

Anticonvulsant, Anxiolytic and Sedative Activities Of The Methanol Extract of *Abrus Precatorius* (Linn.) Leaves

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ABSTRACT

Abrus precatorius L. is a woody twinning climber, a member of the Fabaceae family indigenous to parts of Asia and Africa. It has been used widely in traditional medicine for curing many conditions such as malaria, sores, chest pain, cough, inflammation and many others. It was also reported that the hot water extract of the leaves and the roots have been used in treating persons suffering from episodes of convulsions in Tanzania. The aim of this present study was to evaluate the anti-convulsant, anxiolytic and sedative activities of the methanol extract of *A. precatorius* (Linn.) leaves in-vivo using murine models of convulsion, anxiety and sedation.

The anticonvulsant activity of ethanol extract of *A. precatorius* leaves was investigated using pentylene tetrazole, strychnine and picotoxin-induced convulsion. Anxiety tests used included head dips and elevated plus maze. Phenobarbitone-induced sleeping test was used to assess the sedative effect of *A. precatorius*. Safety of the extract was determined using the brine shrimp lethality assay.

Preliminary phytochemical screening on the dried and powdered leaves indicated that alkaloids, tannins, flavonoids and saponins, with a yield of 17.40% are present in the leaves. The brine shrimp lethality assay showed a LC₅₀ of 8.189 µg/ml. Results from the *in-vivo* neuropharmacological profile of the crude methanol extract of the leaves at doses of 200, 400 and 800 mg/kg body weight had central effects although not sig-

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nificant ones compared with diazepam which was used as the standard. The results from the convulsion studies expressed as percentage protection indicates that 400mg/kg and 800mg/kg had 25% protection against pentylenetetrazol induced convulsions. The methanol extract of *A. precatorius* leaves might possess anticonvulsant and sedative activities, thus justifying its use in the management of epilepsy.

Keywords: Anticonvulsant, Anxiolytic, sedative, *Abrus precatorius*, epilepsy.

INTRODUCTION

Abrus precatorius L. is a woody twinning climber, a member of the Fabaceae family indigenous to parts of Asia and Africa. It has been used widely in traditional medicine for curing many conditions such as malaria, sores, chest pain, cough, inflammation and many others. It was also reported that the hot water extract of the leaves and the roots have been used in treating persons suffering from episodes of convulsions in Tanzania.

Over 50 million people are estimated to have epilepsy, making it one of the most common non-communicable neurological diseases, with a very large proportion of affected persons living in developing countries of the world, especially in Asia and Africa. A vast majority of these patients rely on traditional medicine almost exclusively because therapy is symptomatic, controlling seizures in only about 25%, and neither effective prophylaxis nor cure is available¹. The characteristics of the disease are periodic and unpredictable seizures due to disordered, synchronous and rhythmic firing of population of the brain's neurons². A study in south western Nigeria identified the cause of epilepsy as psychological stress, head injury, birth or head injury, and supernatural or due to a contagion³.

Over the years, various mechanisms to alleviate the condition have led to the development/production of a wide variety of antiepileptic drugs (AEDs). AEDs are classified majorly into first, second and third generations, with 14 new AEDs licensed for clinical use between 1989 and 2009. Majority of the AEDs have very potent adverse effects and drug interactions, but the third-generation drugs exhibit less interaction compared with the first- and second-generation AEDs⁴. The ideal AED would suppress all seizures without causing any unwanted effects. Unfortunately, none of the current drugs used control seizure activity without frequent unwanted effects which include impairment of the CNS, drowsiness, lethargy, euphoria, dizziness, headache, and convulsion. Anxiety is a common psychological disorder in epileptics⁵. It is described as an unpleasant emotional state with an unidentified, uncontrollable and unavoidable cause.

The hot water extract of the leaves of *Abrus precatorius* (Linn.) is used in traditional medicine of various neurological conditions including convulsion⁶. How-

ever, there is paucity of detailed scientific study of *Abrus precatorius* leaf extract applied on the central nervous system to control convulsions. The aim of this present study was to evaluate the anti-convulsant anxiolytic and sedative activities of the methanol extract of *A. precatorius* (Linn.) leaves in-vivo using murine models of convulsion, anxiety and sedation.

METHODOLOGY

Plant material and preparation of extract

The plant material was collected in May 2016 from the University of Ibadan Botanical Gardens. It was identified and authenticated at the Forest Herbarium Ibadan, Oyo State, Nigeria in the Forestry Research Institute of Nigeria, Ibadan, where a voucher specimen (FHI. 110694.) was obtained and deposited. The leaves of flowering *A. precatorius* were dried properly in air and then pulverized using a blender to obtain a coarsely ground powder which was extracted using cold extraction method with distilled methanol. 178 g of the coarse powder was macerated in a macerating tank for 48 hours exhaustively. The crude extract was decanted off and filtered. It was thereafter concentrated at 20 °C using the rotary evaporator (BUCHI Rota vapor R-205).

Animal

Young male albino Swiss mice (18–25 g) were obtained from the Animal Centre, College of Medicine, University of Ibadan, Nigeria, and were housed in plastic cages at room temperature with a 12:12 h light–dark cycle. They were fed with balanced rodent pellet diet and water ad libitum. The animals were acclimatized for at least 1 week before being used for experiments. The experimental procedures were following the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Drugs and chemicals

Diazepam (Hoffman-La Roche, Switzerland), pentylenetetrazol (Sigma, USA), picrotoxin (Sigma, USA), and strychnine (Shaanxi Xin Sheng Long Industrial Co., Ltd. China).

Experimental design

Thirty mice randomly divided into six groups (n=5) were used for all the tests except the anticonvulsant assays. The groups included two controls (vehicle and standard drug) and four treatment groups for doses 100, 200, 400 and 800 mg/Kg. A similar grouping was used for the anticonvulsant test except that there were 10 animals per group (n=10)

Brine shrimp lethality assay

The stock solution of the crude methanol extract of *A. precatorius* with concentration of 1000 µg/ml was prepared by dissolving 50 mg of the extract in 5ml of natural sea water. Concentrations of 1000 µg/ml, 500 µg/ml, 100 µg/ml, 10 µg/ml and 1 µg/ml were used for the assay by appropriate dilution with sea water in sample bottles. The experiment was carried out in triplicates. Ten viable *Artemia salina* were introduced into each sample bottle and made up to 5ml using natural sea water. After a period of 24 hours, the number of dead shrimps was counted and recorded⁷.

Novelty induced behavior (NIB)

NIB was assessed using the method described by Ajayi and Ukponmwan (1994)⁸ with some modifications. The mice were allowed 6–10 mins epochs during which locomotion, rearing and grooming were observed and scored to allow for characterization of drug-induced alterations. The mice were then returned to their home cages. Each test session involved allowing the mice to acclimatize to the testing environment (a quiet well-ventilated room) for 30 mins. All behavioral testing was carried out between 9 am and 2 pm. Six groups of five mice each were given 10 mL/Kg vehicle; 100, 200, 400 and 800 mg/Kg MEAP; and 3 mg/kg diazepam orally, before placement in the open field arena. The open field is a rectangular arena composed of a hardboard floor (36×36 cm²) with a surrounding wall (30 cm high) made of white painted wood. The extract dosages were chosen guided by brine shrimp lethality assay and literature. (Similar doses were used in other protocols in this study.)

The floor was divided into squares of 9 cm². One hour after administration, each mouse was introduced into the arena; the frequency of grooming (the number of body cleaning with paws picking of the body and pubis with mouth and face washing actions) and rearing frequency (number of times each mouse stands on its hind legs or with its forearms against the wall of the cage or in free air) were scored for 30 mins. The procedure was repeated for all the mice in the different groups. After each session, the floor of the apparatus was wiped with 70% ethanol and dried thoroughly to remove traces of previous path.

Exploratory activity

To determine potential sedative effects, the hole-board test was used. The hole-board is a wooden board (40×40 cm²) with 16 holes (diameter 3 cm) evenly spaced on the floor⁹. Immediately after the NIB test for each mouse, it was placed at the center of the hole-board and the number of head dips into the holes was scored over a 5 mins period. Results obtained were expressed as mean total number

of head dips (Lister, 1987). The procedure was repeated for all the mice in the different groups. After each trial, the floor of the apparatus was wiped with 70% ethanol and dried thoroughly to remove traces of previous path.

Learning and memory

Y-maze was used to assess the effect of the extract on short term memory. The Y-maze is composed of three equally spaced arms (120°; 41×15×5 cm). The parameters assessed were arm entries (locomotor activity) and spontaneous alternation performance (memory). Immediately after the hole-board test for each mouse, it was placed in one of the arm compartments and allowed to move freely for 5 mins. Entry was defined as when the body (excepting the tail) of a mouse completely enters into an arm compartment. The sequence of entry was manually recorded. Alternation is defined as entry into all three arms consecutively. The arms were labeled A, B, and C, and consecutive entries ABC, BCA, and CAB. The maximum number of spontaneous alternations was then calculated as:

[*Total number of arms entered*]- 2;

Percentage alternations was calculated as

$$\left[\frac{\text{Actual alternation}}{\text{Maximum alternation}} \right] (\times 100) \quad (10)$$

The procedure was repeated for all the mice in the different groups. The apparatus was cleaned after each session to eliminate the odor left off the immediately preceding mouse¹¹.

Anxiolytic test

The elevated plus maze model ^{12, 13} was used to assess anti-anxiety effect. Lister (1987) ¹⁴ validated the use of the elevated plus maze in testing anxiolytic effect in mice. First, the mice were assessed for the aversion of the open space and height. For this aspect, the elevated plus maze with two open and two closed arms was used. The plus maze used is made of wood with open arms (30×5×15 cm) and closed arms (30×5×15 cm). The arms extend from the central platform (5×5 cm). The open arms, the central platform, and the floor of the closed arms were painted black. Next, the apparatus was mounted on a wooden base at an elevation of 38.5 cm above floor level. For the open arms, a slight ledge 4 mm high was erected to prevent the mice from slipping and falling off the edge. Immediately after the learning and memory test, each mouse was placed one after another at the center facing one of the closed arms and assessed for 5 mins. The following behavior was scored: open arm entries, closed arm entries, time spent in open arm and time spent in closed arm. The procedure was repeated for all the mice in the different

groups except the group that was given diazepam; the group was replaced with another set of mice that was given 1 mg/Kg diazepam. After the assessment of each mouse, the lingering olfactory cues were cleansed using 70% ethyl alcohol. The doses used fell in the range that did not affect motor coordination ¹⁵.

Activity cage

The locomotor activity of the mice was measured as horizontal and vertical movement using the multiple activity cage apparatus (Ugo Basile 47420) complete with two sets of emitter/sensor arrays for horizontal and vertical activity. A total of six rodents per group was placed in pairs of 2 mice per session in the activity cage for a duration of 5 minutes. The apparatus was preset for 2 mice per session of 5 minutes on two trials according to the manufacturer's manual. Thereafter, the rodents were returned to their home cages.

Anticonvulsant tests

Pentylenetetrazol-induced convulsion: PTZ (85 mg/kg; s.c.) was used to induce clonic-tonic convulsion in mice ¹⁶. The mice were divided into six groups of ten rodents each. The groups were 10 mL/Kg vehicle; 100, 200, 400 and 800 mg/Kg extract; and 40 mg/Kg phenobarbitone. One hour after the administration (p.o.), the convulsant was used to challenge the animals. The percentage of survival was recorded for each group.

Picrotoxin-induced convulsion: Picrotoxin (14 mg/kg; i.p.) ¹⁷ was used to induce limbic seizures followed by status epilepticus in mice. Again, the mice were placed in six groups of ten rodents each. The groups were 10 mL/Kg vehicle; 100, 200, 400 and 800 mg/Kg extract; and 40 mg/Kg phenobarbitone. Sixty minutes after initial administration (p.o.) the rodents were challenged using the convulsant. The survival percentage for each group was then recorded.

Strychnine-induced convulsion: seizures were induced using Strychnine (2 mg/kg; i.p.) ¹⁷. Once more, every mouse was placed in a group of six with ten rodents each. Group one was 10 mL/Kg vehicle; groups two to five got 100, 200, 400 and 800 mg/Kg extract; while group six received 40 mg/Kg phenobarbitone. An hour from administration (p.o.) the convulsant was used to induce the rodents. Finally, the survival rate was recorded for each group.

Statistical analysis

Results of the experiments and observations were expressed as mean \pm standard error of mean (SEM). The significance of differences between groups was determined using one-way analysis of variance (ANOVA) followed by at least one of the following posthoc test: Dunnett's multiple comparison tests, Tukey's

t-test and Student Neuman Keuls test. A level of significance $p < 0.05$ or 0.01 was considered for each test.

RESULTS AND DISCUSSION

Secondary metabolites are the most beneficial active principles derived from various parts of plants and over time, scientific evidence that they possess the pharmacological activities for which the plant is known are being provided. The therapeutic or prophylactic effects of plant materials typically result through additive or synergistic action of the secondary metabolites present in the plant acting at single or multiple target sites associated with a physiological process¹⁸. The combination of secondary metabolites in a particular plant is often taxonomically distinct¹⁹. This could be the basis for the specificity and uniqueness of medicinal actions of plant. Preliminary phytochemical screenings of the crude methanol extract of the leaves of *A. precatorius* (MEAP) show the presence of alkaloids, tannins, saponins and flavonoids which are responsible for the various pharmacological actions of the leaves and extracts from the leaves of the plant. Alkaloids e.g. piperine, raubasine have been shown specifically to possess anticonvulsant activities as well as flavonoids, terpenoids and saponins. Adedapo *et al.*,²⁰ reported that intraperitoneal LD₅₀ of *A. precartoris* in ethanol in mice is less than 0.1 µg/kg, while Ogbuehi *et al.*, 2015²¹ gave oral LD₅₀ in methanol to be 3942 mg/kg. The study revealed that the extract might be cytotoxic with LC₅₀ of 8.189 µg/ml. Compounds or extract with LC₅₀ value less than 1000µg/ml are considered to be cytotoxic while those giving LC₅₀ values greater than 1000 µg/ml are not considered to be²².

Table 1: Table of results from Brine shrimp lethality assay.

Concentrations (µg/ml)	Death in 1 st vial (x/10)	Death in 2 nd vial (x/10)	Death in 3 rd vial (x/10)	Total death (x/30)	% mortality
1000	9	10	10	29	96.7
500	9	7	10	26	86.7
100	6	3	9	18	60
10	6	4	3	13	43.3
1	4	2	6	12	40

Different pharmacological techniques were used to investigate the anxiolytic, anticonvulsant, and sedative effects of MEAP. In activity cage, open field and hole board tests, decreased number of horizontal and vertical movements, rearing and grooming, and head dips respectively reveal that MEAP might possess sedative effect. Increased activities (movements, novelty induced behavior and head dips) of rodents are indicative of their explorative capability, which are also considered to be central excitatory behavior⁸. The reduction of these activities by MEAP could be due to their central inhibitory action on excitatory neural systems such as glutamatergic and dopaminergic systems among others or their possible potentiation of the central inhibitory systems such as γ -aminobutyric acid (GABA).

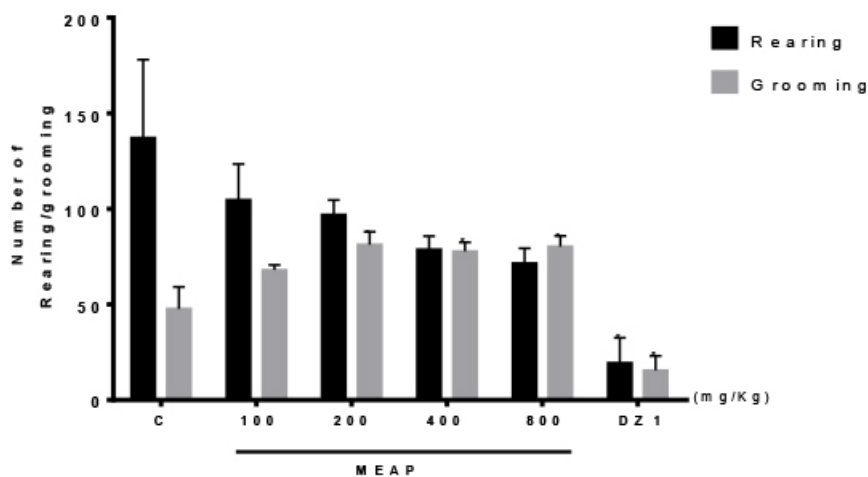


Figure 1: Effects of the methanol extract of the leaves of *A. precatorius* on Novelty-induced rearing and grooming in mice. Bars represent mean values with error bars. One-way ANOVA followed by Dunnet's multiple comparison test. * $p < 0.05$, indicate significant difference from the control (distilled water).

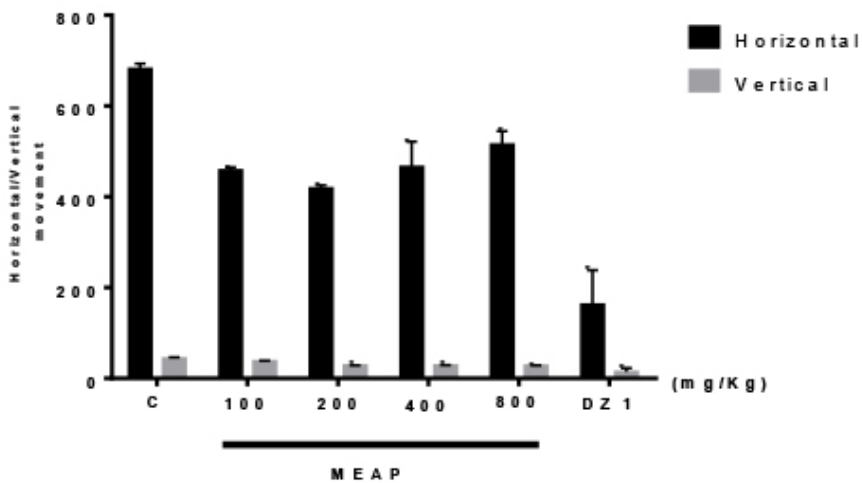


Figure 2: Effects of the methanol extract of the leaves of *A. precatorius* on horizontal and vertical locomotion in mice. Bars represent mean values with error bars. One-way ANOVA followed by Dunnet's multiple comparison test. * $p < 0.05$, indicate significant difference from the control (distilled water).

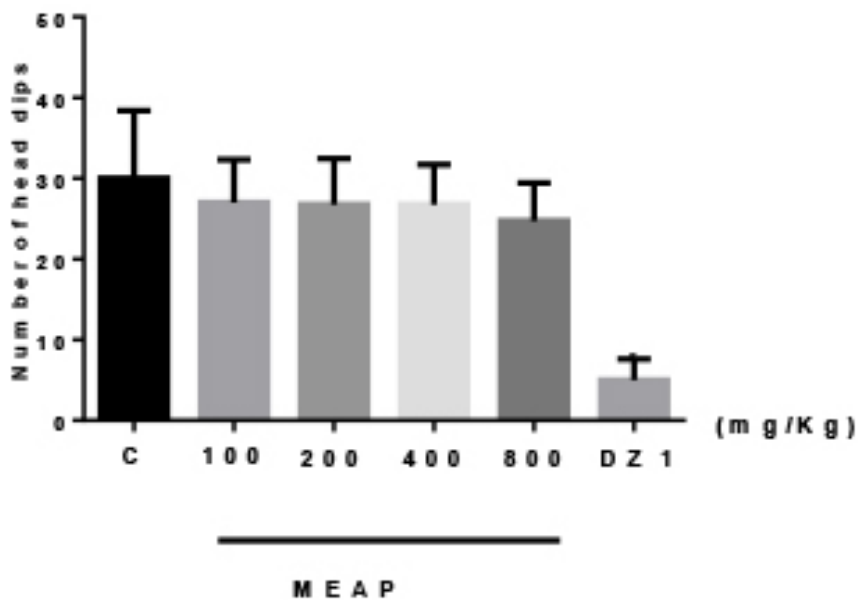


Figure 7: Effects of the methanol extract of *A. precatorius* leaves on exploratory behavior in mice. Bars represent mean values with error bars. One-way ANOVA followed by Dunnet's multiple comparison test. * $p < 0.05$, indicate significant difference from the control (distilled water).

Also, the results from spontaneous alternation test using the Y-Maze showed that MEAP has no significant effect on spatial working memory at doses used in this study.

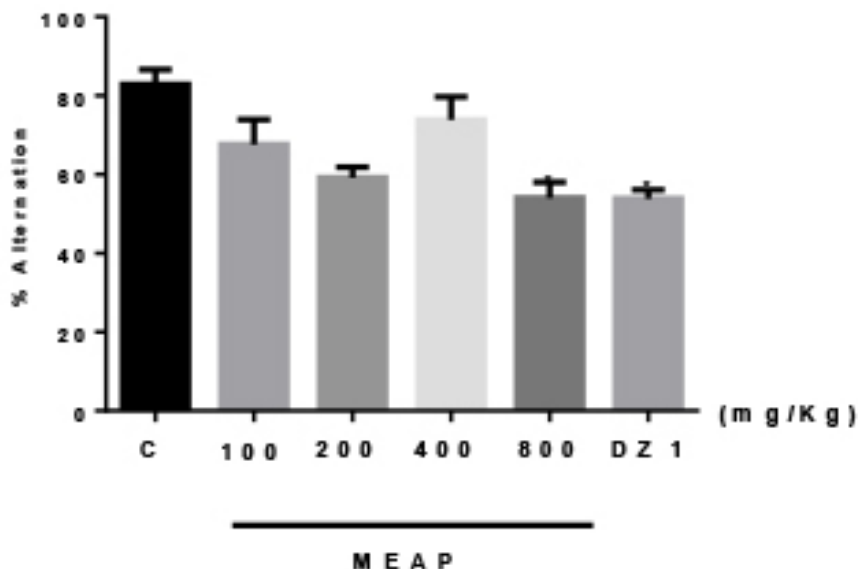


Figure 3: Effects of the methanol extract of the leaves of *Abrus precatorius* on the percentage alternation of mice in the Y-maze. Bars represent mean values with error bars. One-way ANOVA followed by Dunnet's multiple comparison test. * $p < 0.05$, indicate significant difference from the control (distilled water).

EPM favorable for testing of $GABA_A$ -receptors linked anxiolytic drugs ^{29, 30}. Agents, which increase animals' time spent and number of entries into open arms and/or reduce time spent and number of entries in closed arms of EPM, are considered to possess anxiolytic effects ³¹. MEAP significantly decreased time spent in open arms (Figure 4), and increased time spent in closed arms (Figure 5) suggesting that it lacks anxiolytic activity. Avoidance of the open arm portrays a manifestation of fear and anxiety. MEAP increased the index of open arm avoidance (Figure 6). Standard benzodiazepine anxiolytic like diazepam increased time spent in open arm and reduced time in closed arm at 1 mg/kg ³².

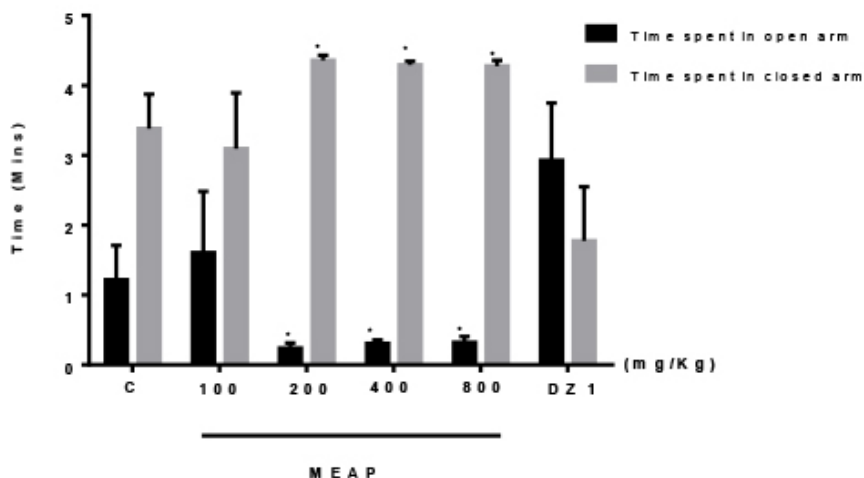


Figure 4: Effects of the methanol extract of the leaves of *A. precatorius* on the time spent in the open and closed arms respectively in the Elevated plus maze test in mice. Bars represent mean values with error bars. One-way ANOVA followed by Dunnett's multiple comparison test. * $p < 0.05$, indicate significant difference from the control (distilled water).

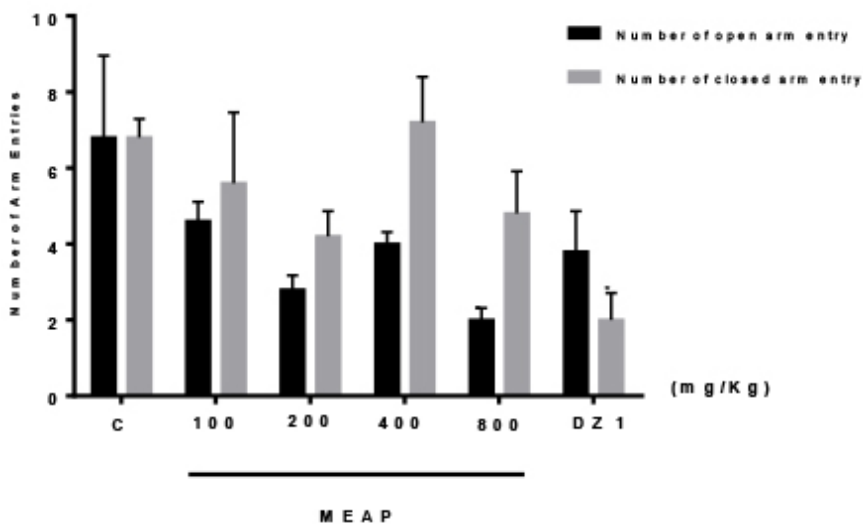


Figure 5: Effects of the methanol extract of the leaves of *A. precatorius* on the number of open and closed arm entries in the elevated plus maze in mice. Bars represent mean values with error bars. One-way ANOVA followed by Dunnett's multiple comparison test. * $p < 0.05$, indicate significant difference from the control (distilled water).

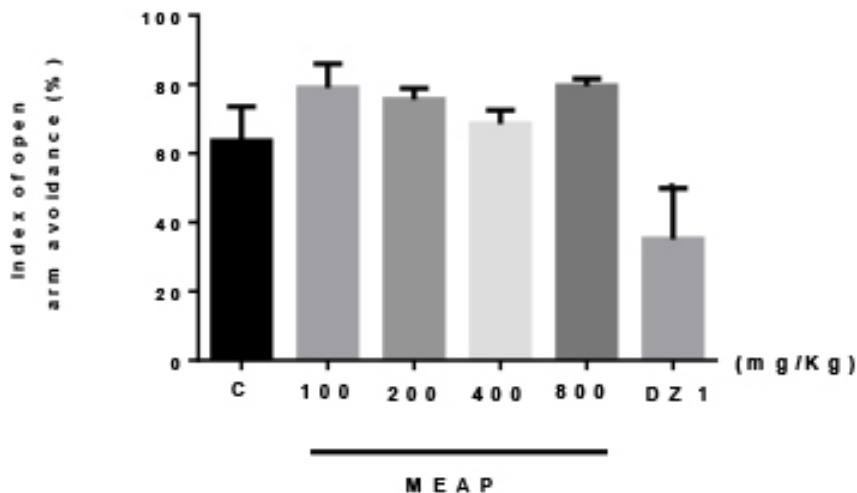


Figure 6: Effects of the methanol extract of *A. precatorius* leaves on the index of open arm avoidance. Bars represent mean values with error bars. One-way ANOVA followed by Dunnett's multiple comparison test. * $p < 0.05$, indicate significant difference from the control (distilled water).

Commonly, anti-seizure drugs are screened using PTZ-induced convulsion^{23, 24}. MEAP protected 25% at 400 and 800 mg/kg in PTZ-induced convulsion, but showed no protection in picrotoxin and strychnine-induced convulsion (Figure 8). Abolishment or increase in the seizure threshold is associated to anticonvulsant activity. This observation suggests that MEAP might possess anti-epileptic effect which is probably mediated by the chloride channel of GABA/benzodiazepine receptor complex and not by the chloride channel of glycine receptors. PTZ evokes convulsions via inhibition of GABAergic neurotransmissions by interfering with GABA_A receptors²⁵. The enhancement and inhibition of the neurotransmission of GABA will attenuate and enhance convulsion respectively^{26, 27}. Phenobarbitone and diazepam exert their antiepileptic effects by enhancing the GABA-mediated inhibition in the brain²⁸. MEAP might possibly antagonize pentylenetetrazol convulsion by interfering with GABA aminergic mechanism(s) to exert its anticonvulsant effect.

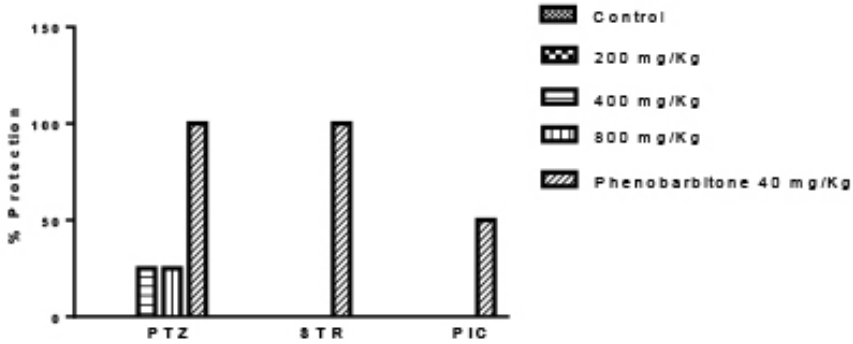


Figure 8: Effects of methanol extract of *A. precatorius* leaves on PTZ, Strychnine and Picrotoxin induced convulsions. Notes: PTZ Pentylene tetrazol 85mg/Kg; STR Strychnine 2 mg/Kg; PIC Picrotoxin 14 mg/Kg

Increasing evidence from several studies indicates that antiepileptic drugs therapy may play a role in intellectual and behavioral impairment in children with epilepsy^{33,34}.

The findings of this study have shown that the methanol extract of *Abrus precatorios* has anticonvulsant and sedative actions ('activity' seems most suitable). These identified activities justify the proposition that the herbal (plant) extract should be subject to further experimental test conditions with human agents to properly determine how effective its use can be in the management of epilepsy.

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