HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2016.01.017

Evaluation of the anticonvulsant activity of the essential oil of *Myrothamnus moschatus* in convulsion induced by pentylenetetrazole and picrotoxin

Emmanuel Randrianarivo[1], Filippo Maggi[2], Marcello Nicoletti[3], Philippe Rasoanaivo[1*]

[1]*Malagasy Institute of Applied Research, Rakoto Ratsimamanga Foundation, Avarabohitra Itaosy, lot AVB 77, Antananarivo, Madagascar*

[2]*School of Pharmacy, University of Camerino, Camerino, Italy*

[3]*Department of Environmental Biology, Sapienza University, Rome, Italy*

ARTICLE INFO

Article history:
Received 24 Nov 2015
Received in revised form 22 Dec 2015
Accepted 4 Jan 2016
Available online 20 Apr 2016

Keywords:
Myrothamnus moschatus
Essential oil
Convulsions
Pentylenetetrazole
Picrotoxin

ABSTRACT

Objective: To evaluate the anticonvulsant effect of the essential oil of *Myrothamnus moschatus* (*M. moschatus*) in convulsion induced by pentylenetetrazole and picrotoxin in rodent models.

Methods: The essential oil of the aerial parts of *M. moschatus* was extracted by steam distillation. Thereafter, it was injected subcutaneously to rats and mice at escalating doses (0.1–0.8 mL/kg). Ten minutes after drug injection, pentylenetetrazole was injected intraperitoneally to rats and picrotoxin was administered to mice by the same route. Diazepam served as the positive control. Every single animal was placed into transparent cage and observed for convulsive behavior for 30 min by using ordinary security cameras connected to a video recorder. Death occurring for a period of 24 h was also recorded.

Results: The essential oil at 0.8 mL/kg completely arrested the pentylenetetrazole-induced convulsion without any sedative effect and delayed its appearance at lower doses, but showed moderate activities on picrotoxin-induced convulsion. For the rats treated with pentylenetetrazole alone, the mortality was 100% within 1 h, but for the rats pre-treated with the essential oil, the mortality was 0%. For the mice treated with picrotoxin, the mortality rate was also 100%, while 20%–100% died in those that had been pre-treated with the oil.

Conclusions: The results confirmed at least partly the traditional uses of the smoke of *M. moschatus* for the management of convulsion, and implied that the essential oil may inhibit the convulsion by GABAergic neuromodulation.

1. Introduction

Epilepsy and convulsive seizures are the most common chronic neurologic disorder that affects approximately 70 million people of all ages worldwide [1]. Nearly 80% of people with these diseases reside in developing countries, where it remains a major public health problem, not only because of its health implications, but also for its social, cultural, psychological, and economic consequences [2]. The prevalence of epilepsy in sub-Saharan Africa seems to be higher than that in other parts of the world with 10 million people affected directly according to World Health Organization estimates [3]. In the central highlands of Madagascar, an epidemiological study on epilepsy estimated the prevalence of the disease to be 2.7% [4]. In addition to genetic and environmental factors, sequels of central nervous system infections, especially meningitis, viral encephalitis, cerebral malaria and neurocysticercosis are the main causes of seizures and acquired epilepsy in the developing world [5,6].

One serious problem in low-income countries is the poor availability and high cost of medications that must be taken daily for a long period of time. The epilepsy and convulsive seizures treatment gap is defined as the proportion of people who require but are not receiving treatment [7]. With an average gap of approximately

*Corresponding author: Philippe Rasoanaivo, Malagasy Institute of Applied Research, Rakoto Ratsimamanga Foundation, Avarabohitra Itaosy, lot AVB 77, Antananarivo, Madagascar.
Tel: +261 340362321
Fax: +261 202230470
E-mail: rafita@moov.mg

All experimental procedures involving animals were conducted in accordance to the guidelines published in: Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Research, Commission on Life Sciences, National Research Council, National Academy Press, Washington, D.C., 1996 and approved by the ethical committee of the University of Antananarivo under the reference No. 048/VPFR/DR/13.

Foundation Project: Supported by the University Research Fund 2014/2015 of the University of Camerino under the grant number FPI000044.

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

75% for low-income countries, and the poorest in Africa reflecting a gap of more than 90%, the situation is quite alarming.

Some patients in Madagascar, even those in well-educated families, view epilepsy and convulsive seizures as manifestations of an evil possession according to their traditional beliefs, and prefer treatments based mainly on prayers and exorcism [8]. Herbal remedies are also used. We have learned from our ethnobotanical field work that local populations use the smoke of *Myrothamnus moschatus* (*M. moschatus*) to expel bad spirits entering the body, which is believed to be responsible for convulsions. We assumed that volatile constituents might be responsible for the anticonvulsant effects. We wish to report here the effects of the essential oil of *M. moschatus* in convulsions induced by the chemoconvulsant agents pentylenetetrazole (PTZ) and picrotoxin (PTX) in mice and rats.

2. Materials and methods

2.1. Plant material and extraction

Aerial parts of *M. moschatus* were collected in the flowering period in January 2010, in the Isalo Region (Southwestern Madagascar). The plant was authenticated by taxonomists at the Botanical and Zoological Park of Tsimbazaza, Antananarivo. A voucher specimen was deposited in the herbarium of the Malagasy Institute of Applied Research, under the accession code MAD0013/RECs. The essential oil used in this study was extracted according to the method described in our previous paper [9].

2.2. Experimental animals

Wistar rats and Swiss mice bred at the animal house of the Malagasy Institute of Applied Research were used in this analysis. All the animals were kept in appropriate cages in an air-conditioned room [(22 ± 2) °C], controlled lighting on 12 h light–dark cycle, and with free access to normal food and water. The experiments took place between 13:00 and 18:00. All experimental procedures involving animals were conducted in accordance to the guidelines published in: Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Research, Commission on Life Sciences, National Research Council, National Academy Press, Washington, D.C., 1996 and approved by the ethical committee of the University of Antananarivo under the reference No. 048/VPFR/DR/13.

2.3. Chemicals

PTZ and PTX were purchased from Sigma Chemical Co. (USA), whereas diazepam was obtained at a local pharmacy. All chemicals were prepared freshly in normal saline solution (0.9%) just before use. The solutions were injected by intraperitoneal (*i.p.*) route and administered in a volume not exceeding 10 mL/kg of body weight.

2.4. PTZ-induced seizure in rats

Wistar rats of either sex (200–250 g) were used for this test. Animals were divided into six groups of four rats each. Group 1 served as the control and received olive oil at 2 mL/kg by subcutaneous (*s.c.*) injection. Group 2 served as the positive control and received diazepam at 5 mg/kg by *i.p.* administration and the other four groups were given escalating doses of essential oil (0.1, 0.2, 0.4 and 0.8 mL/kg) dissolved in an appropriate quantity of olive oil by *s.c.* administration. Ten minutes after administration of the test drug, diazepam or vehicle, PTZ at 60 mg/kg in normal saline solution was injected by *i.p.* route to each rat. Each animal was placed into a transparent cage and observed for convulsive behavior for 30 min post-PTZ administration using ordinary security cameras connected to a video recorder. The time of seizure onset, percentage of seizure, occurrences of tonic-clonic seizure, seizure duration and seizure behavioral scores were recorded. Seizure behavioral scores were as follows [10]: Stage 0: no response; Stage 1: ear and facial twitching; Stage 2: myoclonic jerks without rearing; Stage 3: myoclonic jerks, rearing; Stage 4: turning over into side position, bilateral tonic-clonic seizures; Stage 5: turning over into back position, generalized clonic and tonic seizures. Mortality was also recorded for a period of 24 h. Experiments were carried out in triplicate and results were expressed as the mean ± SD of three determinations.

2.5. PTX-induced seizure in mice

Groups of five mice of either sex with a weight between 20 and 25 g were treated either with escalating doses of the test essential oil (0.1, 0.2, 0.4, and 0.8 mL/kg, *s.c.* route) dissolved in an appropriate quantity of olive oil or the standard control (diazepam, 5 mg/kg, *i.p.* route). The control group received olive oil at 2 mL/kg by *s.c.* injection. Ten minutes after administration of the essential oil or diazepam, the animals were injected with 6 mg/kg of PTX by *i.p.* route and were observed during the next 30 min for the same patterns of seizures as described above. The patterns were then classified as follows: (i) animals that did not convulse within 30 min were considered to be protected, (ii) the number of mice protected in each group was expressed as a percentage, (iii) in unprotected animals, the latency to first convulsion and the durations of convulsions were recorded. The animals were also observed for the mortality for 24 h after administration of PTX. Experiments were carried out in triplicate and results were expressed as the mean ± SD of three determinations.

2.6. Statistical analysis

Latencies to first seizure, seizure intensity and seizure duration were compared by One-way ANOVA followed by the Student–Newman–Keuls test (pb0.05). The number of animals that seized and the number that survived were calculated as percentages. Differences were considered to be statistically significant ($P < 0.05$).

3. Results

3.1. PTZ-induced seizures in rats

As shown in Table 1, essential oil at the dose of 0.8 mL/kg by *s.c.* route and diazepam (5 mg/kg, *i.p.*) completely protected the rats against the seizure elicited by PTZ (60 mg/kg, *i.p.*). At lower doses (0.2 and 0.4 mL/kg, *s.c.*), significant increased latency period as well as reduced frequency and intensity of convulsion were observed. Furthermore, the essential oil at the doses of 0.2, 0.4 and 0.8 mL/kg and diazepam (5 mg/kg, *i.p.*) protected all animals from mortality, observed for 24 h post-treatment.