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RESEARCH ARTICLE

Anticonvulsant Activity of the Aqueous Leaf Extract of *Croton zambesicus* (Euphorbiaceae) in Mice and Rats

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ABSTRACT

To determine the anticonvulsant activity of the leaf extract of *Croton zambesicus* in mice and rats, and in order to verify the traditional use of the plant in the treatment of epilepsy, the pentyleneterazole (PTZ) and the maximal electroshock seizure (MES) models were used for assessing the anticonvulsant effects of the aqueous leaf extract in mice and rats. In the PTZ test, the leaf extract (1000-2000 mg/kg p.o.) produced a significant ($p < 0.05$) increase in the onset of seizures in rats and mice compared with the control group. The aqueous extract (1500 and 2000 mg/kg p.o.) produced some protection (42.9%) in rats, while 1000 mg/kg p.o. of that produced significant protection (71.4%) against PTZ-induced convulsion in mice. In the MES test, the aqueous extract (500-1500 mg/kg p.o.) produced a significant ($p < 0.05$) increase in the onset of seizures compared with the control group. At 1500 mg/kg p.o., the extract also produced significant protection (71.4%) against MES-induced convulsions in mice. The results obtained from this study indicate that the aqueous leaf extract of *Croton zambesicus* may be beneficial in both absence and tonic clonic seizures.

Keywords: *Croton zambesicus, Mice, Rats, Anticonvulsant, Pentylenetetrazole, MES test*

Croton zambesicus muell Arg. (Euphorbiaceae) syn C. amabilis muell. Arg., syn. C. gratissumus Burch, is an ornamental tree grown in villages and towns of Nigeria. It is a Guinea-Congolese species widely spread in tropical Africa [1]. The leaf decoction is used in Benin Republic as antihypertensive and antimicrobial (urinary infections) [2]. The Ibibios in urunan area of Akwa Ibom state of Nigeria use the leaf traditionally as a remedy for malaria [1]. Antidiabetic activity of the ethanolic leave extract has also been reported [3].

The ent-trachyloban-3β-ol, a trachylobane diterpene, isolated from dichloro-methane extract of the leaves has cytotoxic activity on Hela cells [4]. The alkaloidal fractions of the leaf have been reported to possess weak antimicrobial activity [5]. While the essential oil found in the leaves contain p-cymene are linalool and beta-caryophyllene [6]. Mekkawi [7] also reported that the constituents of the essential oil found in the flowering tops include; pinene, limonene linalool, menthol, carvone, thymol, alpha-humulene and ceisnerolidol.

In Jos, Plateau State of Nigeria, the decoction of the leaves is used in the prevention and treatment of epileptic seizures (Dr. Azija, personal Communication). This study aims to evaluate the anticonvulsant activity of aqueous leaf extract of *Croton zambesicus* using pentylenetetrazole (PTZ) and the maximal electroshock seizure (MES) tests.

MATERIALS AND METHODS

Institutional Approval

The work was conducted in the Department of Pharmacology, Faculty of Pharmaceutcal Sciences, University of Jos, Nigeria and was duly approved by the Faculty Postgraduate Board.

Plant Material

The leaves of *Croton zambesicus* were collected from Bauchi Road, in Jos, Plateau State, Nigeria in July 2006. The plant was identified by Mr. I.A. Kareem, at the Federal College of Forestry, Jos and confirmed at Forestry Research Institute of Nigeria (FRIN), Ibadan. The fresh leaves of the plant were shade dried for 8 days and then powdered using mortar and pestle. Fifty grams (50 g) portion of the powdered leaves was extracted by

Table 1. Effect of aqueous extract *of Croton zambesicus* on pentylenetetrazole-induced seizures in rats

Treatment	Onset of convulsion (seconds)	Number convulsed /number used	Mortality (%)	Protection (%)
Distilled water	44.3 ± 1.69	7/7	100	0
Phenobarbitone *(30 mg/kg)*	-	-	0	100
C. zambesicus extract				
500 mg/kg	45.3 ± 0.47	7/7	100	0
1000 mg/kg	45.6 ± 3.22	5/7	71.4	28.6
1500 mg/kg	55.0 ± 1.22*	4/7	57.1	42.9
2000 mg/kg	129.5 ± 6.90*	4/7	57.1	42.9

Results are expressed as mean ± S.E.M. and as % mortality and protection (n=7). *$p < 0.05$ compared with control. One-way ANOVA followed by Duncan post test and Chi square test.

macerating with distilled water for 24 hours and then boiled for 15 minutes, allowed to cool and filtered. The extract was evaporated to dryness at a temperature of 40-45^{o}C. A yield of 3.90 g was obtained and kept at 4 ^{o} C prior to use.

Animals

Albino mice (20-25 g) and albino rats (60-80 g) of either sex were obtained from the animal house of the Department of Pharmacology, University of Jos, Nigeria.

Drugs

The drugs used were supplied from the stock of Department of Pharmacology laboratory, University of Jos, Nigeria and include; pentylenetetrazole (Sigma) and phenobarbitone (Merck).

PTZ-induced convulsion in rats

Six groups, each containing seven rats were used to test for the effect of aqueous extract on PTZ-induced seizures. They were treated as follow;

Group I (control): distilled water (0.5 ml p.o.).

Group II: phenobarbitone (30 mg/kg i.p.).

Groups III-VI: graded doses of aqueous extract (500, 1000, 1500, and 2000 mg/kg p.o.) was administered.

After a pretreatment time of 60 minutes, PTZ (85 mg/kg i.p.) was administered to the six groups of animals. The onset of convulsion, number of animals that convulsed and number of animals that were protected were recorded [8].

PTZ- induced convulsion in mice

A total of forty-two mice were divided into six groups of seven animals each. They were treated as follow;

Group I (control): distilled water (0.5 ml p.o.).

Group II: phenobarbitone (30 mg/kg i.p.).

Groups III-VI: graded doses of aqueous extract (500, 1000, 1500, and 2000 mg/kg p.o.) was administered.

After a pretreatment time of 60 minutes, PTZ (85 mg/kg i.p.) was administered to the six groups of animals. The onset of convulsion, number of animals that convulsed and number of animal that were protected were recorded [8].

Electrically–induced seizure in mice

Thirty-five male mice were allotted into five groups of seven animals each and treated.

Table 2. Effect of aqueous extract *of Croton zambesicus* on pentylenetetrazole-induced seizures in mice

Treatment	Onset of convulsion (seconds)	Number convulsed/ number used	Mortality (%)	Protection (%)
Distilled water	42.0 ± 0.85	7/7	100	0
Phenobarbibitone *(30 mg/kg)*	-	0/7	0	100
C. zambesicus extract				
500 mg/kg	47.9 ± 0.89	7/7	100	0
1000 mg/kg	120.5 ± 0.50	2/7	28.6	71.4*
1500 mg/kg	62.5 ± 2.50*	4/7	57.1	42.9
2000 mg/kg	62.5 ± 2.50*	4/7	57.1	42.9

Results are expressed as mean ± S.E.M. and as % mortality and protection (n=7). *$p < 0.05$ compared with control. One-way ANOVA followed by Duncan post test and Chi square test

Table 3. Effect of aqueous extract on maximal electroshock-induced seizures in mice

Treatment	Onset of convulsion (seconds)	Number convulsed/ number used	Mortality (%)	Protection (%)
Distilled water	9.86±0.67	7/7	100	0
Phenobarbitone (30 mg/kg)	-	0/7	-	100
C. zambesicus				
500 mg/kg	25.0±1.15*	3/7	42.9	57.1*
1000 mg/kg	39.0±4.36*	3/7	42.9	57.1*
1500 mg/kg	27.0±1.00*	2/7	28.6	71.4*

Results are expressed as mean ± S.E.M. and as % mortality and protection (n=7). *$p < 0.05$ compared with control. One-way ANOVA followed by Duncan post test and Chi square test.

Group I (control): distilled water (0.5 ml p.o.).

Group II: phenobarbitone (30 mg/kg i.p.).

Groups III-V: extract (500, 1000 and 1500 mg/kg p.o.).

After a pretreatment time of 60 minutes, a CFP stimulator (model 8048) was used to deliver a stimulus of 50 Hertz at 20 volts via ear electrodes to the different groups. The animals were observed for 2 minutes. The onset of tonic hind limb extension and number of animals protected was recorded [9].

Statistical analysis

The data are expressed as mean ± S.E.M. The data were statistically analyzed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range post test and Chi square test. Values of $p < 0.05$ were considered significant.

RESULTS

The effect of aqueous extract on PTZ-induced convulsion in rats

Intraperitoneal administration of PTZ induced tonic-clonic convulsions with 100% mortality in the control group. The aqueous extract (1000 and 1500 mg/kg p.o.) significantly ($p < 0.05$) increased the onset of convulsion in rats compared with the control group. Extract (1500 and 2000 mg/kg p.o.) offered 42.9% protection against PTZ- induced convulsion in rats (Table 1).

The effect of aqueous extract on PTZ-induced convulsion in mice

The extract (500-2000 mg/kg p.o.) significantly ($p < 0.05$) increased the threshold of PTZ-induced convulsion in mice compared with the control group. At 1000 mg/kg p.o., the extract produced significant protection (71.4%) against PTZ-induced convulsion in mice (Table 2).

The effect of aqueous extract on MES-induced convulsion in mice

The extract (500-1500 mg/kg p.o.) significantly ($p < 0.05$) increased the threshold of MES-induced convulsion in mice compared with the control group. At 1500 mg/kg p.o., the extract produced (71.4%) protection in mice (Table 3).

DISCUSSION

The aqueous extract of *Croton zambesicus* increased the threshold of PTZ-induced convulsion in rats and offered protection against PTZ-induced convulsion. The protection offered against PTZ-induced convulsion in mice (71.4%) was significant compared to that produced in rats (42.9%). Clonic seizures induced by PTZ are blocked by drugs that reduce T-type calcium currents (ethosuximide) and drugs that enhance inhibitory neurotransmission by GABAA receptors (benzodiazepine, phenobarbital and valproate) [10]. Convulsants whose actions previously were unexplained (including penicillin and PTZ) may act as relatively selective antagonist of the action of GABA [11,12]. The fact that the extract protected animal against PTZ-induced seizures may suggest that the plant extract contains compound(s) that facilitate GABAergic transmission. The extract also increased the threshold of seizures and offered protection in the MES test. It has been found empirically that drugs which inhibit PTZ-induced convulsions and raise the threshold for production of electrically-induced seizures are generally effective against absence seizures, whereas those that reduce the duration and spread of electrically-induced convulsions are effective in tonic-clonic seizures [13].

The anticonvulsant activity of the extract, are similar to those of linalool. The essential oil found in the leaves of *Croton zambesicus* contains p-cymene, linalool and beta-caryophyllene [6]. Psychopharmacological evaluation of linalool in mice revealed that this compound has dose-dependent marked sedative effects at the CNS, including protection against PTZ, picrotoxin ,quinolic acid and electroshock induced convulsions [14].

The results of this study show that the aqueous leaf extract of *Croton zambesicus* possess anticonvulsant properties which are possibly mediated partly via facilitation of GABA transmission. These results suggest that

the leaves of *Croton zambesicus* will be beneficial in the management of absence and tonic-clonic seizures.

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REFERENCES

1. Okokon JE, Ofodum KC, Ajibesin KK, Danladi B, Gamaniel KS. Pharmacolgical screening and evaluation of antiplasmodial activity of *Croton zambesicus* against Plasmodium berghei berghei infection in mice. Ind J Pharmacol 2005; 37:243-246.
2. Adjanohaun EJ, Ajakidje V, de Souza S. Contribution to Ethnobothanical and Floristic Studies in Benin Republic ,1989, Vol. 1. Agency for Cultural and Technical Cooperation.
3. Okokon JE, Bassey AL, Obot J. Antidiabetic activity of ethanolic leaf extract of *Croton zambesicus* muell.(Thunder plant) in Alloxan diabetic rats. African J Trad Complement Alt Med 2006; 3:21-6.
4. Block S, Stevigny C, de Pauw-Gillet MC, de Hoffman E, Llabres G, Ajakidje V. Ent-trachyloban-3β-ol, a New Cytotoxic Diterpene from Croton zambesicus. Planta Medica, 2002; 68:647-8.
5. Abo KA, Ogunleye, JS. Antimicrobial Potential of Spondias mombin *Croton zambesicus* and Zygotritonia crocea. Phytother Res 1999; 13:494-7.
6. Menut C, Lamaty G, Bessiere JM, Suleiman AM, Fendero P, Maidou E. Aromatic Plants of Tropical Central Africa. XXII. Volatile Constituents of Croton aubrevillei, J. Leonard and C. zambesicus Muell. Arg. J. Essen. Oil Res 1995; 7: 419-22.
7. Mekkawi AG. The essential oil of Croton zambesicus. Fitoterapia 1985; 56:181-3.
8. Williamson EM, Okpako DT, Evans FJ. Pharmacological Methods in Phytotherapy Research Vol. I, selection, preparation and pharmacological evaluation of plant material. 1996; 183-7.
9. Vogel HG, Vogel WH. (eds) Drug Discovery and Evaluation, Pharmacological Assays. 1997. Springer-Verlag, Berlin Heidelberg pp. 267-9.
10. White SH. The Epilepsies 2 (ed) Roger JP, David C. Butterworth-Heinemann 1997; 485-7.
11. Macdonald RL, Twyman RE, Ryan-Jastrow T, Angelotti TP. Regulation of GABAA receptor channels by anticonvulsant and convulsant drugs and by phosphorylation. Epilepsy Res Suppl 1992; 9:265-77.
12. Macdonald RL, Oslen RW. GABAA receptor channels. Annu Rev Neurosci 1994; 17:569- 602.
13. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology, 5th Edn, Churchill Livingstone Edinburgh, 2003; 552-3.
14. Elisabetsky E, Silva Brum LF, Souza DO. Anticonvulsant properties of linalool on glutamate related seizure models. Phytomedicine 1999; 6:113-9.

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