Research Article

Anxiogenic Effect of Ethanol Extract of *Adenopus breviflorus* (Roberty) Fruit in Mice

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ABSTRACT

Adenopus breviflorus is a perennial climber used locally for its wide range of neuroactive activities in West Africa. Several studies have reported the gastrointestinal, reproductive and anti-microbial effects of extracts of *Adenopus breviflorus*, but there is dearth of information on its anxiety expression effect. This study was therefore designed to investigate the anxiety expression 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 LD_{50}. The EEAB (62.5 – 2000 mg/kg, *p.o.*) was studied for its anxiety expression effect in mice using the elevated plus maze and hole board tests. The mechanism of action was studied using the following receptor blockers: atropine, cyproheptadine, haloperidol, naloxone, propranolol and yohimbine. Data were analyzed using descriptive statistics and ANOVA at p=0.05. The LD_{50} of the crude extract was found to be 7000 mg/kg *p.o.* All the doses of EEAB induced significant ($p<0.05$) increase in time spent in the closed arms relative to the open arms. All the doses of EEAB (62.5-2000 mg/kg) caused significant ($p<0.05$) reductions in head dips relative to the control. Pretreatment of mice with atropine, cyproheptadine, haloperidol, naloxone, propranolol and yohimbine did not reverse the decrease in head dips induced by EEAB (2000 mg/kg) relative to the control. It can be concluded that *Adenopus breviflorus* fruit extract may possess an anxiogenic effect which probably was not be mediated via muscarinic, 5-HT, dopaminergic, μ-opoid, β and α_2-adrenergic receptors.

Keywords: *Adenopus breviflorus,* Anxiogenic, Elevated plus maze, Hole board, Mice.

INTRODUCTION

Being anxious throughout life has implications not just for subjective wellbeing, but also for physical health and longevity[1]. Anxiety is an unpleasant state of inner turmoil, often accompanied by nervous behavior, somatic complaints and rumination[2], when anxiety becomes excessive, it may be considered as an anxiety disorder, and can critically decrease the quality of life inducing several psychosomatic diseases.

The clinical use of a large number of medicines such as those employed in the treatment of the central nervous system (CNS) disorders is generally limited by the occurrence of undesirable side effects. Therefore, the search and development of new medications possessing effective therapeutic properties without adverse effects on the organism would be of great interest in the scientific community worldwide. Medicinal plants are a well-recognized source of substances with potential to provide alternative remedies for our ailments. However, a proper evaluation of their actual efficacy in the treatment of diseases or the occurrence of unwanted effects must be addressed. For instance, accumulating pharmacological data indicate that certain substances present in plants are capable of interfering with the normal functioning of the CNS, producing psychomotor impairment or depressant activity[3,4].

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 language[5]. 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 veined[6].

Medicinally, the plant is used as a purgative in Tanganyika as well as a vermifuge and cathartic in Nigeria[5]. A decoction from the plant is said to be used in Nigeria for headache[5]. 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 stated[7]. It is used as an anticonvulsant, sedative and pain killer[8]. It is used with other medicinal plants as concoctions to aid parturition in humans[9]. Livestock farmers employ the fruit extract of the plant for the treatment of Newcastle disease and coccidiosis in animals[9]. 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 activity[10] and abortifacient activity[11]. The ethanol extract of its whole fruit has been reported to have a broad spectrum antibacterial activity[12] as well as anti-oxidant and anti-ulcerogenic effects[13]. Its ethanol extract has been reported to have a little toxic effect and a lot of

beneficial effects on the hematological functions and blood chemistry of male Wistar rats[14].

Since this plant has been reported to have a wide range of neuroactive activities[8,5], this study therefore aims to investigate the anxiogenic/anxiolytic effect of ethanol extract of *Adenopus breviflorus* fruits in male mice.

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.

Drugs and Chemicals

The following drugs and chemicals were used: Atropine (Griffin, England), Cyproheptadine (Greenfield Pharm. Ltd), Diazepam (Martindale Pharma®, U.K), Haloperidol (Abbott Laboratories), Naloxone (BDH, England), Propranolol (Sigma), Yohimbine (Sigma).

Toxicity test

The method described by[15] was used to determine the LD_{50}, 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 doses 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) where 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 LD_{50} 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.

Anxiogenic/Anxiolytic Tests

(a) **Elevated plus maze test**

The elevated plus maze (EPM) test was used to evaluate the animal anxiety[16,17]. The EPM for mice consisted of two open arms (30 cm x 5 cm) and two closed arms (30 cm x 5 cm x 15 cm) that extended from a common central platform (5 cm x 5 cm) with an open roof, arranged such that the two arms of each type were opposite to each other.

The floor and the walls of each arm were wooden and painted white. The maze was elevated to a height of 38.5cm above the floor level. Testing was conducted in a quiet room that was illuminated by light.

Each animal was placed in the center of the EPM facing one of the open arms. An entry into an arm was defined as the animal placing all four paws over the line marking that area. The number of entries and time spent in the open and closed arms were recorded during a 5 minutes test period. The percentages of open arm entries (100 x open/total entries) were calculated for each animal. Sixty-four mice were randomly divided into eight groups (n=8). Group I was given distilled water (0.2 mL/20 g, *p.o.*), groups II – VII were given EEAB (62.5 – 2000 mg/kg, *p.o.*), while group VIII was given diazepam (2.0 mg/kg, i.p.).

One hour after treatment with the extract, each mouse was placed in the central square of the maze facing an open arm and its behaviours were recorded for 5 minutes.

The frequency of each of the following behaviours was scored and the duration of each behaviour was recoded:

i. Open arm entries.

ii. Closed arm entries.

iii. Time spent in open arms.

iv. Time spent in closed arms.

The index of open arm avoidance which is interpreted as level of anxiety[16] is calculated as:

$$100 - \left(\frac{\%\ time\ spent\ in\ open\ arms + \%\ entries\ into\ open\ arms}{2}\right)$$

(b) Head dips (Hole board) test

The effect of the extract on the rate of head dipping was determined in the hole board which is made up of a number of holes (usually 16) through which the animal can poke its head.

Sixty-four mice were randomly divided into eight groups (n=8). Group I was given distilled water (0.2 mL/20 g, *p.o.*), groups II – VII were given EEAB (62.5 – 2000 mg/kg, *p.o.*), while group VIII was given diazepam (2.0 mg/kg, i.p.).

One hour after treatment with the extract, each mouse was placed on the hole board and the number of times that each animal dipped (poked) its head into the holes in 5 minutes were counted[18].

The hole board was cleaned with 70 % ethanol at intervals when each animal was removed.

Mechanism of action

In another set of experiments, mice were pretreated i.p. for 15 minutes with neurotransmitter blockers to evaluate the mode of action of the extract on the rate of head dipping.

The following receptor blockers were used: atropine (muscarinic blocker, 0.5 mg/kg), cyproheptadine (5-HT blocker, 0.5 mg/kg), haloperidol (dopaminergic blocker, 0.2 mg/kg), naloxone (μ-opoid antagonist, 2 mg/kg), propranolol (β-adrenergic blocker, 0.2 mg/kg) and yohimbine (α_2-adrenergic blocker, 1.0 mg/kg).

The doses administered are the doses that have been found not to induce behavioural effects of their own in experimental animals and as such they only block the receptors involved.

The mice were then pretreated for another 30 minutes with maximal dose of the extract (2000 mg/kg).

The animals were observed for head dipping responses as previously explained.

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 LD_{50} of the crude extract was found to be 7000 mg/kg p.o.

Treatment of mice with all the treatment doses of EEAB (62.5-2000 mg/kg) did not alter the frequency of open arm entries relative to the control, but diazepam caused a significant ($p<0.05$) increase in the frequency of open arm entries relative to the control.

The extract at all the treatment doses induced significant ($p<0.05$) increase in time spent in the closed arms relative to the open arms, while diazepam (1.0 mg/kg) induced a significant ($p<0.05$) increase in time spent in the open arms relative to the closed arms. The extract at all the treatment doses and the control produced significantly higher index of open arm avoidance when compared to diazepam with a significantly lower index of open arm avoidance (Table 1).

Treatment of mice with all the treatment doses of EEAB (62.5 mg/kg, 125 mg/kg, 250 mg/kg, 500 mg/kg, 1000 mg/kg, 2000 mg/kg) and diazepam (2.0 mg/kg) caused significant ($p<0.05$) reductions in head dips relative to the control (Figure 1).

Pretreatment of mice with atropine, cyproheptadine, haloperidol, naloxone, propranolol and yohimbine did not reverse the decrease in head dips induced by EEAB (2000 mg/kg) relative to the control (Table 2).

Table 1: Effect of EEAB on frequency of arm entries and time spent in the arms of an elevated plus maze

Treatment	Dose (mg/kg)	No of entries into arms		Time spent in each arm (sec)		% Time spent in open arms	Index of open arm avoidance
		Open	Close	Open	Close		
Control	0.2ml/20g	4.80 ± 0.22	7.60 ± 0.76	98.40 ± 3.57	223.40 ± 5.76	30.6	65.4
EEAB	62.5	4.20 ± 0.44	6.60 ± 0.68	76.60 ± 2.68	167.60 ± 4.73*	31.4	64.9
EEAB	125	4.80 ± 0.34	6.40 ± 0.71	87.80 ± 3.92	160.20 ± 3.20*	35.4	60.9
EEAB	250	4.80 ± 0.43	6.20 ± 0.68	84.80 ± 3.69	152.60 ± 4.82*	35.7	60.3
EEAB	500	5.00 ± 0.43	6.20 ± 0.45	105.00 ± 3.82	144.80 ± 4.73*	42.0	56.7
EEAB	1000	5.00 ± 0.47	6.20 ± 0.52	106.60 ± 4.32	126.20 ± 4.66*	45.8	54.8
EEAB	2000	5.00 ± 0.45	6.20 ± 0.34	121.8 ± 4.76	187.20 ± 4.21	39.4	58.0
Diazepam	1.0	7.80 ± 0.71*	3.20 ± 0.26	183.40 ± 5.75*	97.80 ± 3.21*	65.2*	32.0*

The results are expresses as mean ± S.E.M. (n=8). One way ANOVA revealed significant difference [$F(7, 32) = 4.616$, $p<0.05$] between various treatment groups. *Indicates significant difference from control. *$p<0.05$.

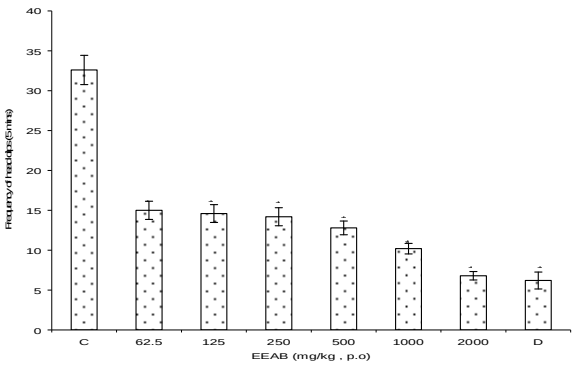

Figure 1: Effect of EEAB on head dips in mice

C: Control, D: Diazepam (2.0 mg/kg, i.p.)

Bars are mean values ± S.E.M. (n=8). One way ANOVA revealed significant difference [F (7, 32) = 40.561, p<0.05] between various treatment groups. *Indicates significant difference from control. p<0.05.

Table 2: Effect of EEAB on head dips in presence of antagonists

Treatment	Dose (mg/kg)	HD/ 5 min
Control	0.2ml/20g	32.60 ± 1.83
EEAB	2000	6.80 ± 0.53*
Atropine	0.5	20.20 ± 1.20*
Atropine +EEAB		7.40 ± 0.57*
Cyproheptadine	0.5	10.80 ± 0.63*
Cyprohetadine + EEAB		3.40 ± 0.60*
Haloperidol	0.2	10.00 ± 0.79*
Haloperidol + EEAB		4.00 ± 0.53*
Naloxone	2.0	10.20 ± 0.37*
Naloxone + EEAB		4.20 ± 0.53*
Propranolol	0.2	9.80 ± 0.39*
Propranolol + EEAB		9.80 ± 0.55*
Yohimbine	1.0	9.80 ± 0.42*
Yohimbine + EEAB		8.20 ± 0.37*

The results are expresses 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; HD: Head dips

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 (LD_{50}/ED_{50}) of drugs and xenobiotics[19]. LD_{50} is the dose at which mortality occurs in 50% population of the experimental animals. The higher the value of the LD_{50} for a substance, the relatively safer the substance is assumed to be. The LD_{50} 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 423[20] for testing acute toxicity is 2000 mg/kg BW; this probably indicates the extract has wide safety margins (low toxicity). Similar result was reported by[21] in *Eichhornia crassipes* extract treated mice.

Anxiety, a state of fear, is characterized by motor tension, sympathetic hyperactivity, apprehension and vigilance syndromes[22]. Anxiety may interfere with intelligence, psychomotor function and memory[23]. Among the models of anxiety disorders that are used in determining anxiolytic or anxiogenic properties of substances is elevated plus maze. The elevated plus maze (EPM) represent one of the most widely used animal models for screening anxiolytic and anxiogenic drugs[17]. This test is able to reproduce anxiolytic or anxiogenic effects in rodents such that anxiolytics produce increase in the time spent in the open arm of the elevated plus maze, while anxiogenic substances produce the opposite effect[16]. In addition, the number of the enclosed entries has been used as a parameter reflecting general motor activity. The extract induced reductions in the number of entries into open arm and time spent in the open arm as well as induced significantly higher index of open arm avoidance when compared to diazepam with a significantly lower index of open arm avoidance which probably indicates that the extract has an anxiogenic effect. This anxiogenic property validates the CNS depressant property of the extract. Similar result was reported by[24] in *Tabernaemontana solanifolia* extracts treated rats.

The anxiogenic activity of the extract can be confirmed by the hole board test. The hole board is a method used to measure the animal's response to a novel environment and to assess emotionality, anxiety and/or responses to stress[25]. In this test, head dipping behaviour may change in response to the emotional state of the animal and an increase in this behaviour could reflect the expression of an anxiolytic reaction of the animal[26]. On the other hand, a decrease in the number of head dipping reveals a sedative or depressant behaviour[27,28]. Results from earlier studies have shown that changes in head-dipping behavior reflect the anxiogenic and/or anxiolytic state of animals-head-dipping is decreased in the anxiogenic state and increased in an anxiolytic state[29,26]. Since the extract caused decrease in head dips, this probably indicates that the extract possess an anxiogenic, sedative or CNS depressant property. Similar result was reported by[30] in *Dracocephalum moldavica* extract treated mice.

It can be concluded that *Adenopus breviflorus* fruit may possess an anxiogenic effect which provides scientific basis to the folkloric claim of the plant as a sedative agent and its anxiogenic activity could not be mediated via muscarinic, 5-HT, dopaminergic, μ-opoid, β and α_2-adrenergic receptors.

Recommendations

The forkloric claim of *Adenopus breviflorus* as a sedative has been explored scientifically in animal models in this study. Hence, it is recommended that people suffering from emotional disturbances 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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