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Effect of Ethanol Extract of *Adenopus Breviflorus* (Roberty) Fruit on Animal Models of Depression

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Abstract

Objective: This study was designed to investigate effect of Ethanol Extract of *Adenopus breviflorus* fruit (EEAB) on animal models of depression.

Methods: Three hundred grams of air-dried *Adenopus breviflorus* fruits were cold macerated in 70% ethanol and concentrated using rotary evaporator. Method described by Lorke was used to determine $LD_{50.}$ Animal models of depression were studied using Forced Swimming Test (FST), Tail Suspension Test (TST), Open Field Test (OFT) and yohimbine-induced lethality test.

Results: 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.* The EEAB (250 and 500 mg/kg) and imipramine (25 mg/kg) caused significant ($p<0.05$) reductions in immobility time in mice in FST relative to the control. The EEAB (1000 and 2000 mg/kg) caused significant ($p<0.05$) increments in immobility time in mice in TST relative to the control. The EEAB (250, 500, 1000, 2000 mg/kg) caused significant ($p<0.05$) reductions in locomotor activity relative to the control. The EEAB (250, 500 and 1000 mg/kg) dose-dependently potentiated yohimbine-induced toxicity in mice relative to the control.

Conclusion: It can be concluded that *Adenopus breviflorus* fruit probably possess both antidepressant-like and depressant-like activities; with the antidepressant-like activity probably mediated via α_2- adrenergic receptor.

Keywords: *Adenopus breviflorus,* Forced swimming test, Immobility time, Mice, Tail suspension test.

INTRODUCTION

Depression is a major psychiatric disorder affecting nearly 21% of the world population and imposes a substantial health burden on society [1]. There are three main kinds of classical antidepressants in clinical practice: tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs) and monoamine oxidase inhibitors (MAOIs). Most of these drugs, however, have undesirable side effects and their mechanisms of action have not been satisfactorily resolved.

A growing number of herbal medicines are being introduced into psychiatric practice, many of which have comparable efficacy to prescription medications with lower side effects. This makes herbal therapies as desirable alternative treatment for severe depression [2].

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 [3]. 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 [4].

Medicinally, the plant is used as a purgative in Tanganyika as well as a vermifuge and cathartic in Nigeria [3]. A decoction from the plant is said to be used in Nigeria for headache [3]. 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 [5]. It is used as an anticonvulsant, sedative and pain killer [6]. It is used with other medicinal plants as concoctions to aid parturition in humans [7]. Livestock farmers employ the fruit extract of the plant for the treatment of Newcastle disease and coccidiosis in animals [7]. The fruit is also used for money-making charms by Yoruba herbalists of South-Western 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 [8] and abortifacient activity [9]. The ethanol extract of its whole fruit has been reported to have a broad spectrum antibacterial activity [10] as well as anti-oxidant and anti-ulcerogenic effects [11]. 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 rats [12].

Since this plant has been reported to have a wide range of neuroactive activities [6, 3], this study therefore aims to investigate the effect of ethanol extract of *Adenopus breviflorus* fruit on animal models of depression.

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 recommendations of the University of Ibadan Ethics Committee 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 where a voucher specimen (FHI 108336) was deposited in their Herbarium.

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 ^{0}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 ^{0}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 by [13] 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 dose for each group. The treated animals were monitored for twenty-four hours for mortality and general behavior. 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.

Antidepressant Tests

(a) Forced swimming test (FST)

The FST has been used as an animal model predictive of antidepressant effect [14] and is the most widely used test to evaluate depression-like behavior exhibited by mice [15]. The procedure developed originally by [16] with a slight modification [17] was employed in this study. Thirty mice were randomly divided into six groups (n=5). 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 imipramine (25 mg/kg, i.p.). One hour after oral administration of the distilled water and extract, and 30 minutes after intraperitoneal injection of imipramine, mice were dropped one at a time and forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), with a depth of 10 cm of water at 25 ± 2°C and observed for 6 minutes. After the initial 2-3 minutes of vigorous activity, the animals showed a period of immobility by floating with minimum movements. Each mouse was judged to be immobile when it stopped struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. The total duration of immobility was recorded during the last 4 minutes of the 6 minutes test with the help of a stop-watch. A decrease in the duration of immobility is indicative of an antidepressant-like effect [14]. The water was changed after each mouse was tested to eliminate the influence of odors from feces and urine excreted by the animal in a previous session. The animals were used only once in each swimming test.

(b) Tail suspension test (TST)

The TST is commonly used as behavioral model for screening antidepressant activity in mice [18]. The tail suspension test was carried out as per the established method [19]. Thirty mice were randomly divided into six groups (n=5). 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 imipramine (25 mg/kg, i.p.). One hour after oral administration of the distilled water and extract, and 30 minutes after intraperitoneal injection of imipramine, mice were suspended individually by the tail from a horizontal bar 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. After 2-3 minutes of vigorous activity characterized by struggling movements, attempts to catch the adhesive tape, or body torsions or jerks, the mice hung passively and completely motionless. Immobility was defined as the absence of any limb or body movements, except for those caused by respiration or when they hung passively and completely motionless. A decrease in the duration of immobility is indicative of an antidepressant-like effect [19]. The total duration of immobility was recorded during the last 4 minutes of the 6 minutes test with the help of a stop-watch.

(c) Open Field Test (OFT)

This test utilizes behavioral changes in rodents exposed to novel environments and is used to confirm that the observed antidepressant effect in the FST paradigm is not due to stimulation of general motor activity. Motor activity was measured in an Open Field Box (OFB) (45 cm x 25 cm x 25 cm) with painted black grids dividing the floor into 16 (7 cm x 7 cm) equal squares. Thirty mice were randomly divided six groups (n=5). 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 imipramine (25 mg/kg, i.p.). One hour after treatment with the extract, each mouse was placed in one of the corners of the box and the number of squares crossed with all four paws was counted for 5 minutes. The cage was cleaned with 70 % ethanol at intervals when each animal was removed [20].

(d) Yohimbine-induced Lethality Test

The antidepressant effect of the extract was further evaluated utilizing the potentiation of yohimbine-induced lethality test in mice as previously described [21]. Sixty mice were randomly divided into six groups (n=10).

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 imipramine (25 mg/kg, i.p.) one hour prior to yohimbine administration (35 mg/kg, i.p.). The number of dead mice in each group was recorded - 24 hours after yohimbine administration and the percent mortality of the test animals was compared with the control animals.

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 EEAB (250 and 500 mg/kg) and imipramine (25 mg/kg) caused significant ($p<0.05$) reductions in immobility time in mice in FST relative to the control, while EEAB (1000 and 2000 mg/kg) did not produce significant ($p>0.05$) changes in immobility time in mice relative to the control (Figure 1).

Treatment of mice with EEAB (1000 and 2000 mg/kg) caused significant ($p<0.05$) increments in immobility time in mice in TST relative to the control, while EEAB (250 and 500 mg/kg) did not produce significant ($p>0.05$) changes in immobility time in mice relative to the control, but imipramine (25 mg/kg) caused significant ($p<0.05$) reduction in immobility time in mice in TST relative to the control (Figure 2).

Treatment of mice with all the treatment doses of EEAB (250, 500, 1000, 2000 mg/kg) caused significant ($p<0.05$) reductions in locomotor activity relative to the control, while imipramine (25 mg/kg) did not produce significant ($p>0.05$) change in locomotor activity relative to the control (Figure 3).

The EEAB (250, 500 and 1000 mg/kg) dose-dependently potentiated yohimbine-induced toxicity in mice relative to the control, while EEAB (2000 mg/kg) did not potentiate yohimbine-induced toxicity in mice relative to the control. Imipramine (25 mg/kg) also potentiated yohimbine-induced toxicity in mice relative to the control (Table 1).

Table 1: Effect of EEAB on yohimbine-induced lethality test in mice

Treatment	Dose (mg/kg)	No of death	% Mortality
Control	0.2 ml/20g	1/10	10
EEAB	250	10/10	100
EEAB	500	5/10	50
EEAB	1000	4/10	40
EEAB	2000	0/10	0
Imipramine	25	7/10	70

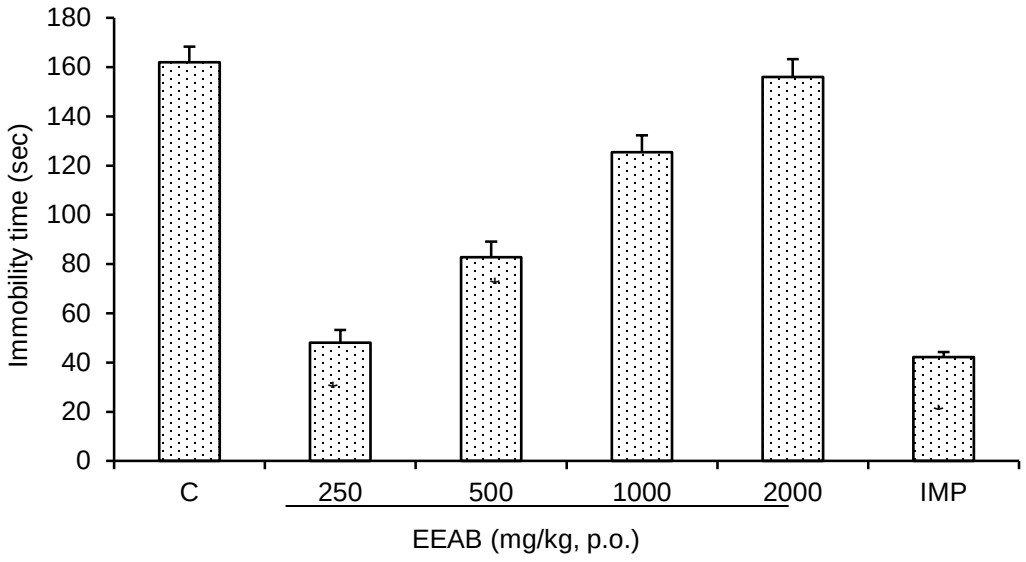

Figure 1: Effect of EEAB on immobility time of Forced Swimming Test (FST) in mice
C: Control, IMP: Imipramine (25 mg/kg)
The results are expressed as mean values ± S.E.M. (n=5). One way ANOVA revealed significant difference [$F(4, 20) = 5.221$, $p<0.05$] between various treatment groups.
* Indicates significant difference from control at $p<0.05$.

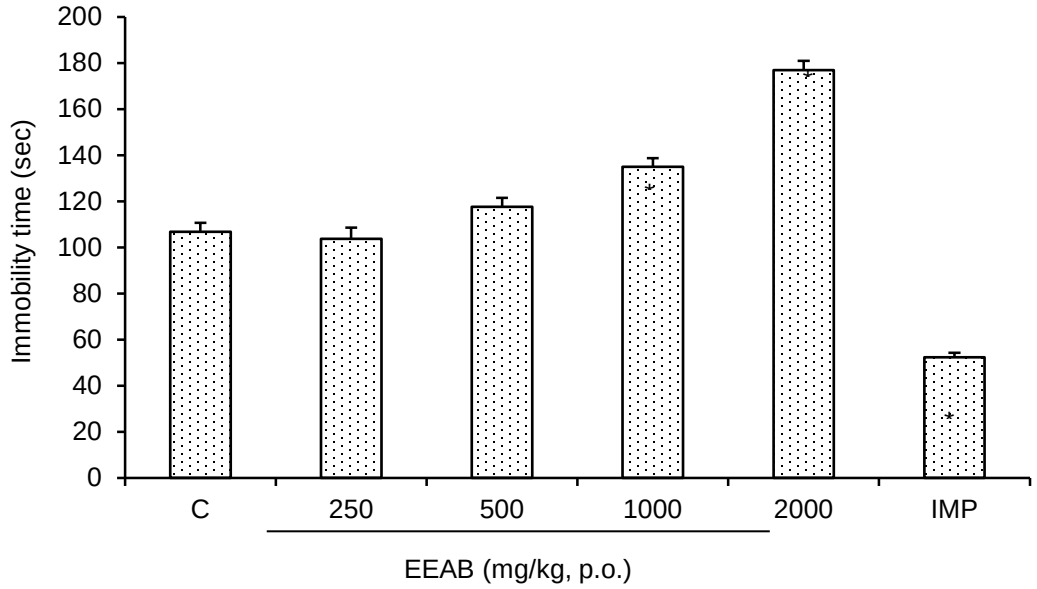

Figure 2: Effect of EEAB on immobility time of Tail Suspension Test (TST) in mice
C: Control, IMP: Imipramine (25 mg/kg)
The results are expressed as mean values ± S.E.M. (n=5). One way ANOVA revealed significant difference [F (4, 20) =14.604, p<0.05] between various treatment groups.
* Indicates significant difference from control at p<0.05.

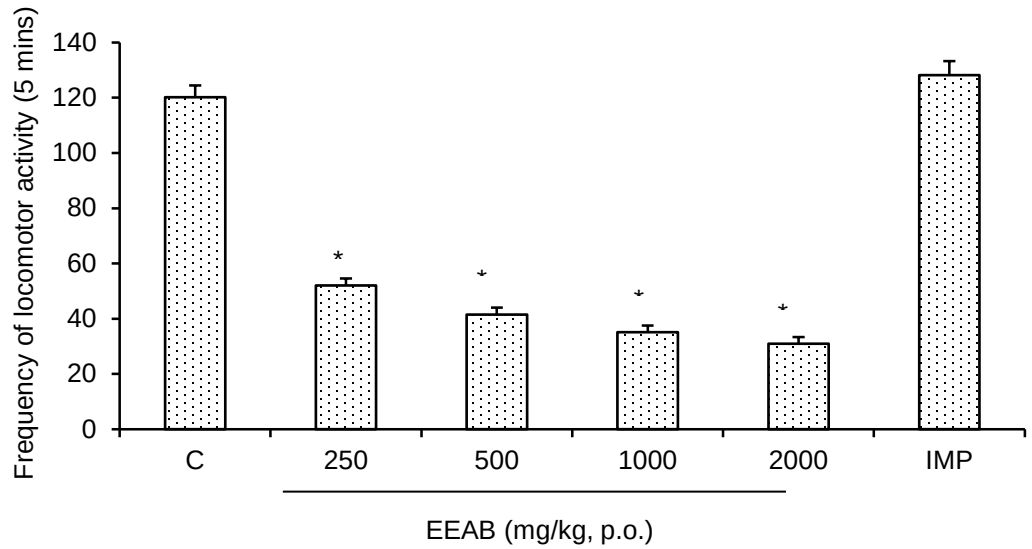

Figure 3: Effect of EEAB on locomotor activity of mice in the Open Field Test (OFT)
C: Control, IMP: Imipramine (25 mg/kg)
The results are expressed as mean values ± S.E.M. (n=5). One way ANOVA revealed significant difference [F (6, 28) =9.347, p<0.05] between various treatment groups.
* Indicates significant difference from control at 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; this probably indicates the extract has wide safety margins (low toxicity). Similar result was reported by [24] in *Eichhornia crassipes* extract treated mice.

Depression is the outcome of an eventual inability to cope with a stream of dissimilar unpleasant stimuli imposed by the environment. Animal models are widely used in pre-clinical antidepressant evaluation and to provide insights into the neuropathology of depression [25]. It is difficult to mimic depression in the laboratory, because depression-related syndromes are associated with multifaceted symptoms which manifest themselves at the psychological, behavioral and physiological levels. The TST and FST were the most widely accepted models for assessing antidepressant-like activity in mice. The immobility taken on in these two tests is understood to reflect a state of 'behavioral despair and variants' or 'failure to adapt to stress' [26, 27]. These two tests are based on the fact that animals will develop an immobile posture when subjected to the short-term, inescapable stress of being suspended by their tail or being dropped into water. These two models are widespread in the laboratory largely due to their ease-of-use, consistency across laboratories, and their ability to detect a broad spectrum of antidepressant agents. Most clinically active antidepressants are effective in the TST and FST, while neuroleptics and anxiolytics produce different effects. Antidepressants can also be distinguished from stimulants because stimulants cause marked motor simulation, in contrast to antidepressants, which do not [27].

The FST remains one of the most common animal models used for screening potential antidepressant agents [28]. This test induces a state of immobility in animals facing an inescapable situation (in a swim tank). Such immobility behavior has been hypothesized to reflect behavioral despair, which in turn may reflect depressive disorders in humans. Therefore, the antidepressant-like activity of a compound is expressed by a decrease in the immobility of animals submitted to forced swimming. This behavioral change is sensitive to major classes of antidepressant drugs even when administered acutely, including monoamine oxidase inhibitors, tricyclics, selective 5-HT reuptake inhibitors, and atypicals [29]. Thus, the significant reduction in immobility time in mice induced by EEAB is suggestive of an antidepressant-like activity. Similar result was reported by [30] in *Rosa damascene* extract treated mice.

Tail suspension test most commonly used for assessing antidepressant activity in animal model of mice. This test is based on the observations that rodents mostly mice although gerbils and rats have been used [31] after initial escape behavior, develop an immobile position when subjected to inescapable stressful situation. Animals are considered as immobile when they are not making any movements of struggling, attempting to hold the adhesive tape, body torsions or jerks i.e. when they hang passively and completely motionless. It has been suggested that acute antidepressants decrease the immobility time in TST. The TST has got an advantage that it can be used to detect broad spectrum of antidepressants irrespective of their mechanism of action, the test is inexpensive, simple, methodologically unsophisticated and easy amendable to automation. The automation has got an advantage that it can help to measure other parameters as well such as power of movement [32]. The use of TST has substantially been increased nowadays for assessing antidepressant activity and has been argued that the TST is less stressful than the FST and has greater pharmacological sensitivity [18]. However, in this paradigm, the extract (EEAB) induced significant increase in immobility time in mice which is suggestive of a depressant-like activity. Similar result was reported by [33] in *Beta vulgaris* extract treated mice.

Agents that enhance locomotor activity in open-field test including psychomotor stimulants, convulsants and anticholinergics, tend to produce a false positive result in FST and TST [34]. Therefore, locomotor activity was assessed in mice in open-field test to rule out any psychomotor stimulant activity [35]. The major difference between the antidepressants and the psychomotor stimulants is that the antidepressants would not cause significant increase in motor activity [27]. Surprisingly, the extract produced a dose-dependent decrease in number of crossings in the OFT in this study. This hypolocomotion effect of the EEAB treated animals when compared with the control group animals in the OFT probably indicates the absence of any psychomotor stimulant activity, thereby supporting the antidepressant-like effect of EEAB observed in the FST. It has been suggested that even if a sedative effect is observed in the open-field test, antidepressant-like activity may be perceived in the FST [36]. Similar results have been reported in which clonidine [37], imipramine [38], desipramine [36], buspirone [38], ipsapirone [38] and gepirone [38] produced significant decrease in immobility time in FST and significant decrease in locomotor activity (number of crossings) at doses similar to those that decreased immobility. Furthermore, fluoxetine, zimeldine, and indalpine significantly reduced immobility time in TST and significantly reduced locomotor activity at doses similar to those that decreased immobility [39]. Collectively, these data indicate that antidepressants can produce decreased locomotor activity in open-field test in rodents.

Potentiation of yohimbine-induced lethality in mice is also used for the assessment of antidepressant drugs [40]. It has been reported that the potentiation of yohimbine-induced toxicity by the compounds can be

predictive of anti-depressant potential [41]. The potentiation of yohimbine-induced lethality has been reported for several antidepressants [42]. Animal models have been developed not only to detect potential antidepressant properties of new substances, but also to identify neurotransmitter systems that may be involved in the mechanisms of actions of antidepressant drugs [43]. Potentiation of yohimbine-induced lethality in mice is used to understand the mechanism involved in the antidepressant activity of potential antidepressant agents [44]. Yohimbine is an alpha 2 adrenergic antagonist, causes increased sympathetic discharge both in the peripheral and central nervous system. The antagonism of alpha 2 receptors also causes an increase in the level of serotonin [45] that may contribute to overall toxicity by activating central serotonergic pathways. Antidepressant drugs by enabling more amines to reach the receptors potentiate yohimbine-induced lethality. This occurs either by their reuptake inhibition or reduced inactivation (Monoamine oxidase inhibitors). The yohimbine potentiation test is therefore sensitive to detect MAO inhibitors, tricyclic antidepressants, noradrenaline (NA), and selective serotonin reuptake inhibitors [21]. The present study has shown that the extract potentiated yohimbine-induced lethality by inducing dose dependent increase in mortality of mice as compared to the control, thereby indicating that biogenic amines may be responsible for the antidepressant effect of EEAB.

It can be concluded that *Adenopus breviflorus* fruit probably possess both antidepressant-like and depressant-like activities; with the antidepressant-like activity probably mediated via α_2- adrenergic receptor.

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