



Anticonvulsant Activity of Ethanol Extract of *Adenopus breviflorus* (Roberty) Fruit in Mice

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ABSTRACT

Adenopus breviflorus is a perennial climber used locally as an anticonvulsant, sedative and pain-killer in West Africa. Several studies have reported gastrointestinal, reproductive and anti-microbial effects of extracts of *Adenopus breviflorus*, but there is dearth of information on its anticonvulsant effect. This study was therefore designed to investigate the anticonvulsant effect of Ethanol Extract of *Adenopus breviflorus* (EEAB) in mice. Three hundred grams of air-dried *Adenopus breviflorus* fruits were cold macerated in 70% ethanol and concentrated using rotary evaporator. The method described by Lorke was used to determine the LD₅₀. The EEAB (250 – 2000 mg/kg, *p.o.*) was studied for its anticonvulsant effect on pentylenetetrazol (85 mg/kg)-, strychnine (2.0 mg/kg)- and picrotoxin (7.0 mg/kg)-induced seizures in mice. The mice were then observed for latency and duration of convulsions and monitored for mortality for 24 hours. Data were analyzed using descriptive statistics and ANOVA at $p=0.05$. The LD₅₀ of the crude extract was found to be 7000 mg/kg *p.o.* The EEAB (250-2000 mg/kg) caused insignificant ($p>0.05$) increase in the onset of convulsions relative to the control as well as significant ($p<0.05$) increase in death time relative to control in PTZ - induced convulsions. The EEAB (250-2000 mg/kg) caused significant ($p<0.05$) increases in the onset of convulsions and death time relative to the control in strychnine-induced convulsions. The EEAB (1000 mg/kg, 2000 mg/kg) induced significant ($p<0.05$) increase in the onset of convulsions relative to the control, while all the treatment doses of EEAB (250-2000 mg/kg) caused significant ($p<0.05$) increase in death time relative to the control in picrotoxin-induced convulsions. However, diazepam (2.0 mg/kg) inhibited the onset of convulsion with 0 % mortality in the three models. It can be concluded that ethanol extract of *Adenopus breviflorus* fruit probably possess anticonvulsant activities which could be mediated via GABAergic and glycine systems.

Keywords: *Adenopus breviflorus*, pentylenetetrazol, strychnine, anticonvulsant, mice.

INTRODUCTION

Epilepsy is a major neurological disorder and up to 5% of the world population develops epilepsy in their lifetime¹. The current therapy of epilepsy with modern antiepileptic drugs is associated with side effects, dose-related and chronic toxicity, as well as teratogenic effects, and approximately 30% of the patients continue to have seizures with current antiepileptic drugs therapy².

Global economic depression as a result of fall in the price of crude oil in the international market with the attendant depreciation in currency values of most African countries relative to the U.S. Dollars coupled with the expensiveness and toxic side effects of most synthetic drugs; reversal to the use of the cheaper and more efficacious herbal remedies cannot be overemphasized by Africans, most of whom are bedeviled by poverty³. Several plants with neuroactive activities have shown efficacies when tested in modern bioassays for the detection of anticonvulsant activities and one of such plant is *Adenopus breviflorus*.

Adenopus breviflorus belongs to the family of Cucurbitaceae. It is commonly called Wild colocynth in English language, "Ogbenwa" in Ibo language and "Tagiri" in Yoruba language⁴. It is a perennial tendril climber. It would usually lie on the ground for want of something to climb and climbs over shrubs and herbs by means of

axillary tendrils. The leaves are simple, alternate and palmately veined⁵.

Medicinally, the plant is used as a purgative in Tanganyika as well as a vermifuge and cathartic in Nigeria⁴. A decoction from the plant is said to be used in Nigeria for headache⁴. It is used in West Africa for a wide range of gastrointestinal disorders and measles in man. In southern Nigeria, its seed-decoction is reportedly given to pregnant women but the purpose is not stated⁶. It is used as an anticonvulsant, sedative and pain killer⁷. It is used with other medicinal plants as concoctions to aid parturition in humans⁸. Livestock farmers employ the fruit extract of the plant for the treatment of Newcastle disease and coccidiosis in animals⁸. The fruit is also used for money-making charms by the Yoruba herbalists of South-West Nigeria because of the cowrie-like inscriptions on its body.

Pharmacologically, it has been reported that the methanol extract of its whole fruit has anti-implantation activity⁹ and abortifacient activity¹⁰. The ethanol extract of its whole fruit has been reported to have a broad spectrum antibacterial activity¹¹ as well as anti-oxidant and anti-ulcerogenic effects¹². Its ethanol extract has been reported to have a little toxic and a lot of beneficial effects on the hematological functions and blood chemistry of male Wistar rats¹³.



Since this plant has been reported to be used medicinally as an anticonvulsant⁷, this study aims to scientifically authenticate the veracity of this claim.

MATERIALS AND METHODS

Experimental Animals

Adult male mice weighing between 20-25 g bred in the Pre-Clinical Animal House of the College of Medicine, University of Ibadan were used.

They were housed under standard laboratory conditions and had free access to feed (Ladokun Feeds Limited, Ibadan, Nigeria) and water; they were acclimatized for two weeks to laboratory conditions before the commencement of the experiments.

All experiments were carried out in compliance with the recommendations of Helsinki's declaration on guiding principles on care and use of animals.

Plant Material

Fresh samples of *Adenopus breviflorus* fruit were bought in Bodija Market, Ibadan, and were authenticated in the Taxonomy Unit of the Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan.

Preparation of Crude Ethanol Extract

Large quantity (7.5 kg) of fresh specimens of the whole fruit of *Adenopus breviflorus* were washed free of debris and pulverized using mortar and pestle and air-dried for eight weeks.

The resultant dried specimens (300 g) were macerated and extracted with 70 % ethanol for 72 hours at room temperature (26 - 28 °C).

The resulting solution was then filtered using a wire-gauze and a sieve with tiny pores (0.25 mm). The 70 % ethanol was later evaporated using steam bath (40 – 45 °C) to give a percentage yield of 8.6 % of the starting sample.

The dried sample was reconstituted in distilled water to make up test solutions of known concentration.

Toxicity test

The method described by¹⁴ was used to determine the LD₅₀, which is the index of acute toxicity. Male albino mice (20-25 g) were used.

This method involved an initial dose finding procedure, in which the animals were divided into three groups of three animals per group. Doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg were administered orally, one dose for each group. The treated animals were monitored for twenty-four hours for mortality and general behaviour.

From the results of the above step, seven different doses (2000 mg/kg, 3000 mg/kg, 4000 mg/kg, 5000 mg/kg, 6000 mg/kg, 7000 mg/kg, 8000 mg/kg) were chosen and administered orally to seven groups of animals of one mouse per group respectively.

The treated animals were monitored for twenty-four hours. The LD₅₀ was then calculated as the geometric mean of the lowest dose showing death and the highest dose showing no death.

Preparation of Stock Solution of EEAB

Ten grams of EEAB were dissolved in 100 mL of distilled water to give a concentration of 0.1 g/mL.

The dosages of EEAB administered in these studies were obtained from the results of the acute toxicity test.

Screening of Anticonvulsant Activity

Effect on pentylenetetrazol (PTZ)-induced convulsions

Pentylenetetrazol (PTZ) at 85 mg/kg (i.p.) was used to induce clonic-tonic convulsions in mice according to¹⁵. Forty-eight mice were randomly divided into six groups (n=8). Group I was given distilled water (0.2 mL/20 g, p.o.), groups II – V were given EEAB (250 – 2000 mg/kg, p.o.), while group VI was given diazepam (2.0 mg/kg, i.p.).

Pentylenetetrazol (85 mg/kg) was administered i.p. to the control and extract treated groups after one hour and to the diazepam treated group after 30 minutes. The mice were then observed for latency and duration of convulsions and monitored for mortality for 24 hours.

Effect on strychnine-induced convulsions

The method described by¹⁶ was used. Forty-eight mice were randomly divided into six groups (n=8). Group I was given distilled water (0.2 mL/20 g, p.o.), groups II – V were given EEAB (250 – 2000 mg/kg, p.o.), while group VI was given diazepam (2.0 mg/kg, i.p.).

Strychnine (2.0 mg/kg) was administered i.p. to the control and extract treated groups after one hour and to the diazepam treated group after 30 minutes. The mice were then observed for latency and duration of convulsions and monitored for mortality for 24 hours.

Effect on picrotoxin-induced convulsions

For this model,¹⁷ method was employed. Forty-eight mice were randomly divided into six groups (n=8). Group I was given distilled water (0.2 mL/20 g, p.o.), groups II – V were given EEAB (250 – 2000 mg/kg, p.o.), while group VI was given diazepam (2.0 mg/kg, i.p.).

Picrotoxin (7.0 mg/kg) was administered i.p. to the control and extract treated groups after one hour and to the diazepam treated group after 30 minutes. The mice were then observed for latency and duration of convulsions and monitored for mortality for 24 hours.

Statistical Analysis

The mean and standard error of mean (S.E.M) were calculated for all values. Comparison between the control and experimental groups was done using one – way analysis of variance (ANOVA) with Duncan's Multiple Range Test. Differences were considered statistically significant at p<0.05.



RESULTS

The LD₅₀ of the crude extract was found to be 7000 mg/kg p.o.

Treatment of mice with all the treatment doses of EEAB (250-2000 mg/kg) caused insignificant ($p > 0.05$) increase in the onset of convulsions relative to the control as well as significant ($p < 0.05$) increase in death time relative to control with 100 % mortality in PTZ - induced convulsions, while diazepam (2.0 mg/kg) inhibited the onset of convulsion with 0 % mortality (Table 1).

Treatment of mice with all the treatment doses of EEAB (250-2000 mg/kg) caused significant ($p < 0.05$) increases in

the onset of convulsions and death time relative to the control with 100 % mortality in strychnine-induced convulsions, while diazepam (2.0 mg/kg) inhibited the onset of convulsion with 0 % mortality (Table 2).

Treatment of mice with EEAB (1000 mg/kg, 2000 mg/kg) induced significant ($p < 0.05$) increase in the onset of convulsions relative to the control, while all the treatment doses of EEAB (250-2000 mg/kg) caused significant ($p < 0.05$) increase in death time relative to the control with 100 % mortality in picrotoxin-induced convulsions. Diazepam (2.0 mg/kg) inhibited the onset of convulsion with 0 % mortality (Table 3).

Table 1: Effect of EEAB on Pentylentetrazol (PTZ)-induced convulsions in mice

Treatment	Dose (mg/kg)	Onset of convulsion (sec)	Death time(sec)	% Protection	% Mortality
Control	0.2ml/20g	44.80 ± 2.95	124.56 ± 6.46	0.0	100.0
EEAB	250	48.20 ± 2.20	188.28 ± 8.53*	0.0	100.0
EEAB	500	49.40 ± 3.95	217.20 ± 883*	0.0	100.0
EEAB	1000	50.60 ± 2.18	261.60 ± 9.32*	0.0	100.0
EEAB	2000	51.60 ± 1.17	268.92 ± 9.83*	0.0	100.0
Diazepam	2.0	-	-	100	0.0

The results are expressed as mean ± S.E.M. (n=8). One way ANOVA revealed significant difference between various treatment groups.* Indicates significant difference from control. * $p < 0.05$.

Table 2: Effect of EEAB on strychnine-induced convulsions in mice

Treatment	Dose (mg/kg)	Onset of convulsion (sec)	Death time(sec)	% protection of convulsion
Control	0.2ml/20g	129.00 ± 5.83	137.40 ± 5.96	0.0
EEAB	250	138.00 ± 5.96	181.20 ± 7.02*	0.0
EEAB	500	163.80 ± 6.24*	222.60 ± 7.86*	0.0
EEAB	1000	175.20 ± 6.93*	252.00 ± 8.43*	0.0
EEAB	2000	211.20 ± 7.54*	258.60 ± 9.53*	0.0
Diazepam	2.0	-	-	100.0

The results are expressed as mean ± S.E.M. (n=8). One way ANOVA revealed significant difference between various treatment groups.* Indicates significant difference from control. * $p < 0.05$.

Table 3: Effect of EEAB on picrotoxin-induced convulsions in mice

Treatment	Dose (mg/kg)	Onset of convulsion(sec)	Death time (sec)	% Protection	% Mortality
Control	0.2ml/20g	307.80 ± 12.03	727.20 ± 14.30	0.0	100.0
EEAB	250	310.20 ± 12.21	775.80 ± 15.93*	0.0	100.0
EEAB	500	337.20 ± 12.48	867.60 ± 16.64*	0.0	100.0
EEAB	1000	374.40 ± 13.11*	1005.00 ± 17.62*	0.0	100.0
EEAB	2000	766.80 ± 14.72*	1308.60* ± 18.22*	0.0	100.0
Diazepam	2.0	-	-	100.0	0.0

The results are expressed as mean ± S.E.M. (n=8). One way ANOVA revealed significant difference between various treatment groups.* Indicates significant difference from control. * $p < 0.05$.

DISCUSSION

Acute toxicity test gives clues on the range of doses that could be toxic to the animal; it could also be used to estimate the therapeutic index (LD₅₀/ED₅₀) of drugs and xenobiotics¹⁸. LD₅₀ is the dose at which mortality occurs in

50% population of the experimental animals. The higher the value of the LD₅₀ for a substance, the relatively safer the substance is assumed to be. The LD₅₀ determination for the extract in mice via the oral route was 7000 mg/kg, which was not toxic to the animals, and since the recommended single high dose by OECD guidelines 423¹⁹



for testing acute toxicity is 2000 mg/kg BW; this probably indicates the extract has wide safety margins (low toxicity). Similar result was reported by²⁰ in *Eichhornia crassipes* extract treated mice.

Currently available anticonvulsant drugs are able to efficiently control epileptic seizures in about 50% of the patients, another 25% may show improvement whereas the remainder does not benefit significantly. Furthermore, undesirable side effects from the drugs used clinically often render treatment difficult so that a demand for new types of anticonvulsants exists. One of the approaches to search for new antiepileptic drugs is the investigation of naturally occurring compounds, which may belong to new structural classes²¹. The anticonvulsant potential of a drug is not only determined by its ability to prevent convulsion and mortality, but also by its ability to delay the onset of convulsion, shorten the frequency and duration of tonic – clonic seizure²².

Pentylenetetrazol (PTZ) – induced convulsions represent the petit – mal type of seizures and this has been primarily utilized as animal model to evaluate antiepileptic drugs. PTZ is known to block the postsynaptic GABA_A receptor mediated Cl⁻ conductance and thus produces seizures²³. The extract caused increase in the onset of convulsions as well as death time in PTZ – induced convulsions, this suggests that its anticonvulsant activity could be mediated through the GABAergic modulation. GABA is an important endogenous inhibitory neurotransmitter widely distributed throughout the central nervous system. A reduction in GABA function in the brain is associated with psychiatric and neurological disorders, including anxiety, depression, insomnia, and epilepsy²⁴. Numerous natural and synthetic compounds interact with GABA_A receptor at distinct, yet incompletely defined sites²⁵. These compounds include barbiturates, benzodiazepines, neurosteroids and picrotoxin²⁶. The postsynaptic GABA_A receptors are implicated in the inhibitory mechanisms. GABA_A receptor agonists as well as drugs, which allosterically modulate the GABA_A receptor channel complex, are therapeutically effective anticonvulsant agents²⁷. Similar result was reported by²⁸ in *Valeriana officinalis* extract treated mice.

In the strychnine – induced seizure model, it is known that strychnine a potent spinal cord convulsant, blocks glycine receptor selectively to induce excitatory response in the CNS²⁹. The extract caused increase in the onset of convulsion and death time in strychnine convulsions, this suggests that its anticonvulsant activity could be through suppression of the action of strychnine on glycine inhibitory mechanism or through the enhancement of glycine inhibitory mechanism. Similar result was reported by³⁰ in *Benincasa hispida* fruit extract treated mice.

Picrotoxin, a potent selective GABA_A receptor antagonist produces seizures by blocking the effect of GABA at central GABA_A receptors which have been associated with epilepsy³¹. Postsynaptic GABA_A receptors are functionally linked to benzodiazepine receptors, barbiturate receptors

and chloride ion channels to form GABA – chloride ionophore complex, which is intimately involved in the modulation of GABAergic neurotransmission epilepsy³². The extract induced increase in the onset of convulsion and death time in picrotoxin – induced convulsions which suggests that its anticonvulsant activity could be via the enhancement of GABAergic neurotransmission by increasing chloride ion flux through the chloride ion channels at GABA_A receptor sites. It has been reported that 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 brain³¹. Similar result was reported³³ in *Vitex negundo* extract treated mice.

It can be concluded that *Adenopus breviflorus* fruit probably possess an anticonvulsant effect which provides scientific basis to the folkloric claim of the plant in the management of convulsion and its anticonvulsant activity could be mediated via GABAergic and glycine systems.

Recommendations

The folkloric claim of *Adenopus breviflorus* as an anticonvulsant has been explored scientifically in animal models in this study. Hence, it is recommended that people suffering from convulsion may use the extract of *Adenopus breviflorus* fruit in the nearest future after isolation and characterization of the active component(s) and clinical trials.

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