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Study of anticonvulsant effect of citronellol, a monoterpene alcohol, in rodents

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Abstract

Citronellol is one monoterpene alcohol, which is present in the essential oils of various aromatic plant species. This study evaluated the neuroprotective activity of citronellol on pentylenetetrazol- and picrotoxin-induced convulsions and maximal electroshock-induced seizures in mice. Administration of citronellol significantly reduced the number of animals of convulsion induced by pentylenetetrazol and eliminated the extensor reflex of maximal electroshock-induced seizures test in about 80% of the experimental animals. In addition, administration of citronellol showed protection in the pentylenetetrazol and picrotoxin tests by increasing the latency of clonic seizures. We also investigated the effect of citronellol in the rat isolated nerve using the single sucrose-gap technique. We showed that the amplitude of the compound action potential decreased more than 90% when the monoterpene was incubated for 30 min at 6.4 mM and we did not verify any effect on the repolarization of the compound action potential. Taken together, our results demonstrated an anticonvulsant activity of the citronellol that could be, at least in part, explained by the diminution of the action potential amplitude.

Keywords: Essential oils; Citronellol; Monoterpene; Anticonvulsant; Compound action potential

The epilepsy is one of the most common neurological conditions that show a prevalence rate in 1–2% of the world population. Even knowing that epilepsy is characterized by a group of brain disorders whose symptoms and causes are diverse they do share a common manifestation: the seizure. Seizures result from the abnormal discharge of groups of neurons within the brain, usually within a focal point, that can result in the recruitment of large brain regions into epileptiform activity [8]. It has been proved by a number of studies that ion channels are involved in the epileptogenesis and evidences are being obtained that support the notion that the behavior of some types of ion channels could be altered during these episodes [19]. Although a considerable number of antiepileptic drugs are available for the treatment of epilepsy there is still an urgent need for development of new drugs as alternatives [13].

The essential oils are natural products that exhibit a variety of biological properties, such as analgesic [1], anticonvulsant [2] and anxiolytic [3,24]. Those effects are attributed to the monoterpenes which are the major chemical components of these essential oils. For instance, the monoterpene linalool, has been reported to have anticonvulsant activity in mice [7]. Similarly, limonene, beta-myrcene and citral presented significant increases in the latency of pentylenetetrazol-induced convulsions [25].

Citronellol is an acyclic monoterpene alcohol prevalent in essential oils of various plants, such as *Cymbopogon winterianus* Jowitt [21] and in other aromatic plant species [17]. This monoterpene occurs naturally as two isomeric optical forms. The *R*-(+)-isomer is a common constituent of plant essential

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oils, especially in the Rutaceae. Conversely, the *S*-(−)-isomer is much less common and it is present in geranium and citronella oils. Some of the pharmacological actions of citronellol have been studied [10–12,26]. Citronellol produced also an increasing effect on the response rate during the alarm period in the conflict test suggesting that it possesses an anti-anxiety effect [24].

In the present study, experiments were performed to evaluate the profile of citronellol anticonvulsant activity in three seizure models. In addition, the effects of citronellol on the nerve compound action potential (CAP) were also examined.

Adult male albino Swiss mice weighing 24–30 g, with three months of age were used throughout this study. The animals were randomly housed in appropriate cages at 24 ± 2 °C on a 12 h light cycle with free access to food (Purina – Brazil) and tap water. They were used in groups of eight animals each. All animals were acclimatized before the experiments. For the single sucrose-gap method we used adults Wistar rats weighing 350–450 g, which were sacrificed by cervical dislocation. Experimental protocols and procedures were approved by the Laboratório de Tecnologia Farmacêutica Animal Care and Use Committee (CEPA/LTF-UFPB #0503/05).

Pentylenetetrazole (PTZ), phenytoin (PHE), picrotoxin (PIC), cremophor and diazepam (DZP) were purchased from Sigma Chemical Co. (USA). (+)-Citronellol was purchased from Dierberger (Brazil). All drugs were injected intraperitoneally (i.p.).

The detailed method has been previously described [4,15]. Animals were divided into five groups ($n = 8$), the first group served as control and received saline with one drop of cremophor, while the second group was given diazepam (3 mg/kg, i.p.). The remaining groups received an injection of citronellol (100, 200 and 400 mg/kg, i.p.). After 60 min of citronellol administration, the mice were challenged with picrotoxin at a dose of 8 mg/kg (i.p.). Immediately after the picrotoxin injection, mice were individually placed in plastic boxes and observed for the onset of clonic seizures, percentage of clonic seizures and mortality rate. Mortality was noted until 48 h after the injection of picrotoxin. Diazepam at 3 mg/kg (i.p.) was used as positive control.

In another series of experiments we used the maximal electroshock (MES) protocol to produce convulsions characterized by tonic hindlimb extension [20]. Electroconvulsive shock (130 V, 150 Hz, for 0.5 s) was delivered through auricular electrodes (ECT UNIT 7801 – Ugo Basile). Mice were divided into four groups ($n = 8$), the first group served as control and received saline with one drop of cremophor, while the second group was treated with phenytoin (25 mg/kg, i.p.) and the other groups received an injection of citronellol (200 and 400 mg/kg, i.p.). After 60 min all groups received the electroconvulsive shock. The number of animals showing tonic convulsions, characterized by the presence of tonic hindlimb extension, was carefully observed. The animals that did not exhibit tonic hindlimb extension were considered protected [23]. Phenytoin and saline were used as positive and negative controls, respectively.

Considering that Na^+, Ca^{2+} and K^+ channels have a central role in generation of the action potential as well as in producing hyperexcitability such as that associated with seizure discharges [27] we performed experiments to measure nerve compound action potentials in order to test if citronellol would have an effect on nerve excitability. Procedures for these experiments were similar as described in a previous paper [5]. Briefly, the sciatic nerves from rats were carefully removed and desheathed. One nerve bundle was positioned across the five compartments of the experimental chamber, which contained vaseline at the partitions to electrically isolate them. Compartments 1 and 2, at one end of the nerve bundle, were used to apply supramaximal stimulation, which consisted of 100 μs isolated rectangular voltage pulses, delivered by a stimulator (CF Palmer, Model 8048, UK), triggered manually. These parameters were chosen to selectively stimulate fast-conducting myelinated fibers (Aα). All compartments were filled with physiological solution with the following composition (in mM): NaCl 150; KCl 4.0; $CaCl_2$ 2.0; $MgCl_2$ 1.0; [*N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid] (HEPES) 10, adjusted to pH 7.4 with NaOH, except for the fourth compartment, which was filled with isotonic (280 mM) sucrose solution that was continuously renewed, to electrically isolate the neighboring recording compartments. Citronellol, at different concentrations, was introduced into the test (central) compartment. The potential difference between the test and the fifth (last) compartment was recorded every 10 min. Data were converted to digital form by a microcomputer-based 12-bit A/D converter at a rate of 10.5 kHz and later analyzed using a suite of programs (Lynx, São Paulo, Brazil). To quantify the effects of citronellol we used the amplitude (which is the potential difference between the baseline and the maximal voltage of the compound action potential), and the time constant of repolarization (τ) that was calculated by the equation $V = V_0 * \exp(t/\tau)$ using non-linear regression analysis applied to the repolarization phase of the compound action potential.

The behavioral data obtained were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test. The incidence of clonic or tonic convulsions were evaluated by Fisher's Exact Test. For CAP recordings the significant level was obtained using the two-tailed Student's *t*-test. Differences were considered to be statistically significant when $p < 0.05$.

As shown in Fig. 1A, citronellol (100, 200 and 400 mg/kg, i.p.) significantly increased, in a dose-dependent manner, the time for the convulsions onset (defined here as latency which means the time to begin the first complete clonic convulsion) in the PTZ model. In this model, diazepam (3 mg/kg), considered as a standard drug for such maneuvers, produced a similar increase of the latency (from 187 ± 36 s for control to 815 ± 70 s for diazepam and 780 ± 75 s for citronellol at 400 mg/kg) when compared to citronellol data. However, citronellol is less potent than DZP. Citronellol was also tested on convulsions chemically induced by picrotoxin and the results turned out to be quite similar as described for the PTZ model. As illustrated in Fig. 1B, citronellol dose-dependently increased the time for the convulsions onset. The maximal effect was observed using citronellol at 400 mg/kg which almost doubled the latency from 388.5 ± 20.4 s for control to 734.8 ± 98.9 s for citronellol (400 mg/kg).

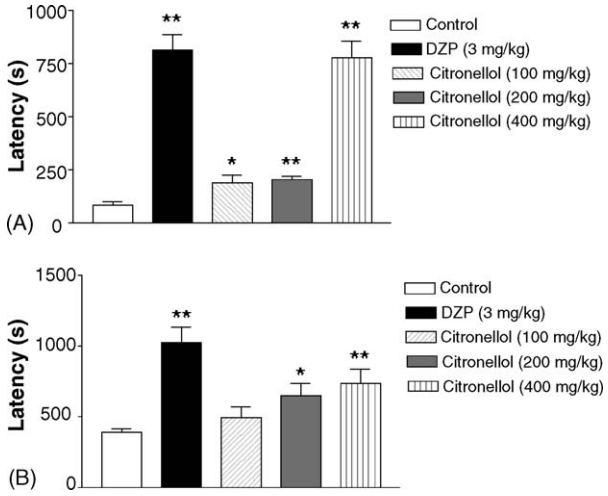

Fig. 1. Effect of citronellol on the latency of the first post-injection convulsion induced by pentylenetetrazol (A) or picrotoxin (B). The bars indicate mean ± S.E.M. ($n = 8$). Statistically significant differences $^{*}p < 0.05$ and/or $^{**}p < 0.01$ with respect to control according to one-way ANOVA.

In studies using MES seizure model, the citronellol (400 mg/kg) produced protection from tonic extension of about 80%, Fig. 2A. The number of animals that experienced convulsions provoked by PTZ injection was also diminished by citronellol (400 mg/kg) administration but without a clear dose-dependence relationship (Fig. 2B).

The above results suggest that citronellol is acting as a neuroprotective drug. It is well established that during the seizures neuronal excitability is definitely enhanced. We then asked the question if citronellol was preventing the seizures because it was actually blocking the action potential discharges. To test this hypothesis, we measured the CAP induced by electrical stimulation. The CAP was recorded with the single-sucrose gap method. Fig. 3A illustrates a typical example of the effects of citronellol on the CAP waveform. The CAP was elicited by supramaximal stimulation (6–8 V, 100 μs) every 10 min. After

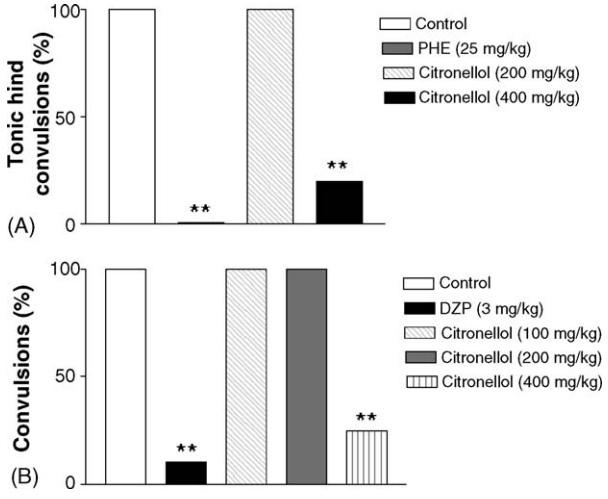

Fig. 2. Effect of citronellol on tonic convulsions induced by electroconvulsive shock (A) or pentylenetetrazol injection (B). The bars indicate percentage values. Statistically significant differences at $^{**}p < 0.01$ (Fisher's exact test).

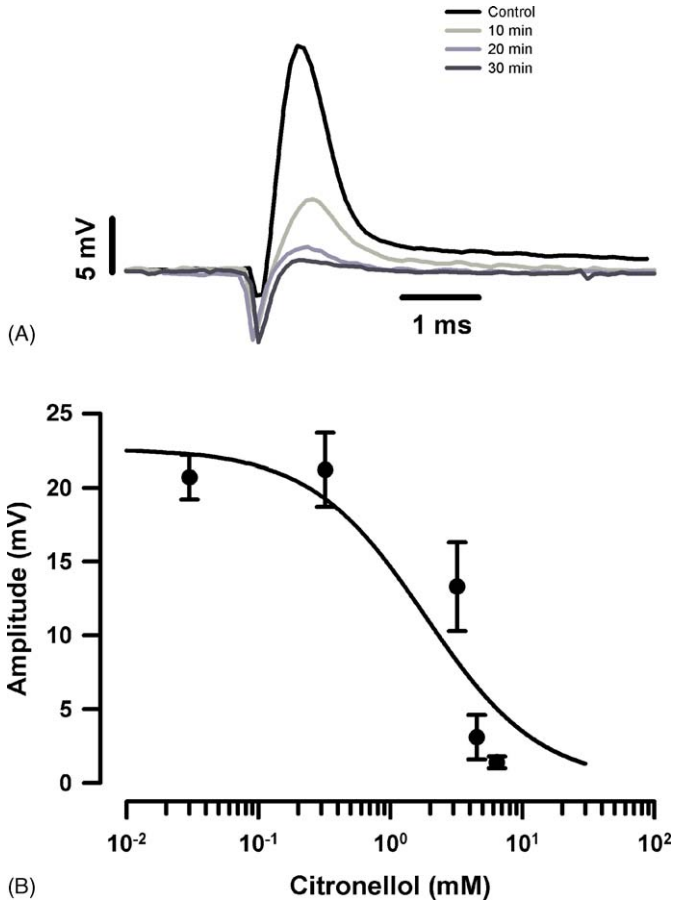

Fig. 3. Citronellol blocks the whole nerve compound action potential (CAP). Panel (A) shows representative superimposed CAP recorded every 10 min in the presence of citronellol (6.4 mM). Stimulation parameters: 6 V/100 μs. Panel (B) depicts the dose–response relationship. Each point represents the mean ± S.E. of four nerve bundles taken at 30 min. The data were fitted by the Hill equation (see text).

stabilization (usually 20–25 min under continuous sucrose perfusion) the control action potential was acquired. Fig. 3A shows superimposed CAP recordings in the absence and presence of citronellol (6.4 mM). Our results show that citronellol produced a significant time-dependent blockade of CAP reaching its maximal effect after 30 min (Fig. 3A and Table 1). There was no significant change in the time constant of repolarization (data not shown, $n = 4$). In order to determine if there was a relation between citronellol concentration and the inhibition of CAP amplitude, we performed a series of experiments in which we varied the extracellular citronellol concentration. It is important to note that we used different nerve bundles for each concentration tested. Fig. 3B (see also Table 1) depicts the dose–response relationship where the continuous line represents the best fit to the data with a Hill equation to obtain an IC_{50} of 2.2 mM and a Hill coefficient (n) of 0.8. The equation used has the following form:

$$f = \frac{(\text{Max} - \text{Min})}{[1 + [\text{citronellol}]/(\text{IC}_{50})^{n}]} + \text{Min}$$

The goal of the present study was to evaluate the anticonvulsant potential of citronellol, a monoterpene present in essential

Table 1
Time-course of citronellol on CAP amplitude blockade

Citronellol (mM)	Control (mV)	Amplitude after 10 min (mV)	Amplitude after 20 min (mV)	Amplitude after 30 min (mV)
0.03	22.1 ± 1.6	22.1 ± 1.2	21.3 ± 1.1	20.7 ± 1.5
0.32	23.6 ± 2.3	22.6 ± 1.9	22.3 ± 2.2	21.2 ± 2.5
3.2	22.1 ± 0.8	16.1 ± 2.2[#]	14.4 ± 2.5[#]	13.3 ± 3.0[#]
4.5	21.9 ± 2.2	17.4 ± 2.0	6.3 ± 1.3[#]	3.1 ± 1.5[#]
6.4	20.4 ± 1.7	6.4 ± 0.7[#]	2.2 ± 0.7[#]	1.4 ± 0.4[#]

Each value represents the mean ± S.E. of at least four nerve bundles.
[#] $p < 0.05$ (statistical significance).

oils. Surprisingly, the combined results demonstrate that citronellol inhibited not only the action of pentylenetetrazol and picrotoxin (chemical-convulsions), but also protected the mice against MES-induced seizures. This feature may be of interest when one thinks in a good candidate for a drug designed to cause neuroprotection in response to various pro-convulsive agents.

The current developments in the availability of new anticonvulsant drugs requires the appropriate choice of animal models of epilepsy for the identification of pharmacological and toxicological activities as well as new mechanisms of action [14]. Therefore, seizure models in laboratory animals are still the most important prerequisite in preclinical search for new anticonvulsant drugs [18].

The MES and PTZ procedures are of predictive relevance regarding the clinical spectrum activity of the test compounds [14,22]. Furthermore, both tests are assumed to identify the efficacy of anticonvulsant drugs [14,18]. Results from the present study show that citronellol may be effective in blocking generalized tonic-clonic partial and generalized clonic seizures.

On the other hand, the genesis of the convulsion originated due to picrotoxin action involves the antagonistic effect of this drug in $GABA_A$ receptors which would reduce the inhibitory synaptic transmission to promote excitatory neurotransmission [9,16]. As reported here citronellol confers protection against seizures induced by chemo-convulsants, including $GABA_A$ receptor antagonists pentylenetetrazol and picrotoxin. Therefore, it is reasonable to suggest that part of the anticonvulsant activity exerted by citronellol may be associated to modulatory effects on GABAergic neurotransmission (Figs. 1 and 2) [6]. Whether citronellol activates $GABA_A$ receptors directly and/or indirectly is unknown and needs further investigation.

Although citronellol is modestly effective when compared to the standard drugs used in our study (e.g., diazepam and phenytoin), the observed effects appear to offer a potential advantage over most of anticonvulsant. For example, diazepam is especially effective in preventing the clonic convulsions induced by pentylenetetrazol but *does not block* generalized clonic-tonic convulsions induced by MES. In contrast, the most significant effect of phenytoin is its ability to modify the pattern of MES-induced seizures. In fact, while the characteristic tonic phase can be completely abolished the clonic seizures may be worsen by phenytoin [20].

The inhibition of the neuronal excitability is associated to the blockade of the voltage-dependent Na^+ channels or a facilitation of the inhibitory synaptic input by simply activating $GABA_A$ receptors. Citronellol depressed the CAP amplitude leading to the suggestion that the anticonvulsant effect observed in the different models used in this work is likely to occur as a result of the voltage-dependent Na^+ channel blockade. In conclusion, citronellol possesses significant anticonvulsant activity probably due to the reduction of neuronal excitability mainly through the voltage-dependent Na^+ channels.

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