Research Article

Sedative-Hypnotic and Skeletal Muscle Activities 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 sedative-hypnotic effect. This study was therefore designed to investigate the sedative-hypnotic and skeletal muscle 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 (250 – 2000 mg/kg, *p.o.*) was studied for its hypnotic-sedative effect by monitoring the pentobarbital-induced sleeping time and sleep latency in mice, EEAB effect was also studied on rectal body temperature and skeletal muscle. 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 doses of EEAB (250-2000 mg/kg BW) caused significant ($p<0.05$) reductions in sleep latency as well as significant ($p<0.05$) increase in sleeping time relative to their respective controls. All doses of EEAB (250-2000 mg/kg) did not produce significant ($p>0.05$) changes in rectal body temperature relative to the control. All doses of EEAB (250-2000 mg/kg) did not produce significant changes ($p>0.05$) in muscle coordination activity relative to the control. It can be concluded that *Adenopus breviflorus* fruit extract may possess a sedative-hypnotic effect which could be mediated via interaction with the GABAergic system.

Keywords: *Adenopus breviflorus,* Sedative, Hypnotic, Skeletal muscle, Mice.

INTRODUCTION

Insomnia is an extremely common symptom both de novo and in the context of other medical and psychiatric disorders. It is estimated that more than 27% people in the world suffer from insomnia with difficulty in initiating or maintaining sleep and this figure is expected to grow by the middle of the 21st century and about 3–10% of all people are chronic and frequent users of hypnotics[1-3]. However, it is well known that the most extensively used benzodiazepines show many unpleasant reactions, such as drug dependence, tolerance, rebound insomnia and amnesia. The new type of hypnotics, such as zolpidem, zolpiclone etc, also showed some extent of side effects[4,5].

Sedatives are drugs that decrease activity and have a calming, relaxing effect. At higher doses, sedatives usually cause sleep. Drugs used mainly to cause sleep are called hypnotics. The difference between sedatives and hypnotics, then, is usually the amount of the dose; lower doses have a calming effect and higher doses cause sleep[6]. Recent studies have shown that herbal drugs exert good sedative and hypnotic effect on the central nervous system[7].

A muscle relaxant is a drug which affects skeletal muscle function and decreases the muscle tone. It may be used to alleviate symptoms such as muscle spasms, pain and hyperreflexia.

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[8]. 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[9].

Medicinally, the plant is used as a purgative in Tanganyika as well as a vermifuge and cathartic in Nigeria[8]. A decoction from the plant is said to be used in Nigeria for headache[8]. 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[10]. It is used as an anticonvulsant, sedative and pain killer[11]. It is used with other medicinal plants as concoctions to aid parturition in humans[12]. Livestock farmers employ the fruit extract of the plant for the treatment of Newcastle disease and coccidiosis in animals[12]. 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[13] and abortifacient activity[14]. The ethanol extract of its whole fruit has been reported to have a broad spectrum antibacterial activity[15] as well as anti-oxidant and anti-ulcerogenic effects[16]. Its ethanol extract has been reported to have a little toxic effect and a lot of

beneficial potentialities on the hematological functions and blood chemistry of male Wistar rats[17].

Since this plant has been reported to be used medicinally as a sedative[11], 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 $^{\circ}$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 $^{\circ}$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: Pentobarbital sodium (Sigma), Diazepam (Martindale Pharma®, U.K).

Toxicity test

The method described by[18] 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.

Pentobarbital-induced Sleeping Time and Sleep latency

The effect of extract on pentobarbital-induced sleeping time and sleep latency in mice was measured as described by[19].

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.).

This was followed one hour later by i.p. administration of pentobarbital sodium (40 mg/kg).

The sleep latency and sleeping time were recorded.

The sleep latency was measured as time in minutes after treatment with pentobarbital sodium and the loss of right reflex.

While the time in minutes between losses and regaining of righting reflex was taken as sleeping time.

Effect on Rectal Body Temperature

The recording of the rectal body temperature was carried out using a thermoprobe inserted 1.5 cm into the rectum of each mouse.

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.).

The temperatures of the animals were recorded immediately before treatment (0 minute) and 30, 60, 90, 120 and 180 minutes after treatment.

The pre-treatment results served as the reference point for the determination of temperature changes[20].

Skeletal Muscle Relaxant Activity (Traction Test)

The ability of a mouse hanging with its fore paws on a small twisted wire rigidly supported above the bench top and placing at least one hind foot on the wire within 5 seconds was determined[21].

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.).

One hour after treatment, each animal was suspended by means of their fore paws and the time of holding the wire was recorded. The number of animals in each group that could not touch the wire with their hind paws within 5 seconds after placement was also recorded.

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 (250-2000 mg/kg) and diazepam (2.0 mg/kg) caused significant ($p<0.05$) reductions in sleep latency relative to the control (Figure 1) as well as significant ($p<0.05$) increase in sleeping time relative to the control (Figure 2).

Treatment of mice with all the treatment doses of EEAB (250-2000 mg/kg) did not produce significant ($p>0.05$) changes in rectal body temperature relative to the control, while diazepam (2.0 mg/kg) induced significant ($p<0.05$) reduction in rectal boy temperature relative to the control (Table 1).

Treatment of mice with all the treatment doses of EEAB (250-2000 mg/kg) did not produce significant changes ($p>0.05$) in muscle coordination activity relative to the control, while diazepam (4.0 mg/kg) caused significant ($p<0.05$) decrease in muscle coordination activity relative to the control (Figure 3).

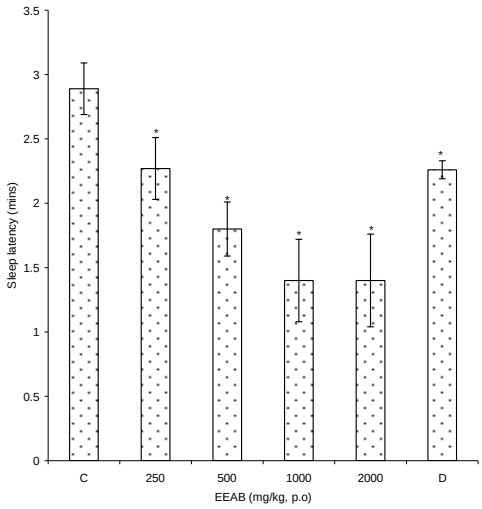

Figure 1: Effect of EEAB on pentobarbital – induced sleep latency in mice

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 between various treatment groups. *Indicates significant difference from control. $p<0.05$.

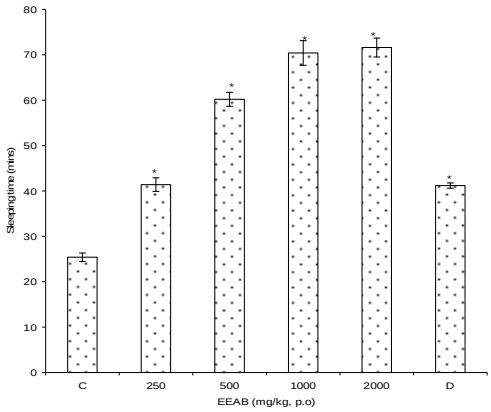

Figure 2: Effect of EEAB on pentobarbital – induced sleeping time in mice

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 between various treatment groups. *Indicates significant difference from control. $p<0.05$.

Table 1: Effect of EEAB on rectal body temperature in mice

Treatment	Dose (mg/kg)	0 min	30 min	60 min	90 min	120 min	180 min
Control	0.2ml/20g	37.64 ± 0.24	37.34 ± 0.13	37.24 ± 0.12	37.34 ± 0.20	37.38 ± 0.10	37.32 ± 0.21
EEAB	250	37.46 ± 0.45	36.82 ± 0.07	37.26 ± 0.21	37.06 ± 0.11	37.18 ± 0.22	37.10 ± 0.11
EEAB	500	37.12 ± 0.28	37.00 ± 0.83	37.34 ± 0.25	37.08 ± 0.15	37.42 ± 0.08	37.62 ± 0.24
EEAB	1000	37.36 ± 0.43	37.16 ± 0.11	37.34 ± 0.21	37.38 ± 0.23	37.42 ± 0.19	37.16 ± 0.13
EEAB	2000	37.30 ± 0.24	37.34 ± 0.22	37.60 ± 0.25	37.42 ± 0.15	37.42 ± 0.15	37.44 ± 2.0
Diazepam	2.0	37.62 ± 0.11	35.40 ± 0.55*	35.54 ± 0.15*	35.50 ± 0.14*	35.34 ± 0.05*	35.56 ± 0.17*

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. *$p<0.05$.

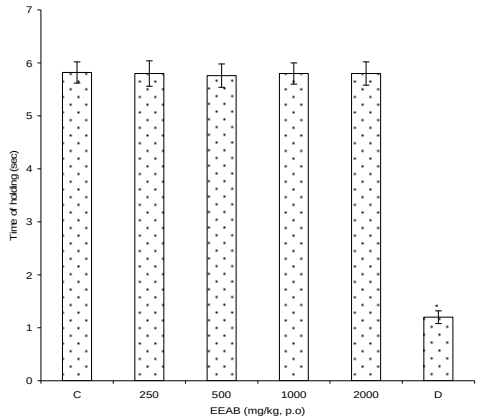

Figure 3: Effect of EEAB on skeletal muscle relaxant activity in mice

C: Control, D: Diazepam (4.0 mg/kg, i.p.)

Bars are mean values ± 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 (LD_{50}/ED_{50}) of drugs and xenobiotics[22]. 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[23] for testing acute toxicity is 2000 mg/kg BW; this probably indicates the extract has wide safety margins (low toxicity). Similar result was reportred by[24] in *Eichhornia crassipes* extract treated mice.

Sleep dissatisfaction and insomnia have a relatively high prevalence in the world[25]. Chronic sleep disorders give rise to some health problems such as emotional disturbances, slower reactions and poor memorizing[26,27]. Despite its high prevalence, insomnia has not received sufficient clinical attention. Currently, benzodiazepines are the most widely used medications. However, the clinical uses of benzodiazepines are accompanied with unpleasant side effects such as drug dependence, tolerance, rebound insomnia, amnesia, psychomotor impairment and potentiating of other central depressant drugs[28]. Hence, there is an urgent need for new hypnotic agents without the aforementioned side effects.

Pentobarbital sodium is a barbiturate that induces sleep in both rodents and humans[29]. The classic method of pentobarbital induced sleeping in mice were often used to screening sedative-hypnotic drugs[30]. Pentobarbitone – induced (animals) is used to test centrally acting effect of agents[31]. The interval between loss and recovery of righting reflex was as taken the index of hypnotic effect[32]. Two parameters were measured in this experiment, sleep latency and sleeping time. Sleep latency is defined as the time in minute from injection time to loss of righting reflex (unconsciousness) while sleeping time is defined as the total time in minute from loss of righting reflex (loss of consciousness) to regain of righting reflex (recovery of consciousness)[33]. Studies have shown that the potentiation of barbiturate hypnosis is an index of CNS depression[34]. Pentobarbital is a short-to-intermediate acting barbiturate that exerts its pharmacological effect on the central nervous system by enhancing inhibition of GABA-mediated neurotransmission[35]. Therefore, the potentiation of pentobarbital induced sleeping time was used to evaluate the possible sedative-hypnotic effects of the extract. Test compounds that prolong pentobarbital-induced sleeping time are considered as sedative agents[36]. The extract could interact with the GABAergic system to induce its hypnotic effect, since it has been reported that several neurotransmitters and endogenous molecules are involved in regulation of sleep and wakefulness. The sleep–promoting neurons located in the anterior hypothalamus release gamma aminobutyric acid (GABA) to suppress activity of wake–inducing areas of the brain[37]. Pentobarbital is known to act at GABA receptors ionophore complex and favour the biding of GABA. Also benzodiazepine agonists such as diazepam enhance the affinity of GABA for its receptor and hence prolong pentobarbital – induced sleep duration[38]. Similar result was reported by[39] in *Ganoderma lucidum* extract treated mice and rats.

Thermoregulation is a complex physiological process involving both central and peripheral autonomic mechanisms. The primary thermoregulatory center resides in the preoptic area of the hypothalamus and controls the balance between heat gain and heat loss. GABAergic terminals and $GABA_A$ receptors on the neurons of the preoptic area of the hypothalamus have been reported to be involved in the process of thermoregulation[40]. In addition, studies have also shown that systemic administration of either GABA or $GABA_A$ agonist usually produce hypothermia[41]. The extract did not induce significant changes in rectal body temperature which probably indicates that it has no effect on the thermostat regulatory center in the brain. Contrary result was reported by[42] in hops (*Humulus lupulus*) treated mice.

A muscle relaxant is a drug which affects skeletal muscle function and decreases the muscle tone. It may be used to alleviate symptoms such as muscle spasms, pain, and hyper-reflexia. The extract did not produce skeletal muscle relaxant effect in mice *vis-à-vis* it did not affect motor coordination which suggests a centrally mediated actions and non-blockade of neuromuscular transmission by the extract. Contrary result was reported by[43] in *Parthenium hysterophorus* extract treated mice.

It can be concluded that *Adenopus breviflorus* fruit may possess a sedative-hypnotic effect which provides scientific basis to the folkloric claim of the plant as a

sedative agent and its hypnotic-sedative activity could be mediated via interaction with the GABAergic system.

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 sleep disorders and other 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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