Evaluation of the Anticonvulsant Activity of Terpinen-4-ol

Damião P. de Sousa[a], Franklin F. F. Nóbrega[b], Liana C. S. L. de Morais[b], and Reinaldo N. de Almeida[b,*]

[a] Departamento de Fisiologia, Universidade Federal de Sergipe, CEP 49100–000, São Cristóvão, Sergipe, Brazil
[b] Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba, Caixa Postal 5009, CEP 58051–970, João Pessoa, Paraíba, Brazil. E-mail: reinaldoan@uol.com.br

* Author for correspondence and reprint requests

Z. Naturforsch. **64 c**, 1–5 (2009); received June 4/August 7, 2008

Terpinen-4-ol is a monoterpenoid alcohol and component of the essential oils of several aromatic plants. Similarly to terpinen-4-ol, other monoterpenoid alcohols have shown anticonvulsant activity in convulsion animal models. The present study aimed to investigate the anticonvulsant activity of terpinen-4-ol. Treatment of mice with terpinen-4-ol (200 mg/kg) caused a significant decrease in the spontaneous motor activity at 30, 60 and 120 min after administration. Terpinen-4-ol (100 and 200 mg/kg) produced a significant dose-dependent increase in the duration of sleeping in mice. Pretreatment of mice with terpinen-4-ol at doses of 100, 200 and 300 mg/kg significantly increased the latency of pentylenetetrazole -induced convulsions. Terpinen-4-ol (200 and 300 mg/kg) also inhibited the induced seizures of picrotoxin. In another model, maximal electroshock seizure, terpinen-4-ol decreased the tonic hind convulsions percentage at the dose of 300 mg/kg. From the overall results we can conclude that terpinen-4-ol showed a depressant effect on the central nervous system and significant anticonvulsant activity.

Key words: Anticonvulsant Activity, Terpinen-4-ol, Essential Oils

Introduction

Most species of plants are a reservoir of chemical compounds potentially useful not only as drugs, but also as unique templates that could serve as a starting point for synthetic analogues. An increasing number of studies have demonstrated that plant-derived essential oils, and their components, exhibit a variety of biological properties, such as anticonvulsant (De Sousa *et al*., 2007a), analgesic (Amaral *et al*., 2007) and central activities (Silva *et al*., 2007; De Sousa *et al*., 2007b). Generally, their action is the result of the combined effect of both their active and inactive compounds. Several active components might have a synergistic effect. Many of these described activities are frequently attributed to monoterpenes, which are the major chemical components of those essential oils. The monoterpene derivatives also have been shown to have several effects on the central nervous system (CNS), including antinociceptive (De Sousa *et al*., 2004), sedative (De Sousa *et al*., 2006a), and antidepressant (De Sousa *et al*., 2006b) activity.

The identification of the main constituents responsible for the activity of a crude essential oil is of interest. A comparative study of the activity of each compound, even if it does not permit the assessment of the potential synergy and antagonism among the components of an essential oil, could enable to determine structures necessary for their pharmacological action. This information should also allow for the prediction of the biological activity of other structurally related chemical substances and the assessment of their possible modes of action. Terpinen-4-ol is a volatile monoterpenoid alcohol and component of the essential oils of several plants such as *Alpinia zerumbet* (Lahlou *et al*., 2002), *Tanacetum cadmeum* (Ozek *et al*., 2007), *Melaleuca alternifolia* (Dewick, 2001), and other aromatic plant species (Pascual *et al*., 2001). Some of the pharmacological actions of terpinen-4-ol are antiulcer (Matsunaga *et al*., 2000) and antihypertensive (Lahlou *et al*., 2002). Similarly to terpinen-4-ol, other monoterpenoid alcohols have anticonvulsant activity, for example, linalool (Elisabetsky *et al*., 1995), citronellol (De Sousa *et al*., 2006c), and α-terpineol (De Sousa *et al*., 2007a). These facts led us to evaluate the profile of terpinen-4-ol in the CNS and its possible anticonvulsant activity.

0939–5075/2009/0100–0001 $ 06.00

Materials and Methods

Chemicals

(–)-Terpinen-4-ol was purchased from Aldrich (USA), and dissolved in 5% Tween 80 to give an emulsion. Pentobarbital, pentylenetetrazole (PTZ), picrotoxin (PIC), diazepam (DZP), phenytoin (PHT), and polyoxyethylene-sorbitan monolate (Tween 80) were purchased from Sigma (USA).

Animals

Male Swiss mice (28–34 g) were obtained from our research animal facility. The animals were maintained at constant room temperature [(21 ± 1) ºC] and a 12/12-h light-dark cycle (light from 06:00 to 18:00), with free access to food and water. All behavioural observations were conducted between 13:00 and 17:00 and carried out in accordance with the ethical committee approvals by the Ethics Committee on Research Animals of the Federal University of Paraiba 2005/06/08 (protocol number 0503/05).

Statistical analysis

The statistical analysis was performed using analysis of variance, followed by the Dunnett's test. The incidence of tonic convulsions was evaluated by Fisher's exact test. A probability level of 0.05 was regarded as significant.

Locomotor activity

Mice were divided into two groups of eight animals each. Vehicle (control) and terpinen-4-ol (200 mg/kg, ip) were injected, respectively. The spontaneous motor activity of the animals was assessed in an activity cage (controller model 7441 and Grid-Floor Detecting Arrangement Cage model 7432; Ugo Basile, Italy) at 30, 60, and 120 min after administration.

Pentobarbital-induced sleeping time

Sodium pentobarbital at a hypnotic dose of 40 mg/kg (ip) was injected to four groups (n = 8) of mice 30 min after pretreatment with 5% Tween 80 (control) or terpinen-4-ol at doses of 50, 100, and 200 mg/kg (ip), respectively. The duration of sleeping time (loss and recovery of the righting reflex) was recorded (De Sousa *et al.*, 2007b).

PTZ-induced convulsions

Mice were divided into five groups (n = 8). The control and positive control groups received 5% Tween 80 or DZP (4 mg/kg), respectively. The remaining groups received an injection of terpinen-4-ol at doses of 100, 200, or 300 mg/kg. 30 min after drug administration, the mice were treated with PTZ (ip) at a dose of 60 mg/kg and observed for at least 15 min to detect the occurrence of the first episode of forelimb clonus (Swinyard *et al.*, 1989).

PIC-induced convulsions

Animals were divided into five groups (n = 8). The first group served as control and received 5% Tween 80, while the second group was treated with DZP (4 mg/kg, ip). The remaining groups received an injection of terpinen-4-ol (100, 200, or 300 mg/kg, ip). 30 min after drug administration, the mice were treated with PIC at a dose of 8 mg/kg (ip). Immediately after the injection of the convulsant agent, mice were placed individually in plastic boxes and observed for the onset of clonic seizures (Bum *et al.*, 2001).

Maximal electroshock-induced convulsions

The maximal electroshock (MES) protocol to produce convulsions characterized by a tonic hindlimb extension was used. 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 5% Tween 80, while the second group was treated with PHT (25 mg/kg, ip) and the other groups received an injection of terpinen-4-ol (200 or 300 mg/kg, ip). After 30 min all groups received the electroconvulsive shock. The percentage of animals showing tonic convulsions, characterized by the presence of a tonic hindlimb extension, was carefully observed. The animals that did not exhibit a tonic hindlimb extension were considered to be protected (Swinyard *et al.*, 1989). PHT was used as positive control.

Results and Discussion

In the present work, the effects of terpinen-4-ol (Fig. 1) were studied in several behavioural ani-

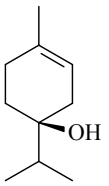

Fig. 1. Chemical structure of terpinen-4-ol.

mal models, such as locomotor activity, pentobarbital-induced sleeping time, PTZ-induced convulsions, PIC-induced convulsions, and MES-induced convulsions. These tests are classical models for screening CNS actions providing information about possible psychopharmacological effects. The present study showed that treatment of mice with terpinen-4-ol (200 mg/kg) caused a significant decrease in the spontaneous motor activity 30, 60 and 120 min after administration (Fig. 2), indicating a central depressant effect (Carlini, 1972). The pentobarbital-induced sleeping time test was also used to confirm, or not, the possible depressive effects observed with terpinen-4-ol. An increase in the sleeping time is classically related to CNS-depressant drugs (Willianson *et al*., 1996). Earlier studies have related the prolongation of barbital hypnosis to pentobarbital metabolic inhibition or action on the CNS involved in the regulation of sleep (Kaul and Kulkarni, 1978). Ours findings

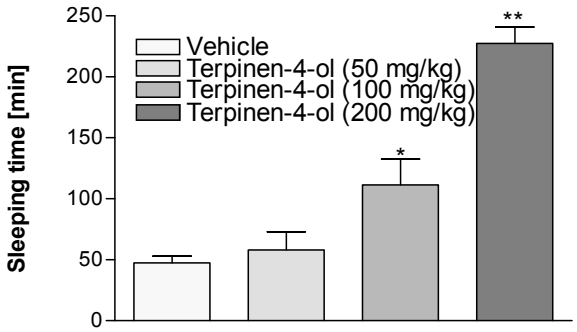

Fig. 3. Effect of terpinen-4-ol on pentobarbital-induced hypnosis in mice. Values are the mean + S.E.M. (n = 8). *p < 0.05, **p < 0.001, when compared with vehicle (control); one-way ANOVA, followed by Dunnett's test.

showed that terpinen-4-ol (100 and 200 mg/kg) produced a significant dose-dependent increase in the duration of sleep (Fig. 3), which possibly confirm the depressant activity on the CNS detected before. These results corroborate those of Fujimori and Cobb (1995), who proposed that the enhancement of barbital hypnosis is a good index of the CNS-depressant activity.

Administration of PTZ caused clonic convulsions in mice. Pretreatment of the mice with terpinen-4-ol at the doses of 100, 200 and 300 mg/kg increased the latency of convulsions significantly,

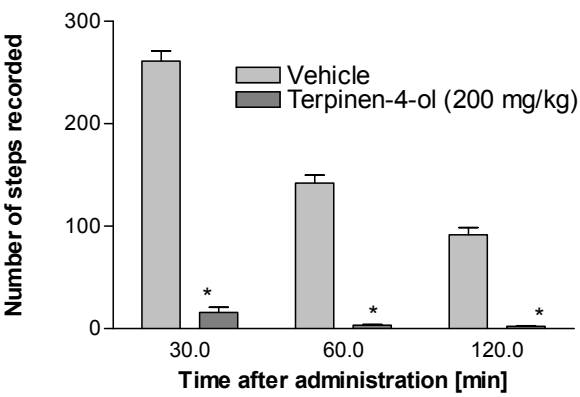

Fig. 2. Effect of terpinen-4-ol on locomotor activity in mice. The parameters evaluated were the total number of pulses measured in the activity cage. Values are the mean + S.E.M. (n = 8). *p < 0.0001, when compared with vehicle (control); one-way ANOVA, followed by Dunnett's test.

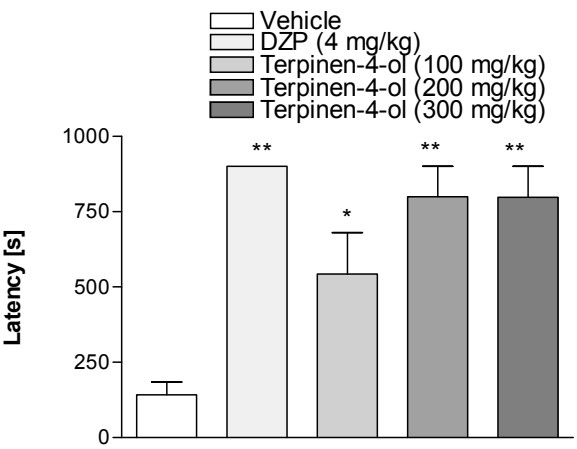

Fig. 4. Effect of terpinen-4-ol on the latency of the first post-injection convulsion induced by pentylenetetrazol. The bars indicate means ± S.E.M. (n = 8). Statistically significant differences at *p < 0.05 and/or **p < 0.001 with respect to control according to one-way ANOVA.

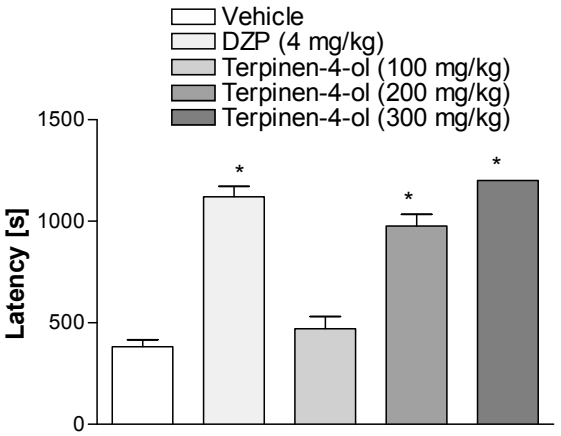

Fig. 5. Effect of terpinen-4-ol on the latency of the first post-injection convulsion induced by picrotoxin. The bars indicate means ± S.E.M. (n = 8). Statistically significant differences at $^*p < 0.001$ with respect to control according to one-way ANOVA.

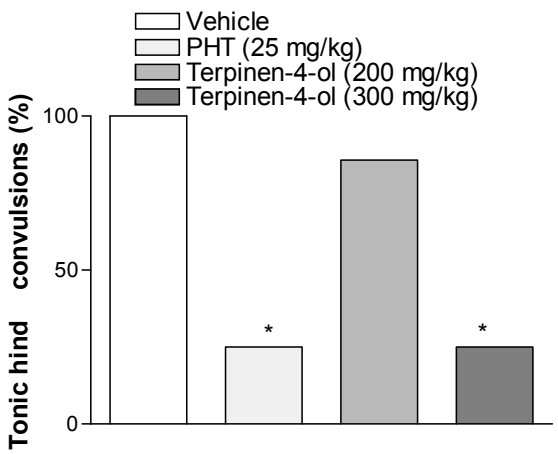

Fig. 6. Effect of terpinen-4-ol on tonic convulsions induced by electroconvulsive shock. Statistically significant differences at $^*p < 0.01$, Fisher's exact test.

close to that of DZP (4 mg/kg), a standard anticonvulsant drug (Fig. 4). The data obtained in this experimental model are in agreement with the results in mice administered with other monoterpenes, such as limonene, citral (Viana *et al.*, 2000), and (+)-carvone (De Sousa *et al.*, 2007c). Terpinen-4-ol (200 and 300 mg/kg) also inhibited the action of PIC (Fig. 5). This is a popular systemic convulsant and is known to be a GABA antagonist exerting its effect by binding to the PIC-binding site which is closely related to the chloride ionophore in the $GABA_A$ receptor complex. Classical anticonvulsants such as carbamazepine, PHT, and DZP have a protective effect against PIC-induced seizures (Deyn *et al.*, 1992). In another model, MES seizure, terpinen-4-ol decreased the tonic hind convulsion percentage at the dose of 300 mg/kg (Fig. 6). The effect was similar that of PHT, a standard anticonvulsant drug. Interestingly, the combined results showed that terpinen-4-ol inhibits not only the action of PTZ and PIC (chemical convulsions), but also protects mice against MES-induced seizures. The results from the present study show that terpinen-4-ol may be effective in blocking generalized tonic-clonic partial and generalized clonic seizures. These data are in agreement with the results obtained in mice administered with α-terpineol, a terpinen-4-ol isomer, which also was effective in PTZ and MES models (De Sousa *et al.*, 2007a).

From the analyses of the results we can conclude that terpinen-4-ol possesses a depressant effect on the CNS and significant anticonvulsant activity probably due to interaction with GABA receptors.

Acknowledgements

This research was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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