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PHYTOCHEMICAL STUDY AND IDENTIFICATION OF PSYCHOPHARMACOLOGICAL PROPERTIES OF THE AQUEOUS EXTRACT OF *MORINDA LUCIDA* LEAVES

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Abstract

This work was carried out in order to identify the chemical families and to evaluate the psychopharmacological and analgesic properties of the aqueous leaf extract of Morinda lucida Benth in rodents (Wistar rats and Balb / c mice). Material and method. Wistar rats weighing 100 to 380 g and male and female Balb / c mice weighing 18 to 32 g were retained. All animals were kept in their natural habitat and cycled. The leaves of Morinda Lucida Benth were harvested in the department of the bowl. The extraction method was masseration. Result and discussion. The analysis of the results shows a variable amount depending on the organ and the différent families. At the level of the aqueous extract of M.L administered per os is well tolerated up to the dose of 1200mg / kg and entails no mortality. M.L's EA at the doses studied does not alter the general behavior and curiosity of animals to touch and sound stimuli. Conclusion. The results of the effects of the EA of leaves of M.L in this study seem to reveal possible psychopharmacological properties, analgesic approaching those of the reference molecules.

Keywords: phytochemical, identification, psychopharmacological, *Morinda lucida*

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INTRODUCTION

In Africa, despite the existence of a health care system for mental disorders, more than 80% of the sub-Saharan population uses medicinal plants for their health care [1,2]. In the Congo the populations resort for their care [3]. These plants because of their therapeutic virtues, are very used in traditional medicine. In addition, very few of these plants listed in the literature have been the subject of a psychopharmacological study, attempting to elucidate their psychotropic profile and to specify their toxicity [4]. In order to contribute to the study of the properties of medicinal plants in rodents, this work was carried out in order to identify the chemical families contained in the plant and to evaluate the psychopharmacological and analgesic properties of the aqueous leaf extract. Morinda lucida Benth in rodents (Wistar rats and Balb / c mice).

MATERIAL AND METHOD

Our study took place in the laboratory of biochemistry and pharmacology of the FSSA and the IRSSA over a period of 3 months: September - November 2018.

Material

1. Animal

Wistar rats weighing 100 to 380 g and male and female Balb / c mice weighing 18 to 32 g were retained. Rats and Balb / c mice were divided into 4 lots each containing 3 and 4 animals respectively. All animals were kept in their natural habitat and subjected to a 12 / 12h light / dark cycle, with free access to water and food. Each batch was fasted for 18 hours before the start of each test.

2. Plant material

The leaves of Morinda Lucida Benth were harvested in the department of the basin (Makoua) on May 3rd, 2018. The ethnobotanical survey was conducted in September 2017. The leaves were identified and registered under the number N °: 8.014.

Methods

1. Preparation of the aqueous extract *of Morinda lucida*

The extraction method used was maceration [3,5]. The leaves of *Morinda lucida* were dried in the sun at 28-32 ° C for 3 weeks. They were reduced to powder using a wooden mortar; 50 g of powder obtained were macerated in 500 ml of distilled water with magnetic stirring for 48 hours. The macerate obtained is filtered and then concentrated to a quarter (1/4) of its initial volume at

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65 ° C in an oven. The resulting concentrated extract (10.15 g) is transferred to a sterile plastic container; hermetically closed and kept in a refrigerator at 4 ° C for psychopharmacological tests.

2. Assessment of Acute Toxicity

To evaluate the degree of curiosity of rodents after administration of the aqueous extract of Morinda lucida leaves at different doses [Adejo et al. 2015].

Procedure

The EA of *Morinda lucida* leaves was prepared at doses of 100, 600, 1200 mg / kg. The Balb / c mice were divided into 4 lots of 2 mice per batch and treated as follows; three (3) batches were injected with the aqueous extract of *M.l* leaves at doses of 100, 600 and 1200 mg / kg and the last of the distilled water at a dose of 10 ml / kg. One hour after administration of the doses, the animals were returned to the observation cage and the general state and degree of curiosity were assessed in the animals, followed by evaluation of the toxicity 24, 48 and 72 hours later.

3. Effect on rectal temperature

For each animal, the temperature was taken before and after administration of different products. Four batches of three wistar rats each are used. Two batches received per os the aqueous extract of *Morinda lucida leaves* at doses of 200 and 400 mg / kg. Lot (3) received 5 mg / kg of haloperidol (Haldol) per os as a positive control and the last 10 ml / kg of oral distilled water as a negative control. One hour after the administration of the products, the rectal temperature of each animal is measured [MIGUEL et al., 2014].

4. Open Field Test (Open Fields)

The goal is to measure levels of general locomotor activity, anxiety, and the ability of rodents to explore [Hall et al., 1934]. The test consists in placing in a motor activity enclosure (dimensions 34cm x 34cm x 20cm and whose floor is divided into 25 equal squares) a naive animal head oriented towards one of the four corners of the Open-field. And measure the number of squares crossed then assess the exploratory capacity in a stressful context for 10 minutes, one hour after administration of different products.

Procedure

Four batches of three wistar rats each are used. Two batches received per os the aqueous extract of *Morinda lucida* leaves at doses of 200 and 400 mg / kg. Lot (3) received 5mg / kg Haloperidol (Haldol) per os as a positive control; and the last 10ml / kg of oral distilled water as a negative control. One hour after the administration of different products, the motor activity of each animal is evaluated using a camera to determine the total number of tiles crossed.

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5. Cross Labyrinth Test

This test consists of measuring the anxiolytic effect of a molecule or the type of anxious behavior developed by rodents who are placed in a labyrinth cross located 50cm above the ground. The experimental device consists of two open arms and two closed arms (50 cm x 10 cm x 40 cm) connected by a central platform (10 cm × 10 cm).

Procedure

Of the four (4) batches of three (3) wistar rats each, two (2) batches received per os aqueous extract *of Morinda lucida* leaves at doses of 200 and 400mg / kg. Lot (3) received 10 mg / kg of oral Diazepam (Valium) as a positive control; and the last 10ml / kg of oral distilled water as a negative control. One hour after the administration of different products, each animal is placed in the center of the device facing an open arm each animal to freely explore the device for 10min. And with the help of a camera the type of anxious behavior developed by each animal was evaluated to determine the following variables: the time and the number of entries spent in the closed arms, open and on the central platform .

6. Hole board discrimination test (Hole board test)

The goal is to evaluate exploratory behavior and curiosity in the mouse using a camera. The method we adopted is the modified one [12].

Procedure

Four batches of four (4) Balb / c mice each were used. Depending on the weight of the animal, each animal received the appropriate administration volume. Two (2) batches received the aqueous extract of Morinda lucida leaves at doses of 200 and 400 mg / kg. Lot (3) received 5mg / kg Haloperidol (Haldol) per os as a positive control; and the last 10ml / kg of oral distilled water as a negative control. An hour after the administration of different products, each mouse is placed in the enclosure facing one of the corners of the floor.

7. Porsolt test (forced swimming test)

The goal is to determine the predictive efficacy of antidepressants in rodents. Procedure

Of the four (4) batches of four (4) Balb / c mice each used, two (2) batches received per os the aqueous extract of Morinda lucida leaves at doses of 200 and 400mg / Kg. Lot (3) received 25 mg / kg Clomipramine (Anafranil) per os as a positive control; and the last 10ml / kg of oral distilled water as a negative control.

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One hour after the administration of different products, each mouse underwent the experiment for 6min. Using a camera the depressive behavior of each mouse is observed to determine the duration of immobility of each animal and the latency time before the first immobilization.

8. Analgesic activity with 2.5% formaldehyde

It involves inducing in rodents inflammatory pain by injection under the left hind paw; one hour after administration of different products; then it is evaluated the licking time of the leg by the animal and the licking frequency for 10 minutes.

Procedure

Four (4) batches of four (4) Balb / c mice each are used and processed as follows; two (2) batches received the aqueous leaf extract of Morinda lucida per os at doses of 200 and 400 mg / kg. Lot (3) received 50mg / kg of oral tramadol (Tremadol *) as a positive control; the last 10ml / kg of oral distilled water as a negative control. One hour after the administration of the products, a required amount of 2.5% formaldehyde is injected into each mouse depending on the weight. With a camera we observed the behavior of each mouse for 10 minutes to determine the frequency and licking time of the paw.

Statistical analysis of the results

The results were expressed as mean ± SEM for a number of experiments. Our database was created with EXCEL 2007.

The significant differences between the groups were determined using the t student statistical analysis test in the Instat Plus software. The differences were considered significant at $p<0.05$.

RESULT

1. Phytochimical study

In table 1 of different chemical families

2. Acute toxicity and observation of the general condition

The doses of EA from leaves of M.l administered did not show any sign of toxicity or resulted in mortality 24 to 72 hours later. No changes in mice with external stimuli were noticed.

3. Effect on rectal temperature

The results are summarized in Figure 1.

4. Effet sur l'activité motrice spontanée

The results of the effect of M.L leaf EA on spontaneous motor activity are shown in Figure 2.

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5. Effet sur le test de labyrinthe en croix

Figure 3 shows the results of the effect of M.L leaf EA on the number of rats entering the open arms of the Labyrinth.

Figure 4 shows the results of the effect of M.L leaf EA

ML foil at 400 mg / kg (9 ± 22.88, P = 0.37) and Diazepam 10 mg / kg (5 ± 6.66, P = 0.37) increase the time spent in the open arms of the Labyrinth.

The results of the effect of the M.L leaf EA on the time spent in the labyrinth open arms are shown in Figure 5.

The results of the effect of ML leaf EA on time spent in labyrinth closed arms are shown in Figure No. 6. At a dose of 400mg / kg (576.33 ± 22.88, p = 0 , 41) ML leaf EA increases the time spent in closed arms

The effect of M.L leaf EA at the 400mg / kg dose (14.67 ± 0.02, p = 0.38) reduces the time spent on the central labyrinth platform. However, the difference is not significant to the control animals.

The results of the effect of the M.L leaf EA on the time spent on the central platform are shown in Figure 7.

6. Effect on the test of Planks with holes

The results of the effect of M.L leaf EA on the number of holes explored are shown in Fig. 8.

7. **Porsolt test**

Clomipramine 25 mg / kg (77 ± 17.5, P = 0.81); and the EA of M.L leaves at a dose of 400 (69.25 ± 3.25, P = 0.67); mg / kg increase latency before immobility. The difference is not significant to the control animals. The results of the effect of EA of leaves of M.L on the latent time in the Porsolt test are shown in FIG. 9.

Clomipramine 25mg / kg significantly decreased the duration of immobility of animals in the Porsolt test (p = 0.04). The EA of leaves of M.L at the dose of M.L 400 mg / kg decreases the duration of immobility.

The results of the EA effect of leaves of M.L on the immobility time in the Porsolt test are shown in Figure 9.

8. Analgesic activity with 2.5% formaldehyde

The results are shown in Figure 10.

Figure 11 shows the results of the effect of M.L leaf EA on paw licking time in 2.5% analdehyde analgesic activity.

Table and figures

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Table 1: Different Chemical Families

	Chimicals Families			
	Alcaloïdes	**Tanins**	**Flavonoïdes**	**Sucres**
leaves	+++	+++	++++	+++
roots	++++	++	++++	+++
barks	++	-	+++	+

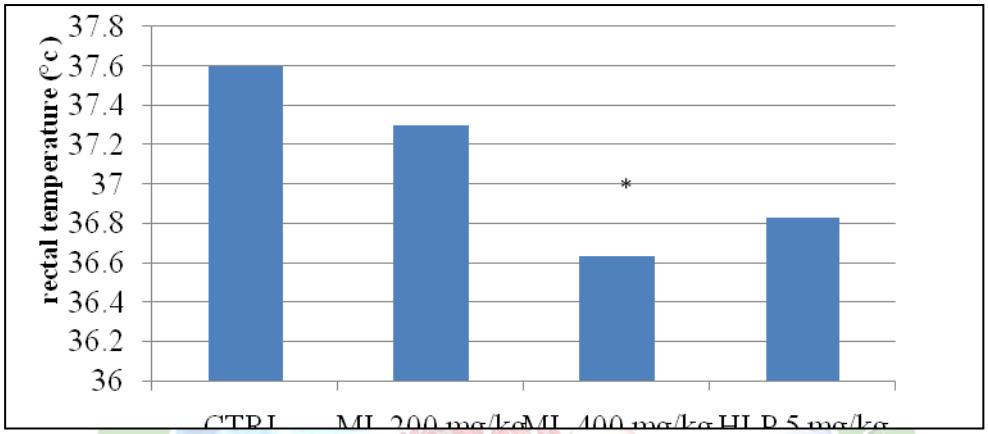

Fig. 1: Effect of M.L leaf EA on rectal temperature *: *p* = 0.05; n = 3; CTRL: control; M.L: Morinda lucida; HLP: Haloperidol

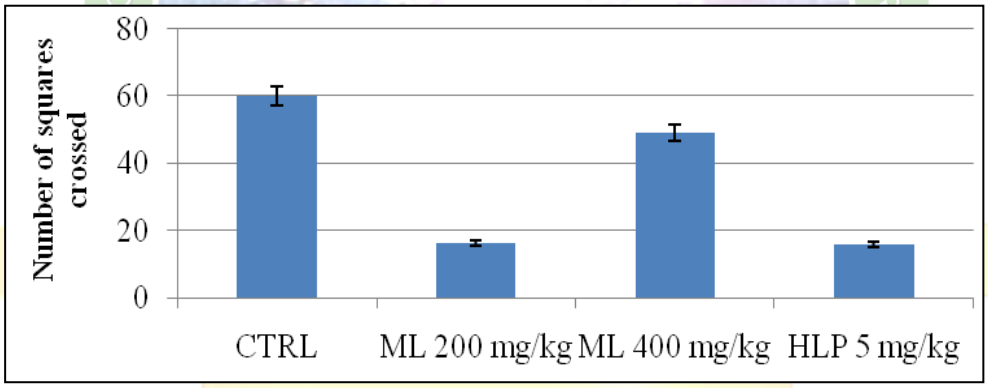

Fig.2: Effect of M.L leaf EA on spontaneous motor activity; n = 3; CTRL: control; M.L: Morinda lucida; HLP: Haloperidol

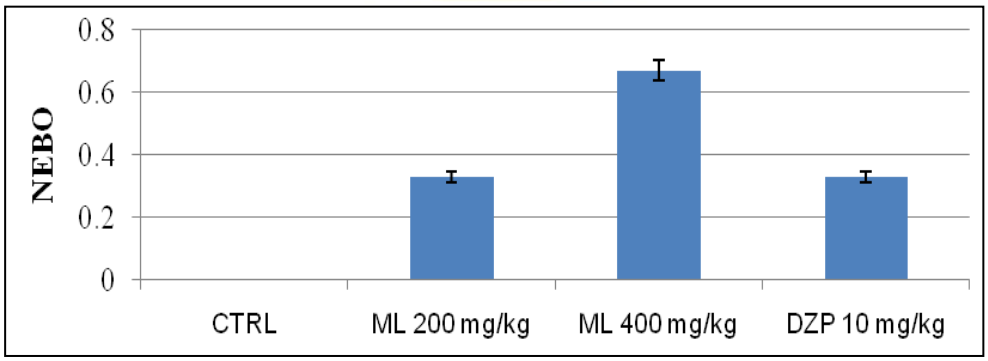

Fig. 3: Effect of the M.L leaf EA on the number of entry into the open arms of the labyrinth; n = 3; CTRL: control witness; M.L: Morinda lucida; DZP: Diazepam.

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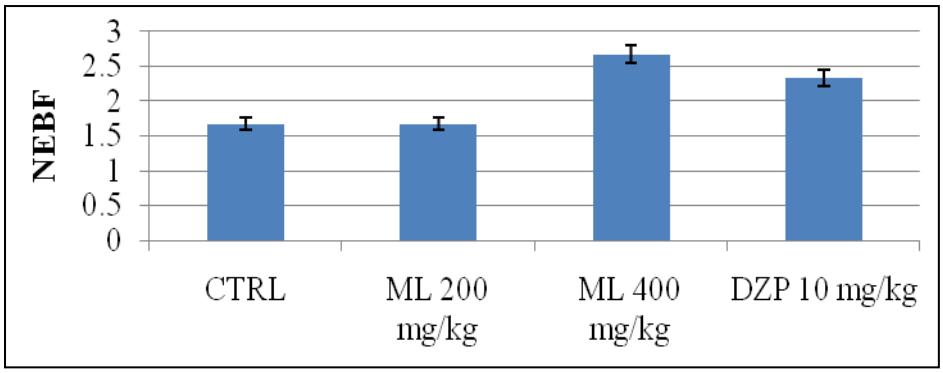

Fig. 4: Effect of the M.L leaf EA on the number of entries in the closed arms of the labyrinth; n = 3; CTRL: control; M.L: Morinda lucida; DZP: Diazepam.

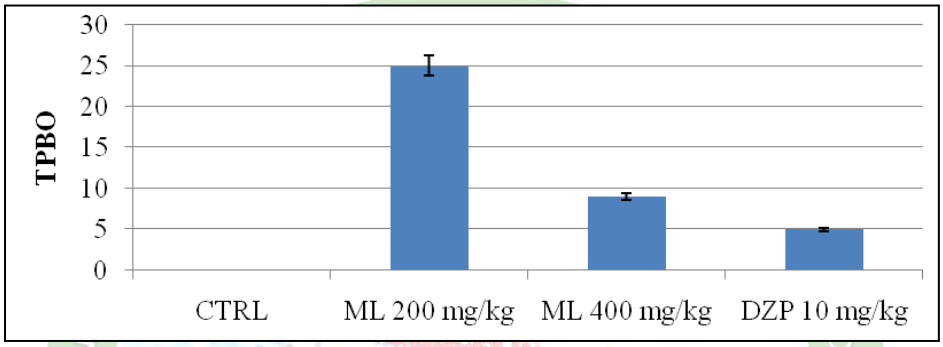

Fig. 5: Effect of M.L leaf EA on time spent in open arms of labyrinth; n = 3; CTRL: control; M.L: Morinda lucida; DZP: Diazepam

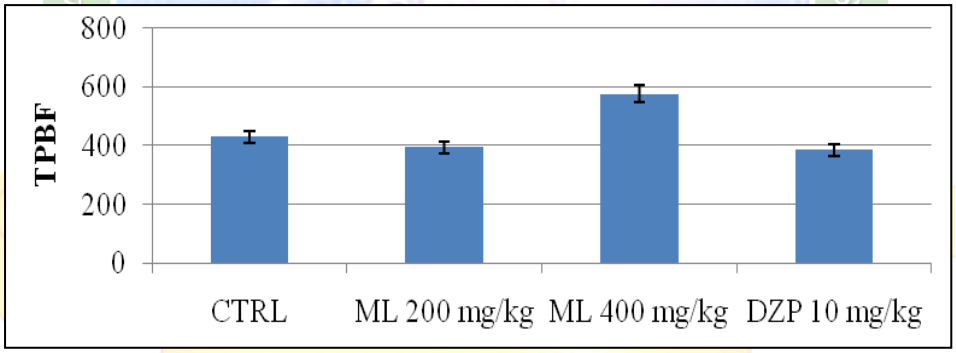

Fig. 6: Effect of M.L leaf EA on time spent in the closed arms of labyrinth; n = 3; CTRL: control; M.L: Morinda lucida; DZP: Diazepam.

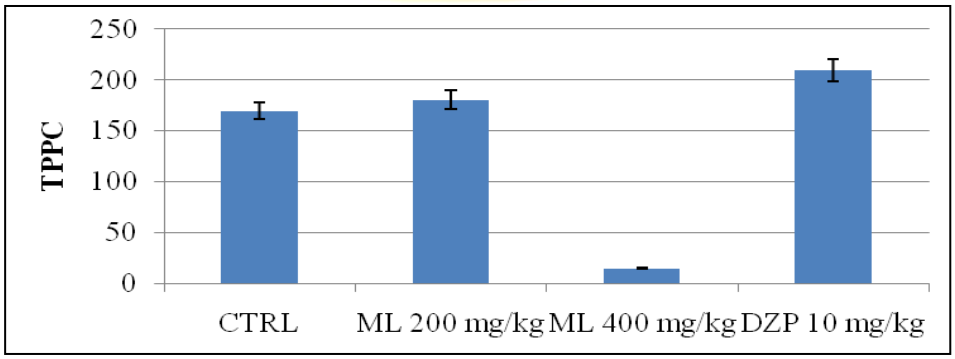

Fig. 7: Effect of M.L leaf EA on time spent on the central platform of labyrinth; n = 3; CTRL: control; M.L: Morinda lucida; DZP: Diazepam

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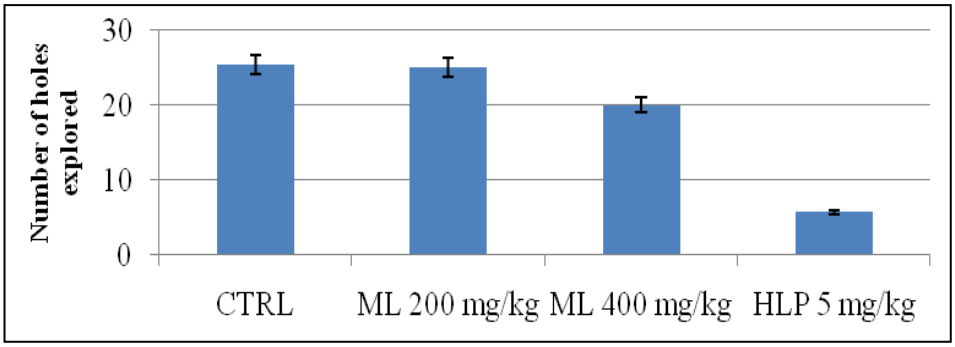

Fig. 8: Effect of the M.L leaf EA on the number of holes explored in the hole board test **: p = 0.005; n = 4; CTRL: control; *M.L: Morinda lucida*; HLP Haloperidol

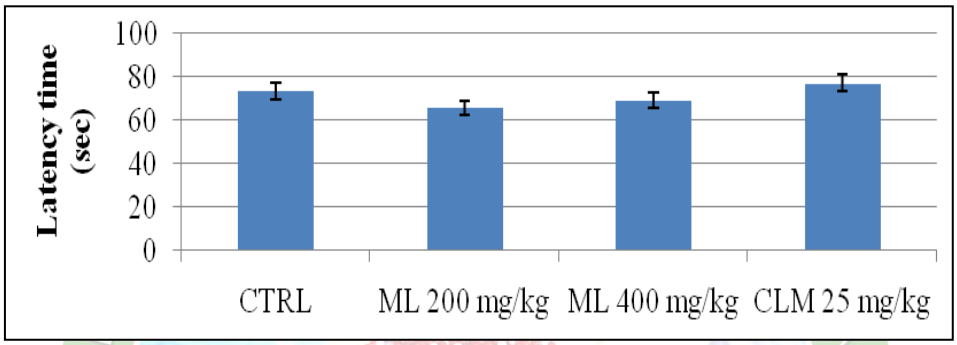

Fig. 9: Effect of EA of leaves of M.L on latency in the Porsolt test; n = 4; CTRL: control; M.L: Morinda lucida; CLM: Clomipramine.

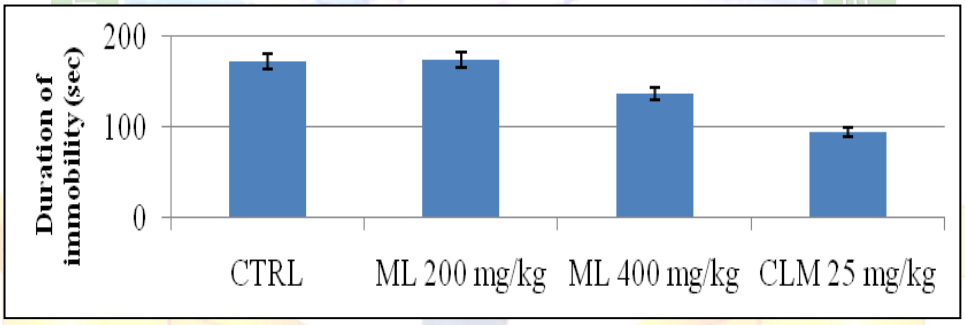

Fig.10: Effect of EA of leaves of M.L on the duration of immobility in the Porsolte test; *: p = 0.04; n = 4; CTRL: control; M.L: Morinda lucida; CLM: Clomipramine.

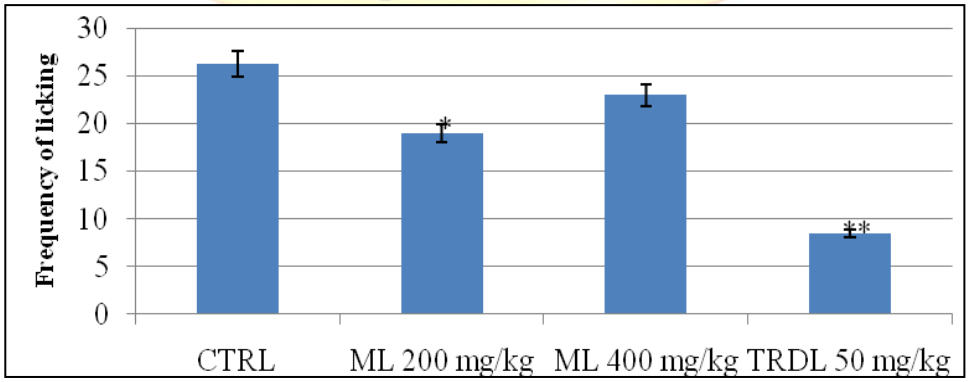

Fig.10: Effect of M.L leaf EA on licking frequency in 2.5% formaldehyde analgesic activity; *: *p* = 0.05; **: p = 0.006; n = 4; CTRL control; M.L: Morinda lucida; TRDL: Tramadol.

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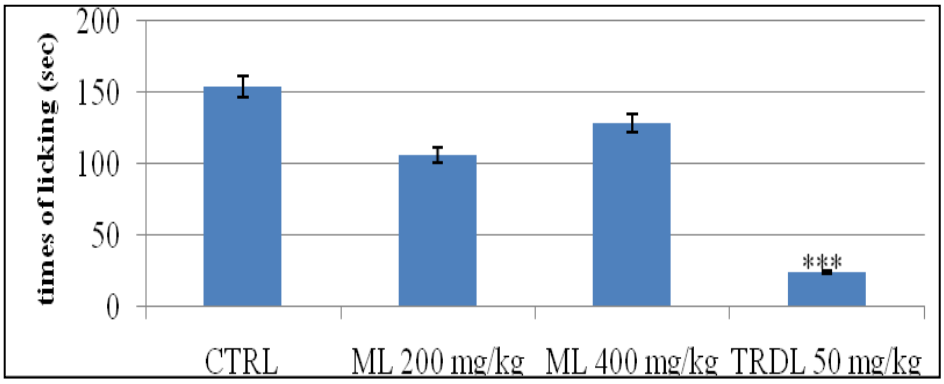

Fig.11: Effect of M.L leaf AE on licking time in formaldehyde antalgic activity at 2.5%; ***: p = 0.0003; n = 4; CTRL: control control; M.L: Morinda lucida; TRDL: Tramadol.

DISCUSSION

The analysis of the results shows that the leaves and roots of Ml have a very abundant amount of flavonoids and abundant alkaloids, tannins and sugars. While the barks in an abundant amount of flavonoids and rather average alkaloids and traces of sugar. The tannins are absent. At the level of the aqueous extract of M.L administered per os is well tolerated up to the dose of 1200mg / kg and entails no mortality. MR's EA at the doses studied does not alter the general behavior and curiosity of animals to touch and sound stimuli [5, 6]. The EA of leaves of M.L at the doses studied not significantly decreases the motor activity, just like haloperidol, a neuroleptic substance which causes psychomotor indifference and sedation [7]. The reduction by EA of M.L leaves at the studied doses of spontaneous motor activity in rats could imply a psychoinhibitory action. In contrast to haloperidol, the EA of M.L leaves at a dose of 400mg / kg significantly decreased the rectal temperature of the rats. These results are consistent with those reported by some authors [8, 9]. Commonly used for the exploration of the neurobiological bases of anxiety and for the screening of anxiolytic substances [15], the Cross Labyrinth test has several advantages in comparison with other experimental models of anxiety [10; 11]. In our study, Diazepam, which is an anxiolytic, reduces the number of animals entering the open arms and increases the time spent in the closed arms, but the difference is not significant for the control animals. These results are in agreement with those of the literature [11; 10]. The EA of leaves of M.L at a dose of 200mg / kg has effects similar to those of diazepam. It is therefore possible that at this dose, the EA of leaves of M.L has anxiolytic properties. The hole board test is used to highlight the inhibitory action of psycholeptics on one of the components of the investigative behavior, the reaction, exploration related to curiosity and the animal's desire to escape [12, 16].

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In contrast to Haloperidol, the EA of 200mg / kg ML leaves similarly reduces the investigative behavior and the exploratory reaction related to the animal's curiosity and desire to escape but the difference is not significant to the control. The forced swimming test is one of the tests used to evaluate the predictive efficacy of an antidepressant molecule [13]. The Porsolt test which subjects the animal to immobility after an initial period of agitation. This immobility reflects a desperate behavior that illustrates a state of mental depression. This condition is reduced by antidepressants [14]. However, the evaluation of the effect of ML aqueous leaf extract on the immobility and latency of animals subjected to forced swimming in our study showed a reduction in the duration of immobility at the dose. 200 mg / kg but there is no significant difference. Classic painkillers inhibit pain at the central or peripheral level. Indeed, Tramadol (Trémadol*) is an opiate that prevents the rise of the impulse generated at the peripheral ends of the C and Aδ fibers by an action on the ascending pathways of pain [15]. Thus at the central level the effect of Tramadol (Trémadol*) and EA of leaves of M.L at the dose of 200 mg / kg were found to be effective against neurogenic pain induced by formaldehyde at 2.5%. However, the analgesic effect of tramadol (tremadol*) is better than that of the EA of leaves of M.L at the dose of 200 mg / kg compared to control control.

CONCLUSION

The results of the effects of the EA of leaves of M.L in this study seem to reveal possible psychopharmacological properties, analgesic approaching those of the reference molecules. Psychopharmacological properties that would require further studies to clarify the neurobiological mechanisms responsible for the effects observed.

REFERENCE

1. Jiofack T., Ayissi I., Fokunang C., Guedje N., Kemeuze V. Ethnobotany and phytomedicine of the upper Nyong Valley forest in Cameroon-African Journal of Pharmacy and pharmacology 2009.3 (4): 144-15
2. Jiofack T., Fokunang C., Guedje N., Kemeuze V., Fongnzossie E., Nkongmeneck Ba., Mapongmetsem Pm. & Tsabang N. Ethnobotanical uses of medicinals plants of two ethnoecological regions of Cameroon - International Journal of Medicine and Medical Sciences 2010. 2 (3): 60-79.
3. https://www.W.H.O. Activités de l'OMS dans la Régions africaine 2016-2017.

DOI: 10.21276/IJIPSR.2020.08.01.852

4. Bouquet A. 1969. Féticheurs et médecines traditionnelles du Congo (Brazzaville). Mémoires O.R.S.T.O.M 36, 305p.

5. Adejo Go, Akintayo Co And Obinna M. Morinda lucida leaf and fruit extracts may attenuate diarrhea arising from ebola. Basic Research Journal of Medicine and Clinical Sciences. 2015. 4(3): 95 – 100.

6. Ebiloma Godwin Unekwuojo, Omale James And Aminu Rhoda Olubunmi1. Suppressive, Curative and Prophylactic Potentials of Morinda lucida (Benth) against Erythrocytic Stage of Mice Infective Chloroquine Sensitive Plasmodium berghei NK-65 SCIENCEDOMAIN international 2011.1(3): 131-140.

7. Miguel L.M., J.B. Mouankie, N. Okemy, E. Mokondjimobe, Diatewa M., A.A. Abena. Effets psychopharmacologiques de l'huile essentielles des fruits de Dacryodes Edulis (G.DON) H. J. LAM. Annales de l'Université Marien NGOUABI. 2014; 15(5) : 61-72.

8. Bero J., Ganfon H., Jonville M-C., Frédérich M., Gbaguidi F., Demol P., Moudachirou M., Quetin-Leclercq J. In vitro antiplasmodial activity of plants used in Beninin traditional medicine to treat malaria. J.Ethnopharmacol 2009.122, 439–444.

9. Patrick B. Memvanga, Gaston L.Tona, Gauthier K.Mesia, Mariano M. Lusakibanza, Richard K. Cimanga. Antimalarial activity of medicinal plants from the democratic republic of Congo:Areview. Journal of Ethnopharmacology 2015. 1-23.

10. Dulawa S.C., Holick K.A., Gundersen B., Hen R. Effects of chronic fluoxetine in animal models of anxiety and depression. Neuropsychopharmacology.**2004**,29(7):1321-30.

11. Hall C., Ballachey E. "A study of the rat's behavior in a field: a contribution to method in comparative psychology". University of California Publications in Psychology 1932. **6**: 1-12.

12. Pellow S. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated Plus maze: a novel test of anxiety in the rat. Pharmacol. Biochem. Behav.**1986**, 2: 525.

13. Makram K. Alaoui, T. Bnabboyha, B. Faridi, Y. Cherrah, A. Zellou. Extraction et activité psychotrope de l'huile essentielle de la verveine odorante Lippia citriodora. Phytothérapie. 2015, 13: 165 – 167.

14. Porsolt R.D., Bertin A., Jalfre M. Le désespoir comportemental chez les souris: un test de dépistage primaire pour les antidépresseurs. Archives Internationales de Pharmacodynamie et de Thérapie 1977. **229** (2): 327-36.

DOI: 10.21276/IJIPSR.2020.08.01.852

15. Lijian Yu, Xiaodan Jiang, Mingneng Liao, Rundi Ma, Tingxi Yu1. Antidepressant-Like Effect of Tetramethylpyrazine in Mice and Rats. Neuroscience & Medicine.2011, 2: 142-148.

16. Monassier. « Les antalgiques non opiacés » Module de Pharmacologie Générale DCEM1, Faculté de Médecine de Strasbourg, Mise à jour : Janvier 2005, 1-6.