Analgesic and Behavioural Effects of *Morinda citrifolia*

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Abstract

The traditional therapeutic indications for the use of *Morinda citrifolia* L. (Rubiaceae) have been investigated. The lyophilised aqueous extract of roots of *M. citrifolia* was evaluated for analgesic and behavioural effects in mice. The extract did not exhibit any toxic effects but did show a significant, dose-related, central analgesic activity in the writhing and hotplate tests; this effect was confirmed by the antagonistic action of naloxone. Furthermore, administration of *M. citrifolia* extract at high dosages decreased all behavioural parameters in the two compartment test, the light/dark choice situation test, and the staircase test; together with the induced sleeping time, these results are suggestive of sedative properties.

Key words

Morinda citrifolia, analgesic activity, sedative effects, behavioural effects.

Introduction

Traditionally, the roots and leaves of *Morinda citrifolia* L. (Rubiaceae) are used in Mauritius, Tangatapu, Vietnam, the Philippines, and India as analgesic or antirheumatic agents as well as for the treatment of dysurea (1, 6, 14, 18, 19). Pharmacological investigations have demonstrated that the roots of *M. citrifolia* exert an antihypertensive action (8, 9, 10, 22) and that an alcoholic extract has an *in vitro* antispasmodic effect on the isolated uterus of the rat (22). Since these first chemical studies, only few substances have since been identified (14) and most of these belong to the anthraquinone class (12, 15).

The initial objective of the present study was to investigate the peripheral and central analgesic properties of *M. citrifolia* by means of both the writhing and hotplate tests (experiment 1). Since central analgesic effects were found, we then proceeded to search for sedative properties by means of a free exploration procedure specially adapted to reveal such effects (experiment 2). As this result was also positive we went on to investigate putative anxiolytic effects by using two experimental models, namely the light/dark choice paradigm described by (7) as modified by (2) and the staircase tests of (20) (experiment 3). In addition, we examined the effect of various doses of *M. citrifolia* on the induction of sleep in mice treated with a subhypnotic dose of pentobarbital (experiment 4) and the acute toxicity of the plant by administering high dosages of *M. citrifolia*.

Materials and Methods

Animals

Male and female Swiss mice weighing 20–22 g (5 weeks old) were used for the acute toxicity tests and male Swiss mice weighing 30–35 g (9 weeks old at the start of the tests) were used for the analgesic and behavioural tests. The animals were obtained from Laboratoire Janvier, Legenest. All animals were conditioned in standard Macrolon boxes (5 mice per box) with laboratory diet (croquettes Extralabo) and drinking water *ad libitum* under a 12/12 h light/dark cycle with additional red light at 1 am in order to observe the animals in their high activity period when the lights were off.

Plant extract

Aqueous extracts were prepared from the decorticated, dried roots of African *M. citrifolia* obtained from the Laboratories of Expansion Aromatique Française (France) by the traditional method: 50 g of dried, powdered roots were decocted during 15 min and macerated for 4 h in 300 ml hot distilled water. After filtration, the aqueous filtrate was concentrated under reduced pressure and then lyophilised; 2.9 mg of lyophilisate corresponded to 30 mg of powdered, dried plant material. All doses are expressed in terms of dried plant material (mg/kg body weight).

Characterisation of the aqueous extract was limited to the evaluation of the anthraquinone compounds by TLC on silica gel with solvent systems (I) benzene-ethyl formate-formic acid (75 : 24 : 1, v/v) and (II) ethyl acetate-formic acid-acetic acid-water (68 : 7 : 7 : 18, v/v); visualisation of spots by ammonia and UV (365 nm). The following R_f values were obtained: solvent system (I) 0.13, 0.15, 0.21, 0.60, 0.75 and solvent system (II) 0.04, 0.24, 0.51, 0.60, 0.85, 0.88, 0.90.

Experiment 1

Writhing test: Prior to testing each animal received the lyophilisate of the aqueous extract of *M. citrifolia* at a dose of 100, 200, 400, 800, and 1600 mg/kg *i.p.* Control animals received 0.9 % NaCl solution *i.p.* 30 min later. For the test, a solution

of 1.2 % acetic acid was injected; morphine (as sulfate, 1.15 mg/kg *i.p.*) was used as a reference analgesic substance. The numbers of contortions and stretchings were then counted over a 30 min period.

Hotplate test: The apparatus comprised a basin filled with hot water containing a glass in which each animal was placed for the testing time. The temperature of the water was 56 °C ± 1 °C in order to cause the animals to lick their forelegs and/or to produce jumping responses. The experimental conditions and substances were the same as those used in the writhing test except that the morphine sulfate dosage was 4.6 mg/kg *i.p.* Each animal served as its own control; before treatment the reaction time of each mouse was determined twice with an interval of 10 min and the mean value used. 30 min after treatment, the reaction time was measured once only. The relationship between the mean time of reaction of each group before and after treatment determines the percentage of variation.

Naloxone (Narcan®, Laboratoire Dupont-Nemours, France), a specific antagonist of morphinometic receptors, was administered at 1 mg/kg *s.c.* 15 min before the plant extract or morphine sulfate in order to determine if the observed pharmaceutical effect has a central origin. Control animals received naloxone under the same conditions.

Experiment 2

Two compartment test: The apparatus consisted of a PVC box (30 × 20 × 20 cm) subdivided into six equal square exploratory units and covered with plexiglass. The box could be divided in half by means of three temporary partitions. The box was kept on a stand in the room in which the mice were housed. During observation, the experimenter always stood next to the box in the same position and observations were made without prior knowledge of the experimental condition of the animal.

Each test subject was placed in one half of the apparatus with the temporary partitions in place for familiarisation. The floor of the box was covered with sawdust and the animal was given unlimited access to food and water. After about 24 h, the animal was exposed to both familiar and novel environments by removal of the partitions without itself being removed from the box. The subject was then observed under red light for 10 min and the numbers of novel and familiar units entered were recorded and defined as locomotion.

Plant extracts were administered 30 min before testing at dosages of 125, 250, and 500 mg/kg. The control group received 0.9 % NaCl solution under the same conditions.

Experiment 3

Light/dark choice procedure: The apparatus consisted of two PVC boxes (20 × 20 × 14 cm) covered with plexiglass. One of them was darkened with cardboard while a 100 watt desk lamp above the other provided the only room illumination. An opaque plastic tunnel (5 × 7 × 10 cm) connected the dark box to the lit one. During observation, the experimenter always sat next to the apparatus in the same position. The animals were tested individually for 5 min periods between 2 pm and 4 pm. The mice were not familar with the apparatus. They were placed in the lit box to start the test session. The amount of time spent in the lit box after the first entry into the dark box was recorded over a 5 min period. When the mouse had placed all four paws in the new box it was considered to have changed boxes.

Staircase test: The apparatus consisted of a PVC enclosure (47 × 10 × 25 cm) with five identical steps. The only room illumination was provided by a 100 watt desk lamp above the staircase. The animals were placed singly on the floor of the box; the number of steps climbed and the number of rearings during a 5 min period were recorded. A step was considered as being climbed only when the mouse had placed all four paws on the next step. The apparatus was cleaned between each test session.

The plant extract was administered *i.p.* 30 min before testing in doses of 100, 200, 400, 800, and 1600 mg/kg. Control animals received 0.9 % NaCl solution.

Experiment 4

Sleep induction test: 0.9 % NaCl solution and the plant extract in doses of 200, 400, 800, 1600 mg/kg *i.p.* were administered 30 min before a 25 mg/kg *i.p.* infra-hypnotic dose of pentobarbital. The latencies of loss and retrieval of the righting reflex as well as the number of animals which fell asleep were recorded.

Acute toxicity

Five groups of 10 mice each (5 males and 5 females) received the *M. citrifolia* extract at doses of 1, 2, 4, and 8 g/kg *i.p.* and 16 g/kg *p.o.* Mortality and different physiological and behavioural effects (skin state, salivation, whimpering, trembling, locomotion, excretion) were noted after 15 min, 1, 4, and 24 h as well as every day for 14 days. Body weights of each mouse were recorded daily.

Statistics

Novelty exploration, light/dark choice, staircase, writhing tests: Statistical significances of differences between control and treated groups were ascertained by a combined analysis of variance and an impaired two-tailed range t-test using the Newman-Keuls method.

Hotplate test: After an analysis of variance, differences between each group before and after treatment were ascertained by Student's t-test.

Sleep induction test: The khi-square test was used in order to compare the number of treated mice which went to sleep with controls.

Results

Experiment 1

Writhing test: The results presented in Fig. 1 show that *M. citrifolia* extracts at doses of 800 and 1600 mg/kg *i.p.* significantly decreased the number of contortions and stretchings of mice induced by acetic acid. Morphine sulfate induced a significant reduction of the noted parameters.

Hotplate test: The results given in Fig. 2 show that *M. citrifolia* extracts increased in a dose-dependent fashion the reaction time of mice; morphine sulfate brought about a significant increase of the reaction time.

Antagonism by naloxone: In the writhing test (Fig. 3) and in the hotplate test (Fig. 4) the analgesic effects of morphine sulfate (1.15 mg/kg and 4.6 mg/kg) and *M. citrifolia* (800 and 1600 mg/kg; 400 and 800 mg/kg respectively), were completely antagonized by naloxone.

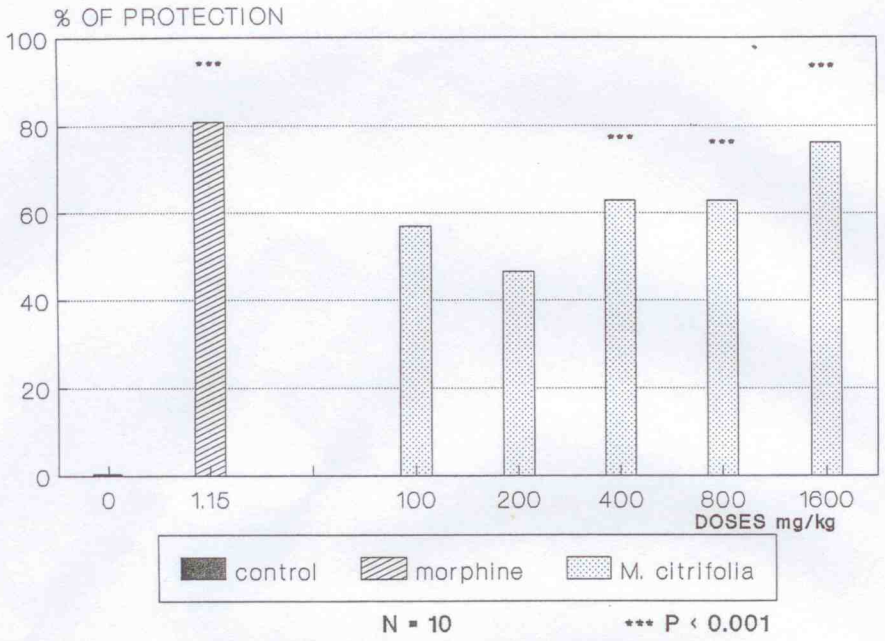

Fig. 1 Analgesic effects of *Morinda citrifolia* and morphine in the writhing test in mice.

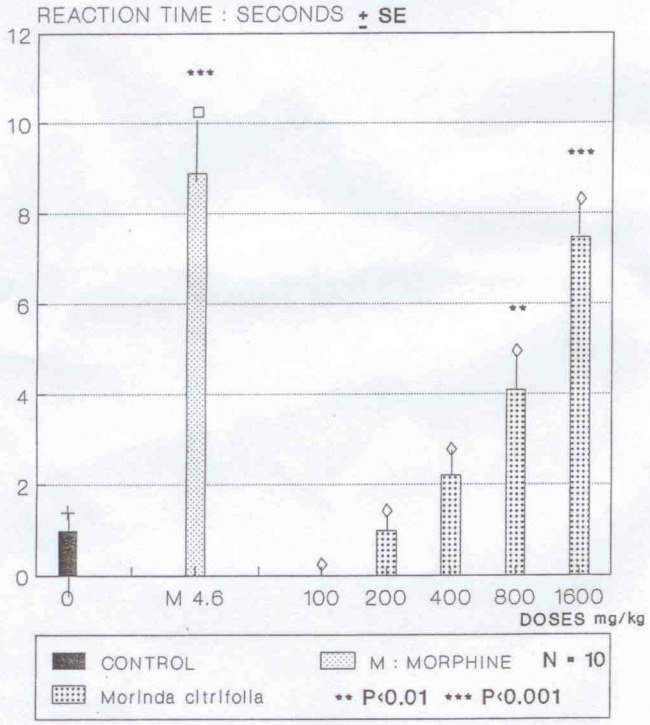

Fig. 2 Analgesic effects of *Morinda citrifolia* and morphine in the hotplate test in mice.

Experiment 2

Two compartment test: As can be seen from Table 1, *M. citrifolia* did not significantly modify the novelty preference of animals but significantly decreased at the dose of 500 mg/kg locomotor activity.

Experiment 3

Light/dark choice situation test: M. citrifolia at 500 mg/kg significantly decreased the time spent by mice in the lit box and the number of transitions (Table 2).

Staircase test: Fig. 4 shows that *M. citrifolia* significantly decreased the number of steps climbed as well as the number of rearings at 800 and 1600 mg/kg.

Experiment 4

Sleeping induction test: Table 3 shows that *M. citrifolia* induced sleep at 1600 mg/kg after administration of an infra-hypnotic pentobarbital dose.

Acute toxicity

M. citrifolia extracts administered *i.p.* and *p.o.* did not induce mortality up to the dose of 8 g/kg *i.p.* and 16 g/kg *p.o.* The treated animals did not present any toxic manifestations on the noted parameters during the first 3 days; the body weights decreased at high doses (4 to 8 g/kg *i.p.* and 16 g/kg *p.o.*) and constipation was observed in treated animals.

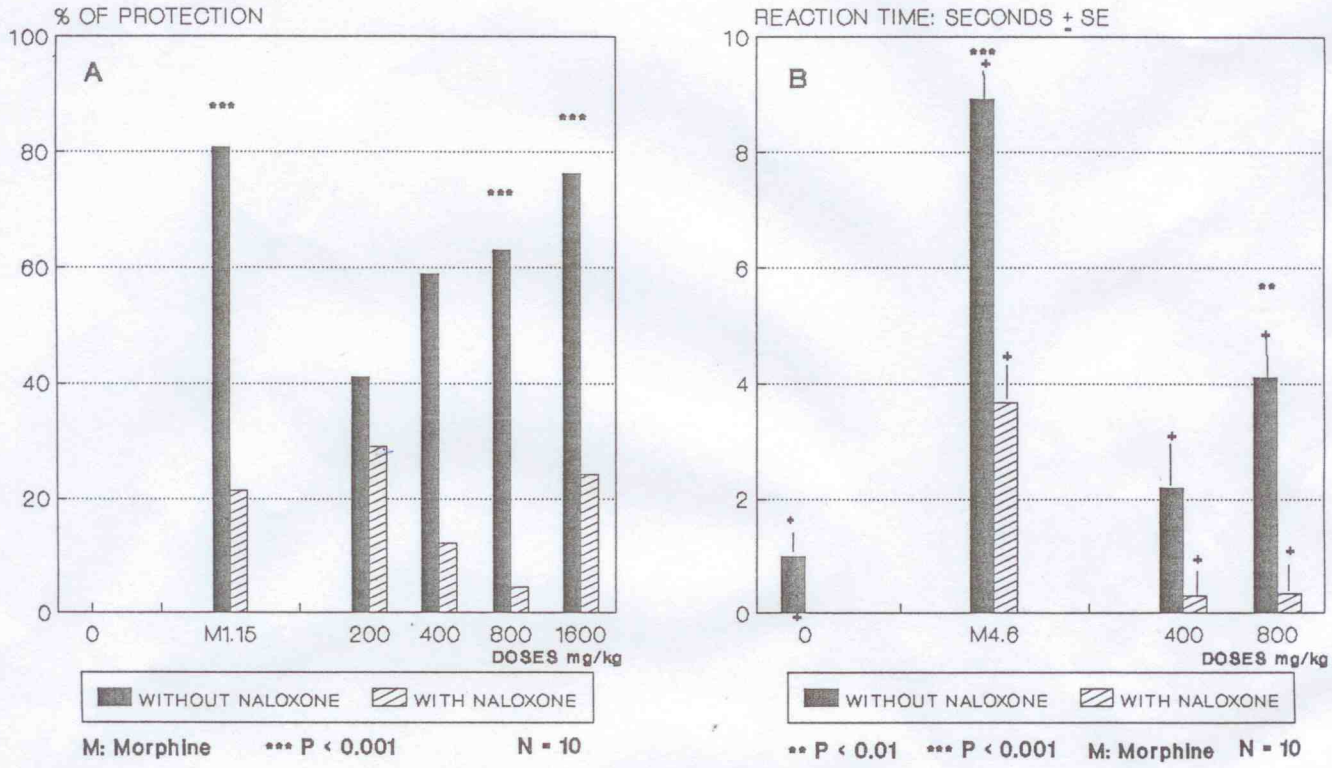

Fig. 3 The antagonistic effect of naloxone on the analgesic activity of *Morinda citrifolia* and morphine in the writhing test (A) and the hotplate test (B).

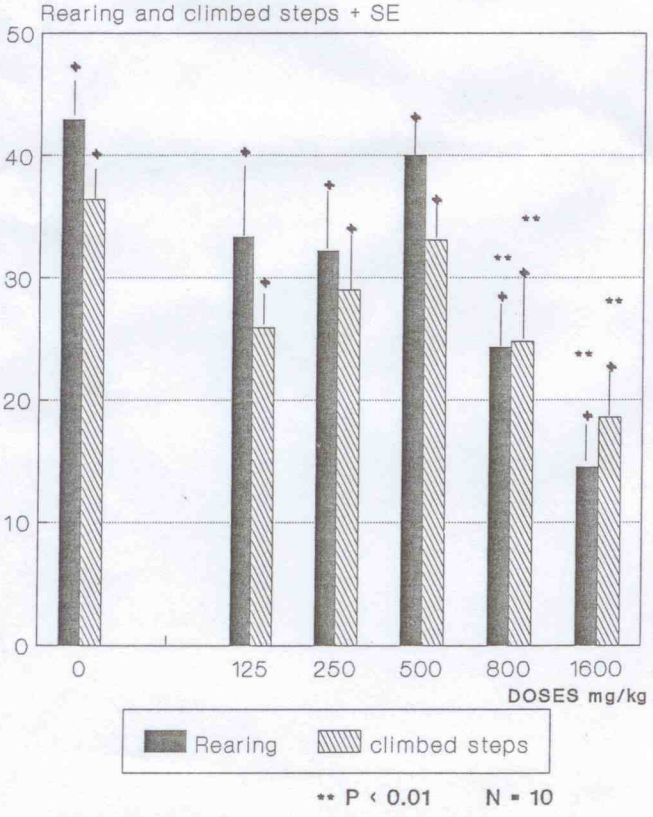

Fig. 4 Effect of *Morinda citrifolia* on the climbed steps and rearing of mice in the staircase test.

Table 1 Effect of *Morinda citrifolia* on novelty preference and locomotion of mice in the two compartment test.

Doses mg/kg	Novelty preference %	Locomotion ± SE	N
Control	75.0 ± 4.4	104.2 ± 8.9	20
125	52.1 ± 6.8	75.9 ± 11.5	10
250	65.5 ± 5.9	80.4 ± 11.5	10
500	54.6 ± 5.2	72.9 ± 7.9*	10

* $P < 0.02$.

Table 2 Effect of *Morinda citrifolia* on time spent by mice in the lit box and the number of transitions between boxes.

Doses mg/kg	Time spent in the lit box/sec	Transition number ± SE	N
Control	89.6 ± 2.8	9.6 ± 0.3	21
125	92.1 ± 3.4	9.0 ± 0.7	10
250	88.4 ± 9.4	9.5 ± 1.1	10
500	62.1 ± 8.7*	7.7 ± 1.0**	10

* $P < 0.02$; ** $p < 0.05$.

Table 3 Effect of *Morinda citrifolia* on sleeping induction after administration of an infrahypnotic dose of pentobarbital (25 mg/kg *i.p.*) in mice.

	Control	*Morinda citrifolia* mg/kg *i.p.*			
		200	400	800	1600
Mice Sleeping %	8	8	42	25	58
N	12	12	12	12	12
P	/	NS	NS	NS	$p < 0.05$

N = number of mice.

Discussion

The present results indicate that the aqueous lyophilised extract of the root material of *Morinda citrifolia* L. appears to have analgesic effects at 800 mg/kg, since this significantly reduced the contortions induced by acetic acid in the writhing test, and increased the reaction time of mice in the hotplate test. The influence of *M. citrifolia* on analgesia in these two tests demonstrated a central analgesic effect, which was confirmed by the antagonistic effect of naloxone, a specific antagonist of morphinomimetic receptors (16).

The results obtained in the behavioural tests show that *M. citrifolia* at 500 mg/kg reduces the locomotor activity of animals, both in the two compartment test (free choice exploratory situation) and in the light/dark choice situation test (non-familiar environment test); the same effect was obtained at 800 mg/kg in the staircase test.

The impairment of parameters and the induction of sleeping time after administration of an infrahypnotic dose of pentobarbital, which appeared with high doses only, suggests that *M. citrifolia* induces a sedative effect. As the opioids are known to reduce behavioural parameters, the sedative effect of *M. citrifolia* could be linked to its central analgesic effect.

These findings validate the traditional analgesic properties of this plant.

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