in all mice in the trials. The duration of tonic hind limb extension seizure was determined in each group.

Effect of extract on motor coordination-rotarod test: The effect on motor co-ordination was assessed using rotarod apparatus (Model 7600, Ugo Basile, Cormerio, Italy) rotating at a speed of 25 rpm. This apparatus consists of a base platform and a rotating rod of 3 cm diameter with a non-skid surface. The rod, 50 cm in length, is divided into five equal sections by six disks. Five mice were tested simultaneously.

Mice were initially selected for their ability to remain on the rotarod for at least two consecutive 60 sec trials before the test day. Mice were randomly divided into seven groups ($n = 6$) and received FEE ($30\text{-}300 \text{ mg kg}^{-1}$, p.o.), diazepam ($0.1\text{-}1.0 \text{ mg kg}^{-1}$, i.p.) or vehicle (10 mL kg^{-1} , i.p.). Thirty minutes (i.p.) or 1 h (p.o.) after the treatments, the latencies to fall from the rod were measured. Mice that stayed on the rotarod for more than 60 sec were given the maximum score, 60 sec.

Statistical analysis: Data are presented as Mean \pm SEM ($n = 6\text{-}10$). Data were analysed using one-way Analysis of Variance (ANOVA) followed by Newman-Keuls post hoc test. GraphPad Prism for Window 5 (GraphPad Software, San Diego, CA, USA) was used for all statistical analysis and $p < 0.05$ was considered statistically significant.

RESULTS

Pentylentetrazole-induced seizure test: The extract showed significant anticonvulsant activity against PTZ-induced clonic seizures. It dose-dependently reduced the duration of the convulsions ($F_{4,25} = 5.010$, $p = 0.0042$) with statistical significance at 1000 mg kg^{-1} ($p < 0.05$). FEE at 1000 mg kg^{-1} also delayed the onset of convulsions ($p < 0.05$). Diazepam (1 mg kg^{-1}), the reference anticonvulsant, prevented the occurrence of PTZ-induced convulsions ($F_{4,25} = 5.010$, $p = 0.0042$) in mice (Table 1).

Table 1: Effect of *Ficus exasperata* leaf extract (FEE) and diazepam on Pentylentetrazole-induced convulsions in mice

Drugs	Dose (mg kg^{-1})	Latency to convulsions (s)	Duration of Convulsions (s)
Vehicle	-	393.00 \pm 59.12	21.00 \pm 3.02
FEE	30	550.70 \pm 124.00	30.33 \pm 6.00
	100	379.60 \pm 67.01	22.67 \pm 5.04
	300	416.70 \pm 48.68	14.52 \pm 4.21
	1000	946.80 \pm 295.20*	5.02 \pm 1.34*
Diazepam	1.0	0***	0***

Values are expressed as Mean \pm SEM ($n = 6$). * $p < 0.05$; *** $p < 0.001$ compared to vehicle-treated control group (one-way ANOVA followed by Newman-Keuls test)

Picrotoxin-induced seizure test: The extract exhibited profound dose-dependent anticonvulsant effect against picrotoxin-induced seizures by significantly increasing latency to convulsions ($F_{3,20} = 9.164$, $p = 0.0005$, Fig. 1a) and significantly reducing the frequency ($F_{3,20} = 5.396$, $p = 0.0069$; Fig. 1a) and duration ($F_{3,20} = 23.64$, $p < 0.0001$; Fig. 1b) of convulsions. Diazepam produced effects similar to that of the extract. It significantly delayed the onset of convulsions ($F_{3,16} = 13.45$, $p = 0.0001$, Fig. 1c) and reduced the frequency ($F_{3,16} = 12.81$, $p = 0.001$; Fig. 1c) and duration ($F_{3,16} = 15.96$, $p < 0.0001$; Fig. 1d) of convulsions.

Maximal electroshock seizure test: The extract caused significant reduction of the duration of maximal electroshock-induced tonic hind limb extension ($F_{3,25} = 4.135$, $p = 0.0164$, Fig. 2a). Carbamazepine which was used as a reference drug, also produced significant reduction in the duration of the maximal

electroshock-induced tonic hind limb extension ($F_{3,29} = 11.01$, $p < 0.0001$, Fig. 2b) and completely prevented its occurrence at 30 mg kg^{-1} .

Rotarod test: FEE and diazepam at doses of 300 mg kg^{-1} and 1.0 mg kg^{-1} , respectively caused significant decrease in the time spent by mice on the rotating rod (both $p < 0.01$; Table 2).

Table 2: Effect of *Ficus Exasperata* leaf extract (FEE) and diazepam on mice on the rotarod

Drugs	Dose (mg kg^{-1})	Time spent on rod (sec)
Control	-	50.25±8.75
FEE	30	43.00±10.01
	100	33.75±6.25
	300	12.50±5.26**
Diazepam	0.1	47.43±7.49
	0.3	30.00±5.83
	1.0	5.83±3.26**

Values are expressed as Mean±SEM (n = 6). ** $p < 0.01$ compared to vehicle-treated control group (one-way ANOVA followed by Newman-Keuls test)

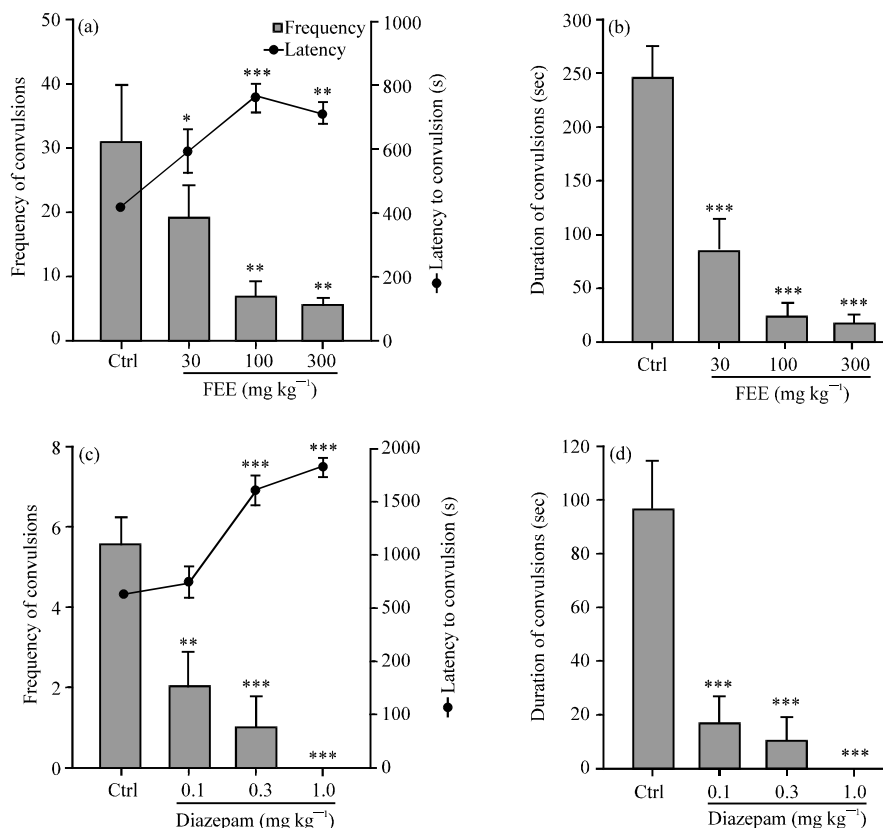


Fig. 1: Effect of FEE (30-300 mg kg^{-1} , p.o.) and diazepam (0.1-1.0 mg kg^{-1} , i.p.) on frequency (a, c), latency to (a, c) and duration (b, d) of picrotoxin-induced convulsions in mice. Each point or column represents the Mean±SEM (n = 6). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to control group (one-way ANOVA followed by Newman-Keuls test)

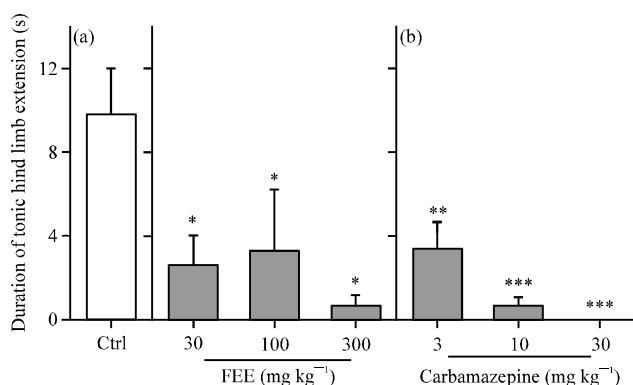


Fig. 2: Effect of (a) FEE (30-300 mg kg⁻¹, p.o.) and (b) carbamazepine (3-30 mg kg⁻¹, p.o.) on the duration of MES-induced tonic hind limb extension. Each column represents Mean±SEM (n = 10). *p<0.05, **p<0.01, ***p<0.001 (one-way ANOVA followed by Newman-Keuls test)

DISCUSSION

The present study has demonstrated that the hydroalcoholic leaf extract of *Ficus exasperata* inhibits pentylenetetrazole-, picrotoxin- and MES-induced seizures in mice. Two most common and predictive screening tests used for characterizing potential anticonvulsant drugs are the pentylenetetrazole- and maximal electroshock-induced seizure tests in laboratory animals (Loscher and Schmidt, 1988; Sayyah *et al.*, 2004). The PTZ-induced seizure test represents a valid model for human generalized myoclonic and absence seizures (Loscher and Schmidt, 1988). Anticonvulsant activity against PTZ seizures also identifies compounds that can raise seizure threshold in the brain (Goodman *et al.*, 1953; Piredda *et al.*, 1985; Mandhane *et al.*, 2007). Since FEE exerted anticonvulsant activity against PTZ seizures, it implies that FEE may contain compounds that have activity against generalized myoclonic and absence seizures. Antagonism of PTZ-induced seizures also suggests that the extract of *F. exasperata* might have effects on GABAergic neurotransmission as PTZ has been shown to interact with the Gamma-aminobutyric Acid (GABA) neurotransmitter (De Sarro *et al.*, 1999). This is further supported by the fact that FEE inhibited picrotoxin-induced seizures. Picrotoxin, a GABA_A receptor antagonist, has been shown to elicit seizures by blocking chloride channels linked to GABA_A receptors (Meldrum and Rogawski, 2007).

The Maximal Electroshock-induced Seizure Test (MEST) is probably the best-validated preclinical test that predicts drugs effective against generalized seizures of the tonic-clonic (grand mal) type (Loscher and Schmidt, 1988; Castel-Branco *et al.*, 2009). Anticonvulsant effect in MEST further indicates the ability of the testing material to prevent seizure spread through neural tissue (Piredda *et al.*, 1985; Castel-Branco *et al.*, 2009). All the

currently available antiepileptic drugs which are clinically effective in the treatment of generalized tonic-clonic convulsions (e.g., phenytoin, phenobarbital, lamotrigine and carbamazepine), are effective in the MEST (Castel-Branco *et al.*, 2009). From the significant activity shown by FEE in the MEST, FEE may be effective against generalized tonic-clonic convulsions.

FEE at doses at and beyond 300 mg kg⁻¹ impaired motor coordination. This may be due to sedative or muscle relaxant effects of FEE. But the effect is most likely to be due to muscle relaxation as a study in our laboratory (unpublished data) shows that FEE antagonizes acetylcholine-induced contractions of chick biventer cervicis muscle.

CONCLUSION

In conclusion, the present study has shown that the hydroalcoholic leaf extract of *Ficus exasperata* possess anticonvulsant properties. However, the mechanisms and the active compound (s) involved in this pharmacological effect are unknown and need to be investigated in further studies.

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