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

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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

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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.

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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.

Table 1
Effect of the essential oil of *M. moschatus* on convulsion induced by PTZ.

Pre-treatment	Doses	Latency period of seizure (s)	Seizure protection (%)	Seizure score	Incidence of tonic-clonic seizure (%)	Duration of epileptic seizure (min)	Mortality in 24 h (%)
Olive oil	–	68 ± 5	0	4.6 ± 0.1	100	115.2 ± 10.4	50
Diazepam (mg/kg)	5	–	100	0.0 ± 0.0	0	0.0 ± 0.0	0
Essential oil (mL/kg)	0.1	115 ± 8	0	4.2 ± 0.4	75	100.7 ± 10.1	25
	0.2	147 ± 11*	0	3.2 ± 0.1*	50	85.4 ± 7.3	0
	0.4	340 ± 9**	50	2.0 ± 0.1*	0	10.2 ± 4.2*	0
	0.8	–	100	0.0 ± 0.0	0	0.0 ± 0.0	0

*, **: Refer to statistical significance in comparison with control values.

3.2. PTX-induced seizure in mice

Table 2 shows that in the PTX model, diazepam (5 mg/kg, *i.p.*) demonstrated 80% protection but the essential oil failed to protect the animals from seizures. However, the essential oil significantly increased seizure latency and reduced seizure duration in the unprotected animals in a dose-dependent manner. While no death was observed for diazepam, 100% death was recorded for mice treated with 0.1 and 0.2 mL/kg of essential oil, and respectively 40% and 20% death for mice treated with 0.4 and 0.8 mL/kg.

Table 2
Effect of the essential oil of *M. moschatus* on convulsion induced by PTX.

Pre-treatment	Doses	Seizure latency (s)	Seizure duration (s)	Protection (%)	Mortality in 24 h (%)
Olive oil	–	360 ± 23	74 ± 7	0	100
Diazepam (mg/kg)	5	1 684 ± 56***	8 ± 1***	80	0
Essential oil (mL/kg)	0.1	489 ± 24	68 ± 7	0	100
	0.2	658 ± 36*	51 ± 4*	0	100
	0.4	876 ± 34**	34 ± 2*	0	40
	0.8	1 369 ± 50***	33 ± 1**	0	20

*: Compared with control values (olive oil) ($P < 0.05$); **: Compared with control values ($P < 0.01$); ***: Compared with control values ($P < 0.001$).

4. Discussion

Diseases possessing a long history of existence have been associated with various kinds of beliefs and practices adopted by local populations in an attempt to abort or attenuate the severity of the disease. One of such diseases is epilepsy, which has been known for thousands of years. The characteristic features of convulsive seizures and sometimes its intensity have led local populations to believe in supernatural origins of the disease, including witchcraft, possession by evil spirits, punishment for not carrying out duties to ancestors, or transgression of taboos [11]. Various empirical practices have also been employed, among these smell and smokes, for example, the historical use of shoe-smell in India for the management of epilepsy [12]. On the other hand, one of the earliest and often overlooked uses of plants is the production of smokes, which has been used since ancient times. Plants have been burned for medicinal and recreational purposes, magico-religious ceremonies, pest control, perfumes, food preservation and flavor enhancer. The use of plant-derived smoke is therefore an important cultural trait. In our ethnobotanical field work, we learned that dry leaves of *M. moschatus* are smoked like a cigar and the smoke is gathered in a rice bag. The convulsing patient then inhales the smoke to expel bad spirits entering his body, which is believed to be responsible for convulsions. *M. moschatus* is known under several vernacular names, which are “maharoaka”, “maroaky”, “maharoaky”, “maha” (capable of) and “roaka_mandroaka” (expelling). Thus, this name refers to the magical ethnobotanical use of the plant being capable of expelling the devil. Furthermore, *Myrothamnus* derived from the Latin word *myron* meaning aromatic and *thamnos* meaning bush, and moschatus from the Greek word moskhos meaning musc. Musc was a name originally given to a substance with a penetrating odor obtained from a gland of the male musk deer. The substance has been used as a popular perfume fixative since ancient times and is one of the more expensive animal products in the world. We assumed that volatile constituents may be responsible for the anticonvulsant activity of the plant. We therefore extracted the essential oil by steam distillation and evaluated its effects on chemically-induced seizures.

PTZ is a central nervous system stimulant with epileptogenic properties which has been widely used to study seizure phenomena and to identify extracts/compounds that may control seizures [13]. PTZ is known as a non-competitive γ-aminobutyric acid (GABA) antagonist at the $GABA_A$ receptor, whereby it is presumed to cause its convulsing effect, but the exact molecular mode of action is not entirely understood, which includes the exact binding site of PTZ at $GABA_A$ receptor complex and other sites of actions [14]. The $GABA_A$ receptor is a chloride channel, which opening by GABA agonistic actions induces major inhibition of the central nervous system, causing sedation, sleep and inhibition of convulsions [15].

PTX is an another chemoconvulsant agent which has been widely used in animal convulsion paradigms. It is also a non-competitive $GABA_A$ receptor antagonist for the GABA agonistic action on the receptor's chloride channel, acting as a channel blocker or allosteric modulator of the channel, which is thought to cause PTX's convulsive effect [16].

At the dose of 0.8 mL/kg, the essential oil of *M. moschatus* completely inhibited the convulsive effects of PTZ. No sedative effect was observed. At lower doses, it demonstrated a significant increase in seizure latency and a significant reduction in seizure duration as compared with the control group. No mortality was recorded.

In quite strong contrast to the strong inhibitory action on PTZ induced convulsions, our data from this study demonstrate that the *M. moschatus* essential oil had only moderate activity on the PTX-induced convulsion in mice.

Our previous studies show that *M. moschatus* essential oil contains a high content of *trans*-pinocarveol and pinocarvone, *cis*- and *trans*-p-mentha-1(7), 8-dien-2-ol, perillyl acetate and β-selinene [17]. It is at present not known which of these compounds is the cause of the anticonvulsive actions, but it is tempting to speculate that one or several major constituents are involved in an action directly on the $GABA_A$ chloride channel complex.

The $GABA_A$ channel is subject to regulation at several different binding sites. It contains two binding sites for GABA, which are also binding sites for the GABA agonists muscimol, gaboxadol, and bicuculline, an allosteric binding site for benzodiazepines that bind benzodiazepine agonists causing channel closing and benzodiazepine inverse agonists causing channel opening, as well as competitive benzodiazepine antagonists which prevent the actions of both the benzodiazepine agonists and inverse agonists. Furthermore, the $GABA_A$ receptor contains allosteric binding sites for a number of other compounds, which include PTZ and PTX. The exact binding modes for the latter are not fully known and are presumably located spatially different from each other and involved in different modes of modulation of the receptor channel. It is known that PTX binding itself is allosterically regulated in the presence of muscimol, bicuculline, and benzodiazepine agonist and inverse agonists and combinations thereof in complex fashion [18]. It is stated that indeed components of *M. moschatus* essential oil cause an action on the $GABA_A$ channel; the difference in efficacy against PTZ and PTX induced convulsions can be explained by a difference in the mode of channel closing caused by the two convulsive agents and the action caused by the *M. moschatus* essential oil component(s). Admittedly, at the moment this is only speculative and must await more direct assessments of actions of the *M. moschatus* essential oil and its components on the $GABA_A$ channel.

However, from our ethnopharmacological observations on the native use of *M. moschatus*, its smoke when inhaled seems to have a very strong action in halting convulsions in human subjects. The essential oil has a very distinctive strong conifer-like and quite pleasant smell. In fact, earlier alternative treatments reported control of seizures by olfactory stimulation [19,20]. The reception and interpretation of smell occurs in the limbic system of the brain, particularly in the temporal lobe and the site of temporal lobe epilepsy which is the most common form of epilepsy and is also often associated with pharmacoresistance. When inhaled, the essential oil stimulates smell receptors in the nose that sends chemical messages through nerves to the brain's limbic system. Animal models support a link between olfaction and seizures, as neurotransmitter deficiencies were detected in the olfactory bulb of genetically epilepsy-prone rats [21], and because olfactory stimulation is capable of attenuating the seizure threshold in amygdala-kindled rats [22]. In addition to animal studies, clinical studies in humans have demonstrated that electrical stimulation of the olfactory bulb is an effective treatment of otherwise intractable epilepsy [23]. Chemical or electrical stimulation of the olfactory system may thus attenuate or arrest convulsive seizures.

Inhalation of the essential oil of *M. moschatus* might find therapeutic applications in the prevention for oncoming convulsions because the absorption by the nose is as fast as an intravenous injection. The essential oil may also be used as adjunct therapy to the existing antiepileptic drugs in refractory epilepsy. Today, the paths of science-based and alternative medicine practices are largely parallel and independent, sometimes antagonistic, sometimes cooperative. Both paths have fostered many divergent roads. Our most significant challenge remains the creation of a meaningful dialogue and exchange of ideas and lessons across and beyond the boundaries of both approaches.

Conflict of interest statement

We declare that we have no conflict of interest.

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