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# ANXIOLYTIC PROPERTY OF HYDRO-ALCOHOL EXTRACT OF MORINGA OLEIFERA AND ITS EFFECT ON BEHAVIORAL ACTIVITIES OF RAT

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## ABSTRACT

**Objective:** The aim of the present work is to evaluate the anxiolytic effect of hydroalcoholic extract of leaves from *Moringa oleifera* 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 *Moringa oleifera*. The extract of *Moringa oleifera* (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 *Moringa oleifera* (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 *Moringa oleifera* leaves may possess an anxiolytic effect.

**KEYWORDS:** *Moringa oleifera*, leaves, rat, anxiety activity.

## INTRODUCTION

Anxiety disorder is a most common psychological behavioral disorder globally.[1] It leaves negative impact on the quality of life with life time prevalence about 15% and spontaneous remission about 14%.[2] This probably raise questions over the efficacy of the current therapeutic treatment or lack of understanding the neurobiological pathophysiology of anxiety disorder.[3] In general, anxiety disorders can be treated with certain psychotherapeutic drugs. Sedatives and hypnotics are medications that can reduce anxiety and induce the onset of sleep and maintain sleep time.[4] Benzodiazepines are commonly used because of their muscle-relaxant, sedative-hypnotic, and anticonvulsant effects.[5] However, the continued use of these currently available sedative-hypnotic treatments has serious side effects, from respiratory, digestive, and immune system dysfunctions to impaired cognitive function, physical dependence, and tolerance.[6] Therefore, finding novel therapeutic agents with fewer complications in the treatment of anxiety disorder, is of major interest to researchers.[7] Medicinal plant with traditional background of use in neurological diseases could be good candidates to find new anxiolytic agents. *Moringa oleifera* Lam. *(M. oleifera*) is a cruciferous plant that belongs to the Moringaceae family. *M. oleifera* is commonly called horseradish tree or drumstick tree by locals and is a popular staple in different parts of the world. *M. oleifera* is consumed not only for its nutritional values but also its medical benefits,[8] It has traditionally been widely used to improve health. Kings and queens used *Moringa* to improve their alertness and to maintain healthy skin. Other traditional uses of the genus are in healing skin infections, anxiety, asthma, wounds, fever, diarrhea, and sore throats.[9] Currently, *M. oleifera* is reported to enhance a broad range of biological functions including anti-inflammatory, anti-depression, anti-convulsivant, anti-diabetes, anti-cancer, hepatoprotective, and neuroprotective functions.[8,10,11,12,13] No scientific report regarding the in vivo anxiolytic activity of *Moringa oleifera* extract 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 *Moringa oleifera* in mice. For this purpose, we used the elevated plus-maze, Hole board and open field tests.

## 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 *Moringa oleifera* 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 healthy adult male Swiss albino mice weighing (20–30 g) were obtained from the animal house of Jean Lorougnon GUEDE University, Daloa. These animals were housed under standard environmental conditions. The mice 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

### Elevated plus-maze test

The elevated plus-maze (EPM) test consisted of two open arms (30 × 5 × 0.25 cm) and two closed arms (30×5×15 cm) emanating from a common central platform (5×5 cm). Two pairs of identical arms were opposite to each other. The entire apparatus was elevated to a height of 40 cm above floor level. At the beginning of the session, a mouse was placed at the centre of the maze, its head facing an open arm and allowed to explore the maze for 5 minutes, and the following parameters were scored: the time spent and number of entries in each type of arms. The plus maze was carefully cleaned with a wet towel after each animal test. The mice were randomly divided into five groups (5 mice/group). The control group received vehicle (saline water 0.1mL/mice). Diazepam (1 mg/kg b.w., IP) was used as the positive control or standard group and *Moringa oleifera* extract at doses of 5,10 and 20 mg/kg body weight, in the three remaining groups. After each trial, the EPM apparatus was wiped clean with alcohol (70%) Solution.

### Open field test

Locomotor activity and exploratory behavior were assessed in an open field 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. Mice were treated with *Moringa oleifera* (5,10 and 20 mg/kg, i.p), diazepam (1 mg/kg, i.p.) was used as the positive control drug or vehicle (i.p.). After 30 min, they were placed individually in one of the corner squares. The number of rearing, assisted rearing (forepaws touching the wall of the apparatus) and squares traveled were counted for 5 min

### 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 mice could peep through the holes. The mice were treated with *Moringa oleifera* (5, 10 and 20 mg/kg, i.p), diazepam (1 mg/kg, i.p.) or distilled water (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.

## RESULTS

### Elevated plus maze test

The saline-treated mice spent 30.8 ± 1.2 s in the open arm and 249 ± 2.6 s in the closed arm, with 11.3 ± 4.5 entries into the open arm and 13.7 ± 2.6 entries into the closed arm. *Moringa oleifera* extract (5 and 10 mg/kg) and diazepam (1 mg/kg) induced significant (**$P < 0.01$) increase in the occupancy in the open arm. *Moringa oleifera* in the dose of 10 and 20 mg/kg did not cause a significant decrease in the time spent in the closed arm, whereas *Moringa oleifera* at a dose of 5 mg/kg and diazepam brought about a significant (**$P < 0.01$) decrease in the time spent in the closed arm. The animals treated with diazepam and *Moringa oleifera* (5 mg/kg) showed a decreased preference for the closed arm and significantly (**$P < 0.01$) increased entries into the open arm. *Moringa oleifera* at 10 and 20 mg/kg did not produce any significant increase in open arm entries. Table1.

**Table 1: Effect of *Moringa oleifera* on animals' stay in the open and enclosed arms of the elevated plus-maze in mice (n=5).**

| 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 | 30.8±1.2 | 249±2.6 | 11.3±4.5 | 13.7±2.6 |
| DZP1mg/Kg | 106±6.3** | 161±4.5** | 22.5±1.9** | 10.7±1 |
| *MO*5 mg/Kg | 117±1.2** | 140.2±2.4** | 20.5±1.7** | 11.5±2.1 |
| *MO*10 mg/Kg | 92.8±12** | 219.8±1.8 | 10.3±4.1 | 12.5±2.4 |
| *MO*20 mg/Kg | 32.5±2.6 | 220.7±2.1 | 12.2±4.2 | 11.7±2.2 |

**Open field test**

The saline-treated mice traversed 87.2 ± 1 squares and showed 12.8 ± 1.2 assisted rearing and 5.5 ± 1.2 self-rearing during the test interval of 5 min. *Moringa oleifera* at 5 and 10mg/kg and diazepam brought about a significant (**$P$ < 0.01) and dose-dependant increase in the number of squares traversed. The assisted rearing and self-rearing was significantly (*$P$ < 0.05 and **$P$ < 0.01, respectively) increased by *Moringa oleifera* (5 and 10 mg/kg) and diazepam; *Moringa oleifera* at 20 mg/kg did not a produce significant effect. Table 2.

**Table 2: Effect of *Moringa oleifera* on rearing and locomotion in open field test model (n=5).**

| 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 | 30.8±1.2 | 249±2.6 | 11.3±4.5 | 13.7±2.6 |
| DZP1mg/Kg | 106±6.3** | 161±4.5** | 22.5±1.9** | 10.7±1 |
| *MO*5 mg/Kg | 117±1.2** | 140.2±2.4** | 20.5±1.7** | 11.5±2.1 |
| *MO*10 mg/Kg | 92.8±12** | 219.8±1.8 | 10.3±4.1 | 12.5±2.4 |
| *MO*20 mg/Kg | 32.5±2.6 | 220.7±2.1 | 12.2±4.2 | 11.7±2.2 |

**Hole board test**

Each mouse was placed individually in the hole-board apparatus and the number of head pokes and the duration of head poking were noted. With the dose of 20 mg/kg, i.p., of *Moringa oleifera* there was no significant increase in number of head pokes when compared with vehicle. *Moringa oleifera* at 5 and 10 mg/kg, i.p., increased the number of head pokes significantly (**$P$ < 0.01) and dose dependently. The duration of head poking was also significantly (**$P$ < 0.01) increased by *Moringa oleifera* at all doses. The reference standard (diazepam, 1 mg/kg, i.p.)-treated group showed significant increase in exploratory activity (** $P$ < 0.01). Table 3

**Table 3: Effect of *Moringa oleifera* on hole board (n=5).**

| Treatment | Duration on head poking (s) | Number of head poking |
|---|---|---|
| NaCl 10ml | 29.8±1.2 | 27.8±1.2 |
| DZP1mg/Kg | 71±6.4** | 75±6.4** |
| *MO*5 mg/Kg | 51±1.2** | 49±1.2** |
| *MO*10 mg/Kg | 75.8±12** | 77.8±12** |
| *MO*20 mg/Kg | 91.5±2.6** | 28.1±2.6 |

## DISCUSSION

The benzodiazepines (BZDs) are relatively safe and are widely used anxiolytic agents. These agents are known to act through the BZD-GABA receptors. The role of GABA in anxiety is well established.[14] The EPM is one of most popular animal tests for research on behavioral pharmacology of anxiety. It involves spontaneous or natural aversive stimuli, i.e., height, unprotected opening, and novelty.[15] 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.[16] In EPM, naove mice will normally prefer to spend much of their allotted time in the closed arms. This preference appears to reflect an aversion towards open arms that is generated by fear of open spaces. Drugs that increase open arm exploration are considered as anxiolytics and the reverse holds true for anxiogenics.[17] In our study, we noticed that *Moringa oleifera* (5 and 10 mg/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. The open-field apparatus provides information on anxiety-related behaviour characterized by natural aversion of rodents to an open brightly lit area.[18] 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 results

obtained in the open field test showed that *Moringa oleifera* administration significantly increased rearing, assisted rearing, and number of squares traversed, which supports the anxiolytic-like activity of *Moringa oleifera*. The anxiolytic activity of some agents have been assessed by using the hole-board test.[19] A significant increase in the exploratory head-dipping behavior was observed after treatment with 5 and 10mg/kg of *Moringa oleifera* extract, thus reinforcing the hypothesis that it has anxiolytic-like activity. These results confirm the anxiolytic effects of *Moringa oleifera*. They are to be compared with the work of Nsour,[20] who in a similar study showed the anxiolytic effect of *Rauvolfia Serpentina*. from Aidee,[21] who highlighted the anxiolytic effects of the ethanolic extracts of *Argemone mexicana*; from Carla,[22] et who 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. The anxiolytic effect of the hydro ethanolic leaves from *Moringa oleifera* could be due to the presence of alkaloids among the compounds of *Moringa oleifera*. Indeed, Aidee,[21] demonstrated that the alkaloids isolated from *Argemone mexicana* extracts increased the percentage of time spent in the open arms of rat EPM, in the same way as diazepam and *Argemone mexicana* extracts.

## CONCLUSION

In conclusion, the results obtained in our study suggest that the extract of the leaves of *Moringa oleifera* possesses anxiolytic activity, which is possibly mediated through the GABA A -BZD mechanism. Thus, *Moringa oleifera* 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.

## REFERENCES


1. Randall LO, Heise GA, Schallek W, Bagdon RE, Banziger RF, Boris A, et al. Pharmacological and clinical studies on Valium (T. M.), a new psychotherapeutic agent of the benzodiazepine class. Curr Ther Res., 1961; 3: 405–25.
2. Kessler RC, Ruscio AM, Shear K, Wittchen HU. Epidemiology of anxiety disorders. Curr Top Behav Neurosci., 2010; 2: 21–35.
3. Rickels K, Gargia-Espana F, Mandos LA, Case GW. Physician Withdrawal Checklist (PWC-20) J Clin Psychopharmacol, 2008; 28: 447–51.
4. Katzung B.G., Masters S.B., Trevor A.J. Basic and Clinical Pharmacology. 11th ed. McGraw-Hill; New York, NY, USA, 2009.
5. Woods J.H. Benzodiazepines: Use, abuse, and consequences. Pharmacol. Rev., 1992; 44: 151–347.
6. Dhawan K., Dhawan S., Chhabra S. Attenuation of benzodiazepine dependence in mice by a tri-substituted benzoflavone moiety of *Passiflora incarnata* Linneaus: A non-habit forming anxiolytic. J. Pharm. Pharm. Sci., 2003; 6: 215–222.
7. Kent JM, Mathew SJ and Gorman JM., Molecular targets in the treatment of anxiety. Biol. Psychiatry, 2002; 52: 1008-30.
8. Anwar F., Latif S., Ashraf M., Gilani A.H. *Moringa oleifera*: A food plant with multiple medicinal uses. Phytother. Res., 2007; 21: 17–25. doi: 10.1002/ptr.2023.
9. Nur Z. A., Khairana H,* Endang K. *Moringa* Genus: A Review of Phytochemistry and Pharmacology Front Pharmacol, 2018; 9: 108.
10. Abdull R.A., Ibrahim M.D., Kntayya S.B. Health benefits of *Moringa oleifera*. Asian Pac. J. Cancer Prev. APJCP., 2014; 15: 8571–8576. doi: 10.7314/APJCP.2014.15.20.8571.
11. Posmontier B. The medicinal qualities of *Moringa oleifera*. Holist. Nurs. Pract., 2011; 25: 80–87. doi: 10.1097/HNP.0b013e31820dbb27.
12. Banji O.J., Banji D., Kavitha R. Immunomodulatory effects of alcbholic and hydroalcoholic extracts of *Moringa olifera* Lam. leaves. Indian J. Exp. Biol., 2012; 50: 270–276.
13. Chumark P., Khunawat P., Sanvarinda Y., Phornchirasilp S., Morales N.P., Phivthong-Ngam L., Ratanachamnong P., Srisawat S., Pongrapeeporn K.U. The in vitro and ex vivo antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of *Moringa oleifera* Lam. leaves. J. Ethnopharmacol, 2008; 116: 439–446. doi: 10.1016/j.jep.2007.12.010.
14. Rang HP, Dale MM, Ritter JN. Pharmacology: Churchill Livingstone, 2003; 483-94.
15. Dhonnchadha BA, Bourin M, Hascoet M. Anxiolytic- like effects of 5-HT 2 ligands on three mouse models of anxiety. Behav Brain Res, 2003, 140: 203-14.
16. Thakur VD, Mengi SA. Neuropharmacological profile of Eclipta Alba L. Hassk. J Ethnopharmacol, 2005; 102: 23-31.
17. Hellion-Ibarrola MC, Ibarrola DA, Montalbetti Y, The anxiolytic-like effects of *Aloysia polystachya* (Griseb) Moldenke (Verbenaceae) in mice. J Ethnopharmacol, 2006, 105: 400-8.
18. Choleris E, Thomas AW, Kavaliers, A detailed ethological analysis of the mouse open field test: Effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. Neurosci Behav Rev, 2001, 25: 235-260.
19. Bhatacharya SK, Satyan KS. Experimental methods for evaluation of psychotropic agents in rodents: Anti-anxiety agents. Indian J Exp Biol, 1997; 35: 565-75.
20. Nsour W. M., Lau C. B., Wong I. C. Review on phytotherapy in epilepsy Seizure, 2001; 9: 96–107.
21. Aideé I. A, Omar D. M., Miguel Á. D. Anxiolytic-like effect of ethanolic extract of *Argemone mexicana* and its alkaloids in Wistar rats, 2016; 6: 476-488.

22. Carla L., Giancarlo C., Gian L. G. anxiolytic effect of an extract of *Salvia miltiorrhiza* roots in rats, 2018; 81: 390-397.
23. Charles K. B., Robert P. B., Donatus W. A, Anxiolytic and Antidepressant Effects of *Maerua angolensis* DC. Stem Bark Extract in Mice., 2018; 5: 1-17.
24. Carnevale G., Di Viesti V., Zavatti M. Anxiolytic-like effet of *Griffonia simplicifolia* Baill. Seed extract in rats. Phytomedicine, 2011; 18(10): 848-851.