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Full Length Research Article

ACTIONS OF HYDRO-METHANOL LEAF EXTRACT OF BRYOPHYLLUM PINNATUM (CRASSULACEAE) ON MOTOR COORDINATION AND EXPLORATORY ACTIVITIES IN MICE

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ABSTRACT

This study was carried out to evaluate the effect of the hydro-methanol leaf extract of Bryophyllum pinnatum(family:Crassulaceae) on motor coordination and exploratory behaviour in mice weighing between 25-30g. The LD50 of the extract was calculated to be 400mg/kg and the various doses administered were 25mg/kg, 50mg/kg, and 100mg/kg. The parameters evaluated were the hole board test for explorative activity, beam-walking assay for motor coordination deficit. The results revealed that the extract showed a statistical significant ($P<0.05$) increase in the number of foot slips and beam latency at 25mg/kg and 50mg/kg. It also showed a decrease in head dips but was not significant.These results showed that the extract possesses some central nervous system (CNS) depressant activity which may be due to the effect or activity of one or more of the phytochemicals present in the plant.

Key words: Bryophyllum pinnatum, Diazepam, Beam walk, Hole board, Phytochemical screening.

INTRODUCTION

In developing countries, remedies from plants play an important role in the health care of millions of people. People still depend largely on traditional healing practices despite the advancement in modern medicine (Nwabuisi, 2002: Ojewole, 2005). An important area in which herbal medicines enjoy usage worldwide is in the management of nervous system disorders. Scientists believe that natural product selection of plants with diverse application in traditional medicine might be encouraged by their easily noticeable central nervous system(CNS) effects (Amos *et al*., 2001). *Bryophyllum pinnatum* is a fleshy shrub, which grows 2-4 feet tall. The plant, which belongs to the family *Crassulaceae*. It is grown for ornamental and medicinal uses (Burkill, 1985). It is a perennial herb growing widely and used in folkloric medicine in tropical Africa, India, China, Australia and tropical America (Engler, 1926; Balzer, 1949). The plant is classified as a weed (Oliver-Bever, 1983), it grows throughout the Southern part of Nigeria (Gill, 1992).It is popular known as "ewe abamoda or odundun" by the Yoruba tribe of Southwestern Nigeria, "odaa opue" among the Igbos, "da bu si" in Chinese (Ghasi *et al.*, 2011; Iwu, 1993) and "sutura" by the Hausa peoples of Nigeria.

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The leaves of *Bryophyllum pinnatum* have been reported to possess antimicrobial (Mehta and Bhat, 1952; Akinpelu, 2000; Oliver-Bever, 1983), antihypertensive (Ojewole, 2002), anti ulcer (Pal and Nag, 1991), anti-inflammatory and analgesic (Pal and Nag, 1989; 1992), and antifungal (Misra and Dixit, 1979)activities.

MATERIALS AND METHODS

Animals

A total of sixty-three (63) adult mice of both sexes weighing between 25 to 30g were used. They were obtained from the Animal House laboratory of the Department of Pharmacology and Clinical Pharmacy, ABU, Zaria, and were kept in the laboratory for 7 days before use. They were placed on standard feed and allowed free access to food and water.

Plant Material Identification

Fresh leaves of *Bryophyllum pinnatum* were collected around Zaria, northern Nigeria. The identification and authentication of the plant was carried out at the Herbarium unit of the Department of Biological Science, Ahmadu Bello University, Zaria, where a specimen was deposited with a voucher number, 1834.

Preparation of the Plant Extract

The fresh leaves of *Bryophyllum pinnatum* were collected and dried under shade and ground into powder. The powder (407.23g) was macerated in 30 % of distilled water and 70 % methanol at room temperature for 24 hours. It was then filtered using a filtered paper (Whatmann size no.1), and the filtrate evaporated to dryness in water bath at 60°C. A greenish residue weighing 11.93g was obtained. This was kept in air tight bottle in a refrigerator until used.

Acute Toxicity Studies of the Extract of *Bryophyllum pinnnatum* (leaves) in Mice

The intraperitoneal median lethal dose (LD_{50}) of the plant extract was conducted and calculated in mice according to the method of Lorke (1983) using thirteen (13) mice. In the initial phase, three (3) groups of three mice each was treated with the extract at doses of 10mg/kg, 100mg/kg and 1000mg/kg body weight intraperitoneally and observed for signs of toxicity and death for 24 hours. In the second phase, animals were divided into four (4) groups each containing a mouse and were injected intraperitoneally with four more specific doses of the extract at 20mg/kg, 40 mg/kg, 80 mg/kg and 160 mg/kg based on the result of the first phase. The LD_{50} was calculated as the square root of the product of the maximum dose for all survival and minimum dose for all death.

Preliminary phytochemical screening

The extract of the leaves *Bryophyllum pinnatum* was subjected to preliminary phytochemical screening tests according to the method described by Trease and Evans (1989).

Chemicals and drugs used

All chemicals and drugs used were of analytical grade. Diazepam was purchased from La Roche Ltd. Basel, Switzerland.

Experimental procedure

Test for exploratory activity (Hole Board Test)

Twenty five (25) mice were randomly divided into five groups of 5 mice each. The first group was administered normal saline which served as a negative control. Mice in the second group received diazepam 1.5mg/kg which served as a positive control, while third and fourth and fifth groups received the extract at the doses of 25mg/kg, 50mg/kg and100mg/kg respectively. The exploratory activities of the extract in mice following intraperitoneal administration was determined using the Hole board test (File and Wardil, 1975). The apparatus consists of a white wooden board (40 x40cm) with four equidistant holes (1cm diameter x 2cmdepth).Each mouse was placed singly at one corner of the board. It was allowed to move about and dip its head into the holes. Poking the nose into a hole is a typical behaviour of the mouse indicating a certain degree of curiosity. The number of dips in five minutes (enough time to exhibit curiosity otherwise) was recorded. The test was carried out 30 minutes after intraperitoneal treatment with the extract at the doses 25mg/kg, 50mg/kg and 100mg/kg.

Test for motor coordination (Beam Walk Assay)

Mice were allowed to walk from a start platform along a ruler (80cm long and 83cm wide) elevated 30cm above the bench by metal supports to a goal box. Several trials were performed for each mouse and designed such that the mice tested are aware that there is a goal box that could be reached. A ruler was used because the mice find it easy to cross and at the same time, it will induce minimum anxiety (Stanley *et al.,* 2005). Twenty five (25) mice were randomly divided into five groups of 5 mice each. The first group received normal saline which served as a negative control. Mice in the second group received diazepam 1.5mg/kg which served as a positive control, while third, fourth and fifth groups received the extract at doses of 25mg/kg, 50mg/kg and 100mg/kg. Once the animals were tested on the ruler, they were moved immediately to the beam test. The beam is made of wood, 8mm in diameter, 60cm long and elevated 30cm above the bench by a metal support. The animals were placed at one end of the beam and allowed to walk to the goal box thirty minutes after treatment with the extract. Mice that fell were returned to the position they fell from with a maximum time of 60 seconds allowed on the beam. The number of foot slips (one or both hind limbs slipping from the beam were recorded with the aid of tally counter. The number of foot slips is a measure of motor coordination deficit (Stanley *et al.,* 2005).

Statistical analysis

The data was statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey's Post-Hoc test.The data obtained from the experiment were expressed as Mean ± SEM. The values of $p < 0.05$ were considered as significant (Duncan *et al.,* 1977).

RESULTS

Preliminary Phytochemical Screening

The preliminary photochemical screening of the hydro-methanol extract of *Bryophyllum pinnatum* conducted indicated the presence of tannins, saponins, flavonoids, cardiac glycosides, glycosides and sugars (carbohydrate)

Table 1. The percentage mortality of the different doses of the extract of Bryophyllum pinnatum (leaves) administered intraperitoneally in mice during the first phase ofacute toxicity study

Group	Dose(mg/kg)	Deaths	Mortality (%).
1	10	0/3	0
2	100	2/3	66.67
3	1000	3/3	100

Table 2. The percentage mortality of the different doses of the extract of Bryophyllum pinnatum (leaves) administered intraperitoneally in mice during the second phase of acute toxicity study

Group	Dose(mg/kg)	Deaths	Mortality (%)
1	20	0/1	0
2	40	0/1	0
3	80	0/1	0
4	160	0/1	0

Acute Toxicity Studies (LD_{50})

Toxicity signs noticed upon administration of the extract include decrease motor activity, decreased food intake and death. The median lethal dose of extract in mice was calculated to be 400mg/kg body weight intraperitoneally.

Table 3. Effect of hydro-methanol leaf extract of Bryophyllum pinnatum on motor coordination (Beam walk assay) in mice

Treatment / Dose		Number of foot slips	Latency (s)
Normal saline	1ml/kg	0.40 ± 0.24	14.20 ± 2.65
Diazepam	1.5mg/kg	9.80 ± 1.62*	50.60 ±2.64*
Extract	25mg/kg	7.20 ± 0.66*	38.40 ± 6.28*
Extract	50mg/kg	7.0 ±1.00*	57.80 ± 2.20*
Extract	100mg/kg	3.0 ± 0.95	31.20 ± 9.16

Table 4. Effect of hydro-methanol leaf extract of Bryophyllum pinnatum on exploratory behaviour (Hole board) in mice

Treatment / Dose		Number of head dips in 5 minutes
Control	1ml/kg	15.60 ± 1.81
Diazepam	1.5mg/kg	10.20 ±3.80
Extract	25mg/kg	8.80 ± 2.60
Extract	50mg/kg	14.80 ± 3.84
Extract	100mg/kg	11.00 ± 2.68

Beamwalk assay

The administration of 25mg/kg and 50mg/kg showed a statistical significant increase ($P<0.05$) in the foot slips when compared with control group that received normal saline. The diazepam group also increased the number of foot slips significantly ($P<0.05$). The extract also showed a statistical significant increase ($P<0.05$) in the time it took the mice to traverse the beam (latency) at doses 25mg/kg and 50mg/kg. The diazepam group also increased the latency period significantly ($P<0.05$). The extract at 100mg/kg increased both foot flips and latency but was not statistically significant when compared to the control.

Explorative Behaviour (Hole board test)

The extract at the different doses decreased the number of head dips in mice when compared to the control. Diazepam also caused a decrease in the number of head dips when compared to the control. Results obtained from the different doses of the extract and Diazepam however did not show any statistical significant decrease in the parameter been investigated.

DISCUSSION

The hydro-methanol leaf extract of *Bryophyllum pinnatum* significantly increased the number of foot slips and also the time taken to traverse the beam (latency) at lower and intermediate doses of 25 and 50mg/kg respectively. The increase or decrease of foot slips observed have been found to be a sensitive measure of determining benzodiazepines-induced motor coordination deficits (Stanley *et al.,* 2005). The increase in the number of foot slips observed in this study suggests that the extract may possess a potent sedative property. The results obtained showed that the extract at the different doses decreased the number of head dips in mice when compared with the control, though it was not statistically significant. File and Wardill (1975) reported that the hole-board experiment is a measure of exploratory behavior in animals. A decrease in this parameter indicates a sedative behaviour and a high propensity for antipsychotic action (Feilding and Lal, 1978; File and Pellow, 1985). Crawley (1985) reported that this parameter has been accepted for the evaluation of anxiety conditions in animals. According to Adzu (2002) and Viswanatha *et al.,* (2006), a decrease in exploratory behaviour in mice is a measure of CNS depression as demonstrated by the reduction in number of head dips. Thus, the decrease in exploratory behavior upon administration of the hydro-methanol leaf extract further supports the neurosedative property of the plant as reported by Salahdeen and Yemitan, (2006) and its possible application in anxiety conditions.

Conclusion

The neurobehavioral investigation of the hydro-methanol leaf extract of *Bryophyllum pinnatum*in mice showed that the extract possesses some CNS depressant activities as observed in the decreased head dipping in the Hole Board test andan increase in the number of foot slips and also an increase in the time taken to traverse the beam in the beam walk assay conducted.

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