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Evaluation of the Anxiolytic Effect of Methanolic Leaves Extract of *Paullinia pinnata* Lin in Mice

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Authors' contributions

This work was carried out in collaboration between all authors. Author MA performed the experiments and wrote the first draft of the manuscript. Author JA designed the study and managed the analyses of the study. Author AHY performed the statistical analysis, wrote the protocol. Author MGM managed the literature searches and did all the corrections mentioned by the reviewers. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

This study was designed to evaluate the anxiolytic effect of methanolic leave extract of *Paullinia pinnata* L. in mice. The elevated plus-maze and staircase paradigm were used to assess the anxiolytic activity of the methanolic leaf extract of *Paullinia pinnata* and diazepam. The results of the elevated plus-maze test showed that the extract at the dose of 50 mg/kg and diazepam significantly ($P<0.05$ and $P<0.005$) increased both the number of entries and time spent in the open arm by mice. In staircase paradigm, the extract produced a significant ($P<0.05$-$P<0.0005$) dose dependent decrease in the number of steps ascended and number of rearing events compared to the control mice. Diazepam significantly ($P<0.0005$) reduced the number of rearing events compared to control. The result of the present preliminary study suggests that methanolic leaf extract of *Paullinia pinnata* may possess an anxiolytic activity.

Keywords: Paullinia pinnata; anxiolytic; elevated plus- maize; staircase; diazepam.

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1. INTRODUCTION

Anxiety, a state of excessive fear, is characterized by motor tension, sympathetic hyperactivity, apprehension and vigilance syndromes [1]. Anxiety may interfere with intelligence, psychomotor function and memory [2].

Anxiety symptoms are common in the community, and anxiety disorders are common in primary and secondary medical care settings [3]. The disorders typically persist for many years, and are associated with significant personal distress, reduced quality of life, increased morbidity and mortality, and a substantial economic burden [4]. Current treatments for anxiety disorders have modest efficacy: many patients do not respond or are unable to tolerate pharmacological approaches (principally antidepressants) and psychological interventions (such as cognitive–behaviour).

Many animal models of anxiety examine the natural behavioural patterns of mice and rats to develop ethologically based behavioural tasks [5]. These include 'approach–avoidance' tasks [6], in which animals are exposed to an aversive/ threatening environment e.g. open, elevated arms of the elevated plus-maze, light arena (light/dark exploration/emergence tests); and open field tests, with anxiety-like behavior (phenotype) in each case, inferred from increased avoidance. Other models include social interaction tests (review by [7]), punishment-based conflict procedures (e.g. punished drinking [8]. (Vogel et al., 1971), defensive burying tests [9], predator stress [10], and the examination of ultrasonic vocalizations induced by stress such as maternal separation [11], while novel techniques include the use of radiotele metry to asses a variety of physiological parameters in real time (e.g. core body temperature [12].

In recent years, various types of herbal medicines have been used as anxiolytic drugs in different parts of the world. The essential oil of *Stachys lavanduifolia* has been reported to possess anxiolytic effects with relatively lower sedative activity than diazepam [13].

Paullina pinnata Lin is a woody or sub-woody climber of damp sites and stream-banks of the forest with tendrils, imparipenate leaves (2 pairs+1 leaf) that is wide spread in tropical Africa and Madagascar.

Paullina pinnata is popularly known as 'Cheese and Butter", "Five finger", "Five leaves"(English),"Yaatsa biyar", "Hannu biyar" Furen amarya", "Goron dorina"(Hausa) "Kakansela" (Yoruba),"Enu-kakanchela" (Nupe), "Okpanam" (Igbo).

The plant has various applications in traditional medicine, For instance, the leaf infusion is used to treat dysentery, diarrhea [14], aches, stiffness, lumbago and rheumatism [15]. It is also used in the treatment of mental disorders, sterility and tonsillitis [16]. *Paullinia pinnata* has also been reported to be used in treatment of tetanus in children, snake bite and paralysis [15].

Interested in investigating the pharmacological properties of *Paullinia pinnata* extracts. The intraperitoneal LD_{50} of the methanolic leaf extract in mice was reported to be 471.2mg/kg and that the extract demonstrated sedative effects in mice [17].

These effects were observed as a pronounced increase in diazepam induced sleeping time in mice and a decrease in exploratory behavioral pattern by the head dip test. Taking the above evidence into account, the aim of the present study was to evaluate the anxiolytic

effects of the methanol extract of *Paullinia pinnata* leaves using the elevated plus-maze (EPM) and staircase tests in mice.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh leaves of *Paullina pinnata* Linn were collected from Samaru village, Zaria, Kaduna state Nigeria. The taxonomical identification of the plant was done by Mal. Musa of the herbarium unit, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. A voucher specimen No 427 was then preserved for future reference.

2.2 Preparation of Plant Extract

Fresh leaves of *Paullinia pinnata* were cleaned and air- dried for seven days under shade. The dried leaves were crushed into course powder using pestle and mortar**.** One kilogram (1kg) of the powdered leaves was cold macerated with 1L methanol at room temperature for 24hrs with shaking. The resultant mixture was then filtered using Whatman's filter paper No. 1 and the filtrate concentrated to dryness using water bath with a yield of 11%w/w as crude methanol extract. Aliqout portions of the plant extract residue were weighed and dissolved in distilled water for use on each day of the experiment.

2.3 Phytochemical Analysis

Phytochemical screening of the methanolic leaf extract of *Paullinia pinnata* was performed according to standard laboratory procedures [18].

2.4 Animals

Swiss albino mice (20-25g) of either sex maintained at the animal house of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria were used. Animals were kept and maintained under laboratory conditions of temperature, humidity, and light and were allowed free access to food (Standard pellet diet) and water *ad libitum.*

2.5 Behavioural Parameters Used to Test Anxiolytic Activity

2.5.1 Elevated plus-maze test (E.P.M)

The EPM consisted of two open arms (35x5cm) crossed with two closed arms (35x5x20 cm). The arms were connected together with a central square of 5x5cm. The apparatus was elevated to the height of 25cm in a dimly illuminated room. Five groups of six mice each were used in this experiment. The first three groups were treated with the extract (25, 50 and 100mg/kg, i.p), the fourth group received diazepam (2mg/kg, i.p) and the last group received normal saline 30 min before being placed individually in the center of the EPM, facing a closed arm. The time spent in both the open and closed arms was recorded for 5min. The numbers of entries into the open and closed arms were also counted during the test. An entry was defined as having all four paws within the arm [19].

2.5.2 Staircase test

The staircase test was carried out according to the method of [20]. It consisted of placing an experimentally naïve mouse in an enclosed staircase with five steps (2.5x10x7.5cm). The apparatus was 45cm in length, with one end 12cm and the other 25cm in height. Five groups of six mice each were used in this experiment. The first three groups were treated with the extract (25, 50 and 100mg/kg, i.p), the fourth group received diazepam (0.25mg/kg, i.p) and the last group received normal saline. After 30 min of drug administration each mouse was placed individually on the floor of the box with its back on the staircase, and then the behavior was videotaped. The numbers of steps climbed and rearing were counted for 3 min. A step was considered to be climbed only if the mouse had placed all four paws on the step. Rearing was recorded when the mouse rose on its hind legs either on the step or against the wall to sniff the air. The number of steps descended was not counted (Plates 1 and 2).

Plate 1. Front View of Stair case Apparatus

2.5.3 Statistical analysis

In all cases results were expressed as mean±SEM and analyzed for statistical significance using Student's *t*-test. A P value ≤0.05 was considered significant.

Plate 2. Side view of stair case apparatus

3. RESULTS

3.1 Phytochemical Screening

Phytochemical screening of the methanolic leaf extract of *Paullinia pinnata* revealed the presence of alkaloids, tannins, flavonoids, carbohydrates and cardiac glycosides, while negative reactions were recorded for saponins, anthraquinones and steroid/triterpenes.

3.2 Elevated Plus-maze Test

Animals treated with normal saline spent 4.60±3.89 sec in the open arm and 162.40±10.58 sec in the closed arm, with 0.40±0.23 entries into the open arm and 10.60±2.16 into the closed arm. Diazepam and extract (50mg/kg) caused significant ($P<0.05$) increase in the occupancy in the open arm. The extract at all doses tested did not cause a significant decrease in the time spent in the closed arm. Mice treated with diazepam and extract 50mg/kg showed a decreased preference for the closed arm and significantly ($P<0.05$) increased entries into the open arm (Table 1).

3.3 Staircase Test

The extract at the doses of 25, 50 and 100mg/kg significantly (P<0.05, P<0.01 and P<0.005, respectively) and dose dependently decreased the number of steps ascended compared to the control. There was however no significant change in the mice treated with diazepam (0.25mg/kg) on the stair climbing parameter. The extract also induced significant (P<0.05 and P<0.0005) and dose-dependent decrease in the number of rearing events while diazepam significantly (P<0.0005) reduced the number of rearing events compared to control (Table 2).

Table 1. Effect of *P. pinnata* on animals' stay in the open and enclosed arms of the elevated plus-maze in mice

Treatment *(*i.p*)*	Time spent in the open arm (sec.)	Time spent in the enclosed arm (sec)	Entries into open arm	Entries into enclosed arm
N/Saline	4.60±3.89	162.40±10.58	0.40±0.23	10.60±2.16
P.P 25mg/kg	11.83±7.33	188.67±12.89	1.33±0.80	11.50±1.48
P.P 50mg/kg	30.00±13.63[a]	154.67±9.55	2.33±0.76[a]	7.67±1.20
P.P 100mg/kg	7.50±6.55	201.13±21.43	0.67±0.49	6.5±1.06
Diazepam 2mg/kg	54.83±15.03[b]	116.17±5.90	6.50±2.36[a]	13.83±1.70

Values are Mean±SEM (n=6), Student's t-test, a=P<0.05, b=P<0.005 compared to control, P.P=Paullinia pinnata.

Table 2. Effect of *P. pinnata* extract in the staircase test in mice

Treatment (i.p)	Stairs climbed (counts/3min)	Rearing (counts/3min)
N/Saline	31.67±6.50	24.83±3.71
P.P 25mg/kg	13.50±3.21[a]	8.00±2.45[c]
P.P 50mg/kg	11.67±1.63[b]	2.67±0.42[d]
P.P 100mg/kg	6.00±2.06[c]	1.50±0.22[d]
Diazepam 0.25mg/kg	34.00±2.10	4.70±1.20[d]

Values are Mean±SEM (n=6), Student's t-test, a=p<0.05, b=p<0.01, c p<0.005, d=p<0.0005 compared to control. P.P=Paullinia pinnata.

4. DISCUSSION

The validity of the EPM test for evaluation of anxiolytic or anxiogenic effects of drugs has been well documented [21]. Exposure to the elevated plus maze test induces behavioural and physiological effects in rodents consistent with fear and anxiety. The animal is placed in the centre of an elevated four-arm maze where only two of the arms are enclosed and the reduction of time spent in the open arms reflects increased anxiety. The benzodiazepines are considered the drug of choice in the treatment of anxiety. Unfortunately, there are several side effects like sedation and myorelaxation that are considered as unwanted effects in an anxiolytic drug [22]. These agents are known to act through the BZD-GABA receptors. The role of GABA in anxiety is well established [23].

Several plants that are used in folk medicine to diminish anxiety are reported to bring about an increase in the exploration of the open arms in the EPM test [24]. In EPM, naïve mice will normally prefer to spend much of their allotted time in the closed arms. Drugs that increase open arm exploration are considered as anxiolytic and the reverse holds true for anxiogenic

[25]. In this study, P.P 50 mg/kg induced significant ($P<0.05$) increases in both the number of entries and time spent in the open arms. As expected, in the present work, the treatment with diazepam, a benzodiazepine anxiolytic drug, led to a significant ($P<0.05$ and $P<0.005$, respectively) increase in the entries and time that mice spent in the open arms of the EMP.

The mouse staircase paradigm is a relatively simple and efficient procedure for screening anxiolytic agents. It combines step-climbing, which serves as an index of exploratory and locomotor activity, and rearing, which serves as an index of anxiety [20]. In line with previous data diazepam induced anxiolytic effect in the stair-case test that was reflected by a decrease in the number of rearings without effect on the number of steps climbed [20]. Another study also reported that diazepam at doses of 0.25 and 0.5 mg/kg did not have inhibitory effect on the locomotor activity of control mice [26].

The results obtained in the staircase test showed that the methanolic leaves extract of *Paullinia pinnata* significantly ($P<0.05$-$P<0.0005$) and dose-dependently reduced both climbing and rearing activities, while diazepam only significantly ($P<0.0005$) reduced the rearing event without affecting the climbing parameter. The reduction of climbing parameter could be explained by the general reduction of locomotion after extract treatment (which is accordance with reduction of rearing numbers).

It is noteworthy that suppression of both rearing and climbing behavior has been induced by non-benzodiazepine psychotropic agents, such as antipsychotics, tricyclic antidepressants, and buspirone, but a dissociation of the two behaviors (i.e., suppression of rearing but not climbing) occurred only in agents with agonistic activity at the $GABA_A$/benzodiazepine receptor/chloride ion channel complex [20]. However such specificity of rearing activity for agents active at benzodiazepine has not been demonstrated in all studies [27,28].

Earlier reports on the chemical constituents of plants and their pharmacology suggest that plants containing flavonoids, saponins and tannins possess activity against many CNS disorders [29]. The anxiolytic effect of methanolic leaf extract of *Paullinia pinnata* may be related to presence of some of these phytochemical constituents as observed in the phytochemical screening.

In conclusion, the preliminary experimental evidence obtained in this study indicates that *Paullinia pinnata* methanolic leaf extract possesses anxiolytic property and has potential clinical application in the management of anxiety disorders. Further investigation of the mechanism (s) of action as well as active substance (s) responsible for biological actions may be necessary.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No.85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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