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Anxiolytic activity of hydroalcoholic leaf extract of *Morinda lucida* in rats using experimental models of anxiety

Bakou Niangoran François[1], BA Abdoulaye[2], Atayi E[3]
[1] Unit of Animal Physiology, Jean Lorougnon GUEDE University, Daloa, Côte d'Ivoire
[2] Laboratory of Neuroscience, UFR Biosciences, Felix HOUPHOUET-BOIGNY University, Abidjan, Côte d'Ivoire
[3] Neurology Service, Functional Exploration Unit of the Nervous System, C.H.U. from Cocody-Abidjan, Côte d'Ivoire

Abstract
Objective: The aim of the present work is to evaluate the anxiolytic effect of hydroalcoholic extract of leaves from *Morinda lucida* in rat.
Materials and Methods: The hole-board test, elevated plus-maze paradigm and open field test, were used to assess the anxiolytic activity of hydroalcoholic extract of leaves from *Morinda lucida*. The extract of *Morinda lucida* (5, 10, and 25mg/kg, i.p.) and diazepam (1 mg/kg, i.p.) were administered 30 min before the tests.
Results: The results showed that extract of R.V(10 and 25 mg/kg, i.p) significantly increased the number and duration of head poking in the hole-board test. In the elevated plus-maze, the extract significantly increased the exploration of the open arm in similar way to that of diazepam. At a dose of 10 and 25 mg/kg i.p. the extract significantly increased both the time spent in and the entries into the open arm by rat. Further, in the open field test, the extract significantly increased rearing, assisted rearing, and number of squares traversed, all of which are demonstrations of exploratory behavior.
Conclusion: The results of the present study suggest that a hydroalcoholic extract of *Morinda lucida* leaves may possess an anxiolytic effect.

Keywords: *Morinda lucida*, leaves, rat, anxiety

1. Introduction
Neuropsychiatric disorders form 10.5% of global burden of disease (disability-adjusted life year) and it is suggested that this could increase to 15% by 2020 [1] It will be the third leading cause of disability in low-income countries [2]. Anxiety is a normal emotion under stressful circumstances but may become maladaptive when it constitutes a psychiatric disorder. Recent trends suggest that anxiety disorders may be more important than originally thought and have become a prominent area of research interest. Benzodiazepines are most commonly prescribed class of compounds for anxiety despite many unwanted side effects such as sedation, muscle relaxation, ataxia, amnesia, and tolerance on chronic use. These may also cause ethanol and barbiturate potentiation [3]. Therefore, finding novel therapeutic agents with fewer complications in the treatment of anxiety disorder, is of major interest to researchers [4]. Despite a phenomenal development of modern drug industry, medicinal plants with traditional background of use in neurological diseases could be good candidates to find new anxiolytic agents. *Morinda lucida* (Benth), a member of Rubiaceae family, is among important medicinal plants in Ivorian traditional medicine. It is widely distributed in West Africa and is used in African folk medicine to treat several diseases [5]. The leaves are bitter and are used by the natives to treat malaria, yellow fever, jaundice, hepatitis, eczema, edema, cough, hypertension, diabetes, and sexual weakness [6, 7, 8]. In some cases, the plant is employed in the treatment of cerebral congestion, dysentery, stomach ache, ulcers, leprosy, and gonorrheal [9].

Literature survey revealed a variety of pharmacological actions such as trypanocidal, antimalarial, hypnotic, analgesic, anti-inflammatory, antipyretic activities for these plants [10, 11, 12, 13]. No scientific report regarding the in vivo anxiolytic activity of *Morinda lucida* extract (*ML*) has been published. That's why, the present study was undertaken to assess the possible anxiolytic effects following single administration of hydro ethanolic extract of leaves from *Morinda lucida* in rat. For this purpose, we used the elevated plus-maze, Hole board and open field tests.

2. Materials and Methods
Plant material
Fresh leaves of the plant were collected from Daloa, (Cote d'Ivoire) in November, 2019. The plant was identified and verified by botanist Professor from Jean Lorougnon GUEDE university of Daloa (Cote d'Ivoire). The collected leaves were dried under a shade during two weeks and pulverized using the crushing assistance (IKAMAG RCT®). The powder of leaves obtained, constituted our sample to be analyzed.

Extract preparation
A 100 g sample of crushed *Morinda lucida* was used for extraction. The sample was soaked overnight in 70% alcohol (30:70) and filtered using Whatman No.1 paper. The process was repeated twice by adding fresh solvent every time. The pooled extract was subjected to flash evaporation followed by lyophilization. The lyophilized sample was further analyzed to determine its anxiolytic property.

Animals

25 males Wistar rats aged 8-10 weeks weighing (145 - 250 g) were obtained from the animal house of Jean Lorougnon GUEDE University, Daloa. These animals were housed under standard environmental conditions. The rats were fed with FACI® (Fabrication d'Aliments de Côte d'Ivoire) pellets, groundnuts and dried fish. They had free access to drinking water ad libitum.

Drugs and chemicals

The standard drugs Diazepam and saline water were collected from Square Pharmaceuticals Ltd., Cote d'Ivoire. Distilled water which was used for dilution purpose was prepared was obtained from Jean Lorougnon GUEDE university of Daloa (Cote d'Ivoire).

Behavioral parameters used to test anxiolytic activity

Open field test

Locomotor activity and exploratory behavior were assessed in an open field by the method described by Souza [14]. The apparatus consisted of a wooden box (60 × 60 × 30 cm^3) with the floor divided into 16 squares (15 × 15 cm^2). The apparatus was illuminated with a 40-W lamp suspended 100 cm above. Twenty-five rats were randomly divided into five groups of five rats each. One hour before test session, rats were treated with graded doses of *ML* (5, 10, and 25 mg/kg, i.p.) while the control received 10 ml normal saline/kg i.p. 30 min later each rat was placed individually in the center of the apparatus and observed for 5 min to record the locomotor (number of squares crossed with four paws) and exploratory activities (indicated by frequency of rearing and assisted rearing) [14].

Elevated plus-maze test

The elevated plus maze is an anxiety paradigm based on the rodent's natural aversion to a novel and potentially dangerous environment represented by the open and elevated spaces [15]. The elevated plus maze apparatus is a plus (+) shaped wooden structure, consisting of two open arms (40×5×10 cm3) and two enclosed arms (40×5×10 cm3) extended from a central platform (10×10 cm2). The maze was elevated 50 cm from the room floor. Rats were randomly divided into five groups. The rats that served as control group received 10 ml normal saline/kg body weight i.p, while the treated rats received *ML* (5, 10, and 25 mg/kg body weight i.p) and diazepam (1 mg/kg body weight i.p.). Thirty minutes after intraperitoneal administration of diazepam, each rat was placed at the center of the maze, facing one of the open arms and allowed to explore the maze freely for a 5-min testing period. The time spent in open and enclosed arms were recorded. The maze was thoroughly cleaned between tests with a tissue paper moistened with 70% ethanol.

Hole board test

The hole board apparatus consisted of a wooden chamber (40 × 40 × 25 cm^3) with 16 holes (each of 3 cm diameter) evenly distributed on the floor. The apparatus was elevated to a height of 25 cm from the ground so that the rat could peep through the holes. The rats were treated with *ML* (5,10, and 25mg/kg body weight i.p), diazepam (1 mg/kg body weight i.p) or saline water (10ml/kg body weight i.p) 30 min prior to test and kept in the apparatus. The numbers and the duration of head poking were recorded during the 5 min observation period

Statistical analysis

Results are expressed as mean ± S.E.M. The statistical analysis of data was done using the one-way analysis of variance (ANOVA) followed by Dunnett's test. A probability level less than 0.05 was considered statistically significant.

3. Results

Open field test

There was significant (*P<0.05) increase in rearing, assisted rearing and number of squares traveled in the *ML* treated (5 to 25mg/kg i.p.) as well as the standard group, compared to the control group, in the open field test. The numbers of assisted rearing in the groups treated with *ML* at 5 and 10 mg/kg was comparable with the standard and at 25 mg/kg, the count was significantly (*P<0.05) higher than the standard group. The number of squares traveled also significantly (*P<0.05) increased when *ML* was administered at 5,10 and 25mg/kg, showing anxiolytic activity of the plant extract. (Table 1)

Table 1: Effect of *ML* on rearing, assisted rearing and squares traveled in open field test (n=5). Values are expressed as mean ± SEM.

Treatment	Rearing	Assisted rearing	Number of square traversed
NaCl 10ml	5.5±1.2	8±1.6	7±1.8
DZP1mg/Kg	8.1±2.3*	14±1.8*	32±2.6*
*ML*5 mg/Kg	8.75±2.6*	15.66±2.1*	14±2.1*
*ML*10 mg/Kg	9.33±5.2*	16.2±1.9*	16.5±2.3*
*ML*25 mg/Kg	10.5±6.4*	16.33±2.3*	17.2±2.6*

Elevated plus maze test

In this test, there was significant (*P<0.05) increase in the number of entries and the time spent in the open arm *ML* (5 to 25 mg/kg, i.p) and diazepam treated groups, whereas there was a significant (*P<0.05) decrease in the time spent in the close arm compared to the control group. Numbers of entries into the open arm in *ML* treated animals with 25mg/kg dose were higher than the standard drug. (Table 2)

Table 2: Effect of *ML* on time spent (s) in open arm, time spent (s) in closed arm, entries in open arm and entries in closed arm in elevated plus maze test (n=5). Values are expressed as mean ± SEM.

Treatment	Time spent in the open arm (s)	Time spent in the enclosed arm (s)	Entries into open arm	Entries into enclosed arm
NaCl 10ml	40±2.5	267±11.2	6.31±1.15	14.21±1.6
DZP1mg/Kg	93±4.14*	58±2.6*	8±1.6*	13.8±1.12
*ML*5 mg/Kg	87±3.6*	247±10.6	7.17±2.01	13±1.13
*ML*10 mg/Kg	89±3.8*	212±11.4	9.31±2.03*	11±2.01
*ML*25 mg/Kg	91±4.01*	79±3.06*	10±2.6*	9.6±2.2*

Hole board test

There was significant (**P<0.01) and dose-dependent increase in the number and duration of head poking after administration of *ML* (10 and 25 mg/kg, i.p) compared to the control group and the results were comparable with the standard drug diazepam. (Table 3)

Table 3: Effect of *ML* on head poking and duration (s) of head pokes in hole board test (n=5). Values are expressed as mean ± SEM.

Treatment	Dose route	Duration of head poking (s)	Number of head poking
NaCl	10ml	32±1.06	37±1.1
DZP	1mg/Kg	58±1.8	65±2.6
ML	5 mg/Kg	38±1.12	40±1.6
ML	10 mg/Kg	62±2.5**	57±1.6**
ML	25 mg/Kg	69±2.7**	67±2.4**

4. Discussion

The open-field apparatus provides information on anxiety-related behaviour characterized by natural aversion of rodents to an open brightly lit area [16]. Animals are thus afraid of the centre and spend more time in the protective corners and in freezing state. Anxiolytics increase total locomotive activity resulting in a reduction of time spent in corners, an increased time spent in the center and a decreased time spent in freezing state. The extract of *ML* at 5,10 and 25 mg/kg body weight increased total locomotive activity and increased rearing of treated rats in our study. The elevated plus maze (EPM) test represents one of the most widely used animal models for screening anxiolytics [15]. This test is able to reproduce anxiolytic or anxiogenic effects in rodents such that anxiolytics produce increase the time spent in the open arm of the elevated plus maze, while anxiogenic substances produce the opposite effect [15, 17]. The indices of anxiety (number of open-arm entries, and time spent in the open arm) are sensitive to agents and are thought to act via the GABAA receptor complex, justifying the use of diazepam (DZP) as a positive control in this study. Diazepam, a benzodiazepine binds to GABAA receptors to increase the frequency of chloride channel openings resulting in hyperpolarization. It increased the frequency of open-arm entries and the time spent in the open arms [18], confirming its anxiolytic effects. In our study, we observed that *ML* (5,10 and 20mg/kg) induced significant increases in the both the number of entries and time spent in the open arms. The number of entries and the time spent in the closed arms were reduced in the extract-treated group as compared to the control group. Hole-board test indicated that the head-dipping behavior was sensitive to changes in the emotional state of the animal, and suggested that the expression of an anxiolytic state may be reflected by an increase in head-dipping behavior [19]. In our study, *ML* (5,10 and 20mg/kg) significantly increased the numbers and duration of head poking compared to the control group. These results confirm the anxiolytic effects of *Morinda lucida*. They are to be compared with the work of Nsour [20], who in a similar study showed the anxiolytic effect of *Rauvolfia Serpentina* [21].; from Aidee, which highlighted the anxiolytic effects of the ethanolic extracts of *Argemone Mexicana* [22]; from Carla, which demonstrated anxiolytic properties of aqueous extracts of *Salvia miltiorrhiza* in rats; Charles[23] and Carnevale[24] who showed anxiolytic properties of extracts of *Maerua angolensis* in mice and *Griffonia simplicifolia* in rat.

5. Conclusion

In conclusion, the results obtained in our study suggest that the extract of the leaves of *Morinda lucida* possesses anxiolytic activity, which is possibly mediated through the GABA A -BZD mechanism. Thus, *Morinda lucida* has potential clinical application in the management of anxiety disorders. Further investigation of the mechanism(s) of action of the plant extract, as well as the active substance(s) responsible for its biological actions, is necessary.

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