Biological and Environmental Sciences

BEST JOURNAL 5(1): 76 - 80
Date received: May, 2007
Date accepted: October, 2007
Printed in Nigeria.

ANTICONVULSANT ACTIVI
EXTRACT OF *ENTADA ABYSS* Steud. Ex. A. Rich

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ABSTRACT

The methanolic extracts of leaves (M_L), stem bark (M_{SB}) and root bark (M_{RB}) of Entada abyssinica were evaluated for its anticonvulsant activities against subcutaneous pentylenetetrazole (Sc-PTZ) and maximal electroshock-induced seizures in mice and chicks respectively. All the extracts proved to be effective in inhibiting maximal electroshock (MES) in chicks, with no protection against Sc-PTZ induced seizures in mice. M_L, M_{SB}, and M_{RB} protected 83.3, 66.7 and 83.3% of the animals from MES induced seizures respectively. Preliminary phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, tannins, saponins, steroids and glycosides. The intraperitoneal (i.p.) LD_{50} for M_L, M_{SB} and M_{RB} was found to be 1587.5, 387.3 and 162.0mg/kg respectively. The observed anticonvulsant activities of the extracts in MES test suggest that these extracts may be useful in the treatment of grandmal epilepsy.

Keywords: Entada abyssinica, anticonvulsant activity, leaves, stem bark, and root bark.

INTRODUCTION

Epilepsy is a collective term used to describe a chronic brain syndrome of various aetiology, characterized by recurrent seizures (convulsion) due to excessive discharges of cerebral neurons, and associated with a variety of clinical and laboratory manifestations. (Theodore and Leonard, 1990). Although modern therapy with synthetic anti-epileptic drugs is effective in approximately 50% of patients, many patients with epilepsy fail to experience adequate control of their seizures, despite optimal use of available anti-epileptic drugs (AED) while others do so only at the expense of significant toxic side effects (Stables and Kupferberg, 1997). Medicinal plants have been used and are still in use for the management of neurological conditions, including epilepsy, in the traditional practices. The plant kingdom is believed to hold cure for the management of debilitating conditions affecting humans. There is therefore a need to intensify research into these medicinal plants with the aim of isolating compounds responsible for the observed effects and development of beneficial ones as phytomedicines for use by the traditional people who cannot afford the high cost of available anti-epileptics.

Entada abyssinica steud ex. A. Rich (Fabaceae) is a small; low branching tree found extensively in Tropical Africa. Locally, the plant is called 'Tawatsa' in Hausa, 'Angaramiri' in Igbo and 'Gbengé' in Yoruba. In West Africa, a decoction of the leaves is taken for fever (Dalziel, 1955). In Central Africa, a decoction of the stem bark is used for the relief of cough (Watt and Breyer-Brandwijk, 1962; Oliver-Breyer, 1986) while a decoction of the root bark is used by the Tangayinkan's as remedy for rheumatism (Watt and Breyer, 1962; Kokwaro, 1976). In Tanzania, an infusion of the dried root is taken for epilepsy (Mathias, 1982).

In this study, the acute toxicity of the methanolic extracts of the leaves, stem bark and root bark of *E. abyssinica* were evaluated in mice to assess its safety. The anticonvulsant activities of these extracts were also investigated to scientifically justify the use of the plant as an anticonvulsant as speculated by the traditional practitioners.

MATERIALS AND METHODS

Collection of Plant Material

The leaves, stem bark and root bark of *Entada abyssinica* were collected along Zaria-Jos Road in the month of November, 2003. The plant was identified by Malam Mohammed Musa of Herbarium Section, Department of Biological Sciences, Ahmadu Bello University, ZariaNigeria. A voucher specimen (NO 900379) was made and deposited in the herbarium.

Extraction Procedure

The collected leaves, stem bark and root bark were air-dried and ground to fine powder separately. 250g each, of the powdered plant material was soxhlet extracted with petroleum ether (60 80°C) for 24 hours. The defatted marc of the plant parts were again extracted with methanol (Soxhlet) for 48 hours. The resulting solutions were filtered and concentrated in-

vacuo to yield greenish brown (8.7g), dark brown (14.3g) and brownish gummy (9.2g) extracts of leaves, stem bark and root bark, respectively. The extracts were coded; M_L (leaves), M_{SB} (stem bark) and M_{RB} (root bark) respectively.

Experimental Animals

Male and female Swiss albino mice weighing 1825g were obtained from the Animal House, Department of Pharmacology and Clinical Pharmacy. Two day old white Ranger cockerels were obtained from National Animal Production Research Institute (NAPRI) Shika, Zaria. All animals were kept under wellventilated conditions, fed on Standard Feeds (Excel Feeds Plc, Ilorin, Nigeria) and allowed water *ad libitum.* All experimental procedures were approved by Ahmadu Bello University, Animal Right Ethic committee.

Phytochemical Screening

The extracts were screened for the presence of various constituents employing standard screening test (Trease and Evans, 1989). Conventional protocols for detecting the presence of alkaloids, tannins, saponins, flavonoids, cardiac glycosides and resins were used.

Acute Toxicity Studies (LD_{50})

LD_{50} determination was conducted using the method of Lorke (1983). Male and female mice were divided into 9 groups of 3 mice each. The first three groups were treated with M_L (intraperitoneally [*i.p*]) at a dose of 400-1600mg/kg, groups 46 received M_{SB} (*i.p.*) at a dose of 400-1000mg/kg, while groups 79 received (*i.p.*) doses of M_{RB} at 100800mg/kg body weight. Animals were observed for general signs and symptoms of toxicity including mortality over a period of 24 hours.

In the second phase 16 mice were divided into 16 groups of one mouse each. Specific doses of M_L, M_{SB}, and M_{RB} were administered and the final LD_{50} calculated.

PHARMACOLOGICAL STUDIES

Pentylenetetrazoleinduced seizures in Mice (Sc-PTZ)

The method of Swinyard *et al.,* (1952) was employed. Ninety albino mice of either sex were randomly divided into 18 groups of five mice per group. The first group which served as negative control was treated with normal saline (*i.p.*). Groups 26 received different doses of ML (100-600mg/kg, *i.p.*); groups 712 received doses of MSB (50-300mg/kg, *i.p.*) while groups 1317 were given varying doses of MRB (25150mg/kg, *i.p.*). Group 18 which served as positive control was treated with 200mg/kg *i.p.* Valproic acid (VPA).

Thirty minutes later, 85mg/kg of freshly prepared solution of leptazole was administered subcutaneously to all the mice. The mice were observed for 30 minutes for the onset and incidence of seizures. An episode of tonic extension of the hind limbs or clonic spasm which persisted for a minimum of 30 seconds was taken as threshold convulsion. Lack of threshold convulsion during 60 minutes of observation was regarded as protection. The number of mice protected was noted and the anticonvulsant properties of the extracts expressed as percentage protection.

Maximal Electroshock-induced Seizures in Chicks (MES)

The methods of Swinyard and Kupferberg (1985); and Browning, (1992) were employed. 108 two day old Cockerels were randomly divided into 18 groups of 6 chicks per group. The first group received normal saline *i.p.*; 26 (100500mg/kg *i.p.*, ML); groups 712 (50-300mg/kg *i.p.* MSB); groups 1317 (25150mg/kg, *i.p.* MRB) while the 18th group received phenytoin (20mg/kg, *i.p*). 30 minutes later, maximal electroshock was delivered to induce seizures in the chicks using the Ugo basile electroconvulsive machine (model 1801) with corneal electrodes placed on the upper eyelid of the chick after dipping them in normal saline. The current, shock duration, frequency and pulse width were set and maintained at 60mA, 0.85s, 100 pulse/seconds and 0.6ms respectively. An episode of tonic extension of the hind limbs of the chicks was considered as full convulsions. Lack of tonic extension of the hind limbs was regarded as protection.

Statistics

Data were expressed as Mean SEM. Statistical analysis was carried out using student's t-test and $P<0.05$ was considered significant.

RESULTS

Phytochemical Screening

Preliminary phytochemical analysis of the extracts revealed the presence of alkaloids, flavonoids, tannins, saponins and glycosides.

Acute Toxicity Studies

The LD_{50} for M_L, M_{SB}, and M_{RB} was found to be 1587.5, 387.3 and 162.0mg/kg *i.p.* respectively. Signs and symptoms of toxicity include stretching, limping, increased respiratory rate, sedation and finally death in some cases.

Anticonvulsant Activities

The various extracts lack significant activity (Table 1) against pentylenetetrazole-induced

seizures. The extracts protected the animals against maximal electroshock induced convulsions, with highest percentage of protections; 83.3, 66.7 and 83.3% at doses of 200mg/kg, 250mg/kg and 100mg/kg for M_L, M_{SB} and M_{RB} respectively (Table 2).

Table 1: Effects of various doses of M_L, M_{SB} and M_{RB} of *E. abyssinica* on the convulsive activities of pentylenetetrazole in mice

Treatment n=5 (	Mean Onset of Convulsion SEM (mins)	Mean Duration of convulsion SEM (mins)	Quantal Protection	Percentage Protection	Percentage Mortality
Normal saline)	12.4 4.39	5.56 1.60	0/5	0	0
M_L (mg/kg)					
100	6.75 2.99	5.05 1.80	1/5	20	60
150	6.80 3.42	4.23 2.83	0/5	0	80
200	7.60 1.60	4.00 1.71	0/5	0	40
400	8.40 3.05	3.60 2.22	0/5	0	80
600	7.40 2.30	2.20 1.00	0/5	0	60
M_{SB} (mg/kg)					
50	8.60 2.70	4.92 2.80	05	0	0
100	9.60 2.88	4.20 3.42	0/5	0	100
150	8.80 1.41	3.68 2.31	0/5	0	80
200	9.90 3.13	2.65 0.83	0/5	0	80
250	9.82 1.30	2.11 1.63	0/5	0	80
300	10.9 2.80	1.80 0.99	0/5	0	100
M_{RB} (mg/kg)					
25	6.80 3.07	4.25 3.36	0/5	0	60
50	8.00 2.83	3.09 1.80	1/5	20	60
100	8.40 1.67	2.36 2.25	0/5	0	60
125	9.22 2.96	3.24 1.50	0/5	0	60
150	9.55 1.10	1.68 1.70	0/5	0	80
Valproic acid (VPA) 200mg/kg	0	0	5/5	100	0

Table 2: effects of various doses of M_L, M_{SB}, and M_{RB} of *E. abyssinica* on maximal electroshock-induced seizures in chicks

Treatment	Dose (mg/kg)	Mean Onset of Convulsion ±SEM(mins.)	Mean Duration of Convulsion ± SEM (mins.)	Quantal Protection	Percentage Protection	Percentage Mortality
Normal Saline	10ml/kg	12.4 ± 4.39	5.56 ± 1.60	0/6	0	0
M_L	100	0.06 ± 0.01	2.42 ± 0.21	0/6	0	0
	150	0.05 ± 0.07	2.40 ± 0.81	0/6	0	0
	200	0.05 ± 0.00	2.10 ± 0.00	5/6	83.3	0
	400	0.06 ± 0.58	1.33 ± 0.40	2/6	33.3	0
	600	7.0 ± 1.10	1.10 ± 0.62	2/6	33.3	0
M_{SB}	50	0.05 ± 0.83	2.22 ± 1.10	2/6	33.3	0
	100	0.05 ± 0.41	1.93 ± 1.30	2/6	33.3	0
	150	0.07 ± 0.26	1.40 ± 0.22	3/6	33.3	0
	200	0.07 ± 0.22	1.05 ± 0.17	3/6	50.0	0
	250	0.07 ± 0.06	0.93 ± 0.50	4/6	50.0	0
	300	1.12 ± 0.20	0.28 ± 0.10	4/6	66.7	0
M_{RB}	25	0.06 ± 0.75	3.20 ± 0.75	0/6	66.7	0
	50	0.07 ± 0.81	2.82 ± 0.41	0/6	0	0
	100	2.10 ± 0.00	1.50 ± 0.00	5/6	0	16.7
	125	0.07 ± 1.10	0.32 ± 0.24	3/6	83.3	0
	150	0.07 ± 1.77	0.30 ± 0.18	3/6	50.0	0
Phenytoin	20	0	0	6/6	50.0	0
					100	0
						0

DISCUSSION AND CONCLUSION

The various doses of M_L, M_{SB}, and M_{RB} of *E. abyssinica* used were not effective in protecting the mice against Sc-PTZ induced seizures when compared with Valproic acid as shown in Table I. This suggests that these extracts are probably not effective in treatment of tonic-clonic seizures primarily in generalized seizures of petitmal type (Loscher *et al.,* 1991b). The observed effects might be in agreement with the findings of Swinyad *et al.,* (1952); Swinyard (1969), that not all antiepileptic drugs have protective value against Sc-PTZ induced convulsions.

On the other hand, the extracts proved to be effective in inhibiting electroshock-induced seizures in chicks. The observed inhibitions of hind limb tonic extensions in chicks were comparable to the effects of phenytoin (100% protection) in this model. MES is a model for generalized tonic-clonic seizure which is highly reproducible with a consistent end-point (Stables and Kupferberg, 1997). The behavioral and electrographic seizures generated in this model are consistent with the human disorders (Swinyard *et al.*, 1989). Ability of the extracts to inhibit the Hind limb tonic extension (HLTE) suggests anticonvulsant activity for the treatment of generalized tonic-clonic and partial seizures

Using the student *t*test there is no significant protection ($P>0.05$) against leptazole-induced seizures in both normal saline, and extract treated groups, while there is significant protection ($p<0.05$) against electroshock-induced seizures in treated groups compared to normal saline.

In conclusion, the methanolic extracts of the leaves, stem bark, and root bark of *E.abyssinica* may be valuable in the treatment of grandmal epilepsy (Loscher *et al.,* 1991a) which supports the speculation for the use of the plant in traditional medicine for the treatment of epilepsy. Further studies will be directed towards isolation and characterization of the biologically active compound (s) which could lead to the discovery of naturally occurring antiepileptic drug (s) from the plant.

Acknowledgement

The authors expressed their sincere appreciation and gratitudes to Mal. Ibrahim Adamu, Mr. Charles Ebute, Mr. John Kono, and Mal. Ya'u, all of the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria Nigeria for their technical assistance in the work.

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