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The anticonvulsant effect of geraniol and inclusion complex geraniol: β-cyclodextrin

[El efecto anticonvulsivo de geraniol y la inclusión de geraniol complejo: β-ciclodextrina]

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Abstract: Geraniol (GR) is an acyclic monoterpene alcohol present in essential oils of aromatic plant species used in Brazilian folk medicine for the treatment of epilepsy. The present study was designed to evaluate the anticonvulsant effect of GR and of the inclusion complex geraniol:β-cyclodextrin (GR:β-CD). Mice were treated with GR or with GR:β-CD (50, 100 and 200 mg/kg) 30 min before pentylenetetrazole (PTZ) or strychnine (STN). GR at 200 mg/kg and GR:β-CD at the doses of 100 and 200 mg/kg significantly increased the latency for the first PTZ-induced convulsion and reduced the percentage of animals that convulsed. The pretreatment of flumazenil did not revert the anticonvulsant effect of GR in the PTZ-induced convulsion model. In the STN-induced convulsion model, the effects of GR were investigated and no difference was found against control. The results demonstrated an anticonvulsant activity of GR in the PTZ-model, which was potentialized by the complexation with β-CD.

Keywords: Epilepsy, Monoterpene, Pentylenetetrazole, Neuroprotective effect.

RESUMEN: Geraniol (GR) es un alcohol monoterpeno acíclico presentes en los aceites esenciales de las especies de plantas aromáticas utilizadas en la medicina popular brasileña para el tratamiento de la epilepsia. El presente estudio fue diseñado para evaluar el efecto anticonvulsivo del GR y de la inclusión de geraniol complejo: β-ciclodextrina (GR:β-CD). Los ratones fueron tratados con GR o con GR:β-CD (50, 100 y 200 mg/kg) 30 minutos antes de pentylenotetrazole (PTZ) o strichinine (STN). GR a 200 mg/kg y GR:β-CD en las dosis de 100 y 200 mg/kg aumentó significativamente la latencia para la primera convulsión inducida PTZ-y redujo la porcentaje de animales que convulsionó. El tratamiento previo de flumazenil no revirtió el efecto anticonvulsivo de GR en el modelo de convulsión inducida con PTZ. En el modelo de convulsión inducida com STN, los efectos de GR fueron investigados y no se encontró ninguna diferencia contra el control. Los resultados demostraron una actividad anticonvulsiva de geraniol en el modelo de PTZ, que fue potenciada por la formación de complejos con β-CD.

Palabras clave: Epilepsia, Monoterpenos, Pentilentetrazol, Efecto Neuroprotector.

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INTRODUCTION

Epilepsy is one of the most common neurological disorders, affecting about 50 million people worldwide. Usually, it is defined as a tendency to recurrent seizures that are caused by an abnormal discharge of cerebral neurons. Presently, seizures are viewed as electromagnetic discharges in the brain in predisposed individuals, attributable in part to putative genetic factors, underlying neurological disorders, and largely unknown neurochemical mechanisms (Loscher, 1998; WHO, 2005).

Traditionally, pharmacological strategies for the treatment of epilepsy have aimed at suppressing initiation or propagation of seizures rather than the processes leading to epilepsy (Loscher, 1998; Loscher & Schimidit, 2002). Currently available antiepileptic drugs have limited efficacy and their adverse effects limit their use and cause difficulties in patient management (Wahab, 2010). Thus, in the last years, a major goal in epilepsy research has been to develop antiepileptic drugs with higher anticonvulsant efficacy and less toxicity than the existing drugs (Loscher, 2002).

Plant extracts can be an important source for the development of more efficient and safer drugs for the treatment of epilepsy (Wahab *et al.,* 2009). The pharmacological use of the plants is mainly attributed to their essential oils' having a great variety of pharmacological activities, including anticonvulsant activity (Quintans-Junior *et al.,* 2008; Almeida *et al.,* 2011; Koutroumanidou *et al.,* 2013; Shareef *et al.,* 2013). The biological properties of essential oils are attributed to the monoterpenes, which are their main chemical constituents (Sousa *et al.,* 2006; Almeida *et al.,* 2011). Previous studies in animal experiments evidenced the anticonvulsant activity of some monoterpenes present in various essential oils, such as linalool (Elisabetsky *et al.,* 1995), citronellol (Sousa *et al.,* 2006), citronellal (Melo *et al.,* 2011), carvacrol, borneol and citral (Quintans-Junior *et al.,* 2010).

Geraniol (GR) is an acyclic monoterpene alcohol (Figure 1) referred to as a mixture of two isomers properly named geraniol (*trans*) and nerol (*cis*). GR is a common constituent of several essential oils and exhibits various biochemical and pharmacological properties (Chen & Viljoen, 2010). However, it is practically insoluble in water, like many of the newly discovered drugs. Water solubility is an important determinant of therapeutic effectiveness of a drug, since it plays an important role in bioavailability (Lapczynski *et al.,* 2008; Zaheer *et al.,* 2011).

Fig. 1

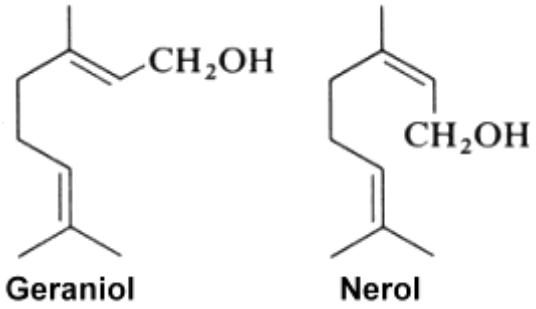

Figure 1
Chemical structures of geraniol and nerol

Cyclodextrins (CD) are cyclic oligosaccharides used in the pharmaceutical industry as complexing agents to increase aqueous solubility of poorly soluble drugs and to increase their bioavaibility and stability (Marreto *et al.,* 2008; Rasheed *et al.,* 2008; Hadaruga *et al.,* 2012; Serafini *et al.,* 2012). The β-cyclodextrin (β-CD) appears to be the most useful pharmaceutical complexing agent because of its complexing efficiency, with extremely low toxicity and capacity of enhancing drug delivery through biological membranes. Furthermore, it has low cost (Loftsson & Masson, 2001).

Since there is no data about the possible anticonvulsant effect of GR, the aim of this study was to evaluate its anticonvulsant activity and of the inclusion complex geraniol:β-CD (GR:β-CD), in two animal convulsion models.

MATERIALS AND METHODS

Animals

One hundred fifty-seven with 2-3 months male Swiss mice (25-35 g) were used. All animals were housed in groups of 8-10 per cage (30 cm x 37 cm x 16 cm) under condictions of acoustic isolation and controlled airflow and temperature (25 ± 1º C), with a 12 h light/12 h dark cycle (lights on 6:00 a.m.). Food and water were available ad libitum. Animals used in this study were handled in accordance with the guidelines of the Brazilian law for the use of animals in research (Law Number 11.794) and all procedures were approved by the local ethics committee (Animal Care and Use Committee of the Federal University of Sergipe - CEPA/UFS Number 84/2011. All efforts were made to minimize animal pain, suffering or discomfort.

Chemicals

Pentylenetetrazole (PTZ), Strychinine (STN), Geraniol 98% (GR), β-cyclodextrin (β-CD), Polyoxyethylene-sorbitan monolated (Tween 80) were purchased from Sigma Chemical Company (St Louis, MO., USA). Diazepam (DZP) and Flumazenil (FLU) was obtained from União Química, Pouso Alegre, MG, Brazil). All the drugs were administered by the intraperitoneal route (ip) at a final volume of 0.1 mL/10 g body weight of mice.

Preparation of the inclusion complex

The inclusion complex, GR:β-CD, was prepared by the slurry procedure. Slurry complexation was carried out by the addition of distilled water until the completion of 126 mL to a beaker containing 1.135 g of β-CD (3:4, v/w) and GR (154 mg), equal to about a 1:1 molar guest- host ratio, was added to the slurry and stirred for 36 h by a magnetic stirring device operating at 400 rpm. Thereafter, the mixture was heated to 70 °C for 2 h in the same device, transferred to an agate mortar, and dried in a desiccator (Menezes *et al.,* 2012).

Convulsions tests

Pentylenetetrazole (PTZ)-induced convulsion test

PTZ, at the dose of 60 mg/kg, was injected in mice for induction of tonic-clonic convulsions (Fisher, 1989). It is well established that GR has low acute toxicity in mammals (Lapczynski *et al.,* 2008; Chen & Viljoen, 2010). Therefore, the selected doses of GR were based in other studies about anticonvulsant effects of monoterpenes (Silva *et al.,* 2009; Quintans-Junior *et at.,* 2010). Mice were divided into five groups (8-12 mice per group), the first group served as control and received vehicle (saline), while the second group was treated with DZP (2 mg/kg, ip). The remaining groups received GR (50, 100 and 200 mg/kg, ip). After 30 min of drug administration, mice were treated with PTZ. Experiments were repeated following the pretreatment with the complex GR:β-CD (50, 100 and 200 mg/kg, ip) or saline, 30 min prior to the administration of PTZ. Animals were transferred to individual cages and (1) the latency for the first convulsion, (2) the number of animals convulsing and (3) the number of the deaths were observed for 15 min. (as in Vasconcelos *et al.,* 2007; Melo *et al.,* 2011). The ability of GR or the inclusion complex GR:β-CD to prevent or delay the onset of convulsions was taken as an indication of anticonvulsant activity. Mice that did not show any tonic-clonic convulsion within 15 min of PTZ administration were considered protected (Vasconcelos *et al.,* 2007; Quintans-Junior *et al.,* 2008).

Strychinine-induced convulsion test

Strychinine (STN) convulsions followed by death were shown to occur in animals by the injection (ip) of 3 mg/kg of the STN (Oliveira *et al.,* 2009). The mice were pretreated with GR (50, 100 or 200 mg/kg, ip) or saline (control group) 30 min before the administration (ip) of STN (n = 6 per group). After that, they were placed in individual cages and (1) the latency for the first convulsion, (2) the number of animals convulsing and (3) the number of the deaths were observed for 15 min.

Mechanisms involved in the anticonvulsant effect of Geraniol

To investigate the possible mechanisms underlying the anticonvulsant activity of GR, the animals were pretreated with flumazenil (FLU, 5 mg/kg, ip) an antagonist of $GABA_A$/benzodiazepine receptors (Savié *et al.,* 2004). The animals were first administered with FLU, 20 min later, with GR (200

mg/kg), and 30 min after GR, with PTZ (60 mg/kg). Behavioral changes (1) the latency for the first convulsion, (2) the number of animals convulsing and (3) the number of the death were observed.

Statistical analysis

The data obtained from latencies for the first convulsion were analyzed using the Kolmogorov-Smirnov test for normal distributions and by Bartlett's test for homogeneity of variances. Then, data were evaluated by analysis of variance (one-way ANOVA) followed by Tukey's test. The incidence (%) of clonic or tonic-clonic convulsions as well as the mortality were evaluated by the Fisher's Exact Test. Results are expressed as mean ± S.E.M. and $p < 0.05$ was considered to reflect significant differences. All statistical analyses were performed using Graph Pad Prism 5.0 (Graph Pad Prism Software Inc., San Diego, CA, USA).

Fig. 2

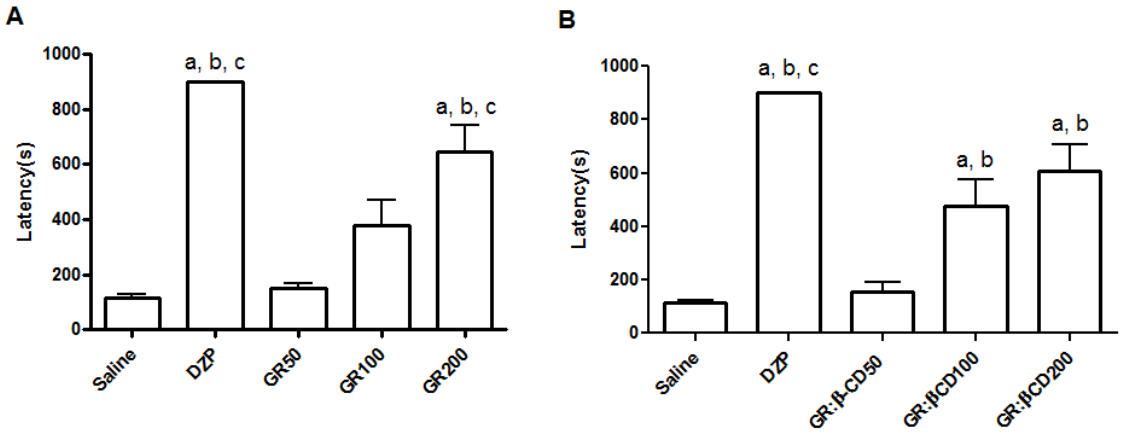

Figure 2

Effect of geraniol (GR) and inclusion complex GR:β-CD on the latency to convulsions induced by pentylenetetrazole (PTZ; 60 mg/kg). (A) Saline, GR (50, 100 and 200 mg/kg), DZP (2 mg/kg), (B) Saline, GR:β-CD (50, 100 and 200 mg/kg), DZP (2 mg/kg) were administered ip 30 min before PTZ. One way ANOVA revealed statistically significant difference between groups. Geraniol increases the latency to PTZ-induced convulsions. Data are expressed as mean ± S.E.M. (n = 8-12 per group). Statistically significant differences $^{a}p < 0.05$ comparing to Saline; $^{b}p < 0.05$ comparing to GR 50 mg/kg; $^{c}p < 0.05$ comparing to GR 100 mg/kg (Tukey's post hoc test)

RESULTS

Geraniol at 200 mg/kg delays onset of PTZ-induced convulsions

In respect to latency to the tonic-clonic PTZ-induced convulsions, one-way ANOVA revealed a significant effect of treatment [$F(4, 47) = 20.90$, $p < 0.001$]. *Post hoc* analysis revealed an increase in the latency to convulsion in the GR 200 mg/kg group when compared to Saline ($p < 0.001$), GR 50 mg/kg ($p < 0.001$) and GR 100 mg/kg groups ($p = 0.047$), but not when compared to the DZP group ($p = 0.127$), see Figure 2A. Fisher's test revealed that a lower percentage of animals convulsed in the DZP and GR 200 mg/kg groups when compared to the Saline group ($p < 0.05$), but not when compared to GR 50 mg/kg and GR 100 mg/kg groups (see Table 1). Moreover, Fisher's test revealed no difference in the frequency of mortality between groups. No deaths were registered in the groups treated with DZP 2 mg/kg, GR 50 mg/kg, GR 100 mg/kg and GR 200 mg/kg, see Table 1.

Geraniol:β-cyclodextrin *at 100 mg/kg delays onset of PTZ-induced convulsions*

In respect to latency to the tonic-clonic PTZ-induced convulsions, one-way ANOVA revealed a significant effect of treatment [$F(4, 47) = 17.95$, $p < 0.001$]. *Post hoc* analysis revealed an increase in latency for seizures in the GR:β-CD 100 mg/kg and GR:β-CD 200 mg/kg groups when compared to Saline ($p =$

0.004 and 0.0001, respectively) and to GR:β-CD 50 mg/kg groups ($p = 0.032$ and 0.001, respectively). No difference was found between the DZP group and GR 200 mg/kg ($p = 0.094$) (see Figure 2B). Fisher's test revealed that a reduced percentage of animals convulsed in the DZP, GR:β-CD 100 mg/kg and GR:β-CD 200 mg/kg groups when compared to Saline group ($p < 0.05$), but not when compared to GR:β-CD 50 mg/kg group (see Table 1). Moreover, Fisher's test revealed that the pretreatment with DZP 2 mg/kg, GR:β-CD 50 mg/kg, GR:β-CD 100 mg/kg and GR:β-CD 200 mg/kg completely protected the animals against the tonic-clonic convulsions induced by PTZ ($p < 0.05$), see Table 1.

Table 1
Effects of Geraniol (GR) and inclusion complex GR:β-cyclodextrin (GR:β-CD) on tonic-clonic seizures induced by PTZ.

Treatment	Dose (mg/kg)	Convulsions (%)	Mortality (%)
Saline	-	100	18
DZP	2	0[a]	0
GR	50	100	0
GR	100	75	0
GR	200	50[a]	0
Saline	-	100	16.6
DZP	2	0[a]	0
GR:β-CD	50	100	0
GR:β-CD	100	66[b]	0
GR:β-CD	200	50[a]	0

N = 8-12, per group
[a] $p < 0.01$ (Fisher's test), significantly different from control
[b] $p < 0.05$ (Fisher's test), significantly different from control

Geraniol has no effect on strychnine-induced convulsions

One-way ANOVA revealed no effect of treatment [$F(3, 19) = 0.39$, $p = 0.75$] in the latencies to first convulsion in the strychnine convulsion model (see Figure 3). All mice in the control group presented convulsions and the pretreatment with GR failed to inhibit seizures and mortality (see Table 2). We did not use the standard control drug, phenytoin, in the in the STN-induced convulsion model because its actions are widely described in the literature. Moreover, as GR did not show positive effect, we believe that the insertion of a group with phenytoin would be an inappropriate use of animals.

Geraniol anticonvulsant action is not antagonized by flumazenil

One-way ANOVA revealed an effect of treatment [$F(2, 28) = 28.26$, $p < 0.001$] in the latencies to first convulsion in the tonic-clonic PTZ-induced convulsions model pre-treated with FLU (5 mg/kg), an antagonist of $GABA_A$/benzodiazepine receptors. *Post hoc* analysis revealed an increase in the latency to convulsions in the GR 200 mg/kg ($p < 0.001$) and FLU + GR 200 mg/kg ($p < 0.001$) groups when compared to the Saline group, but no difference was found between GR 200 mg/kg and FLU+GR 200 mg/kg ($p = 0.226$), see Figure 4.

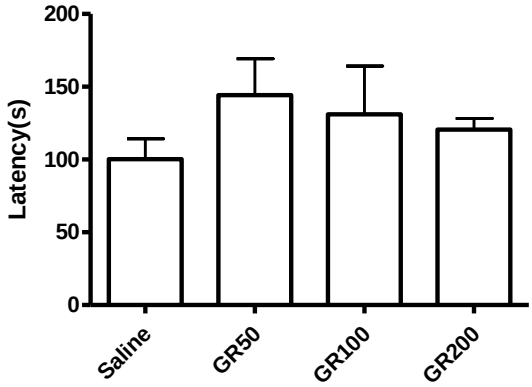

Figure 3
Effect of geraniol (GR) on the latency to convulsion induced by strychnine (STN-3 mg/kg). Saline, GR (50, 100 and 200 mg/kg), DZP (2 mg/kg) were administered ip 30 min before STN. Data are expressed as mean ± SEM (n = 6 per group).

Table 2
Effects of Geraniol (GR) on tonic-clonic convulsions induced by Strychinine.

Treatment	Dose (mg/kg)	Convulsions (%)	Mortality (%)
Saline	-	100	100
GR	50	100	100
GR	100	100	100
GR	200	100	100

n = 6, per group

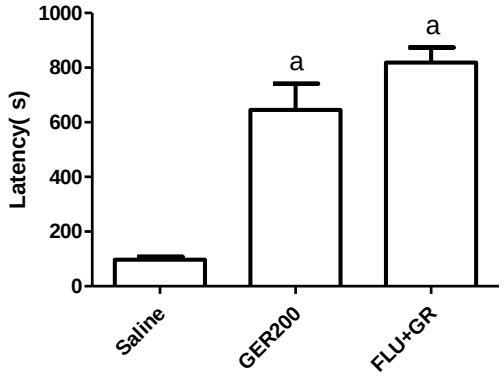

Figure 4
Effect of pretreatment with Flumazenil (FLU) (5 mg/kg) on anticonvulsant effect of geraniol (200 mg/kg). FLU was administered ip 20 min before the GR (200mg/kg) and 50 min before the PTZ. One way ANOVA revealed statistically significant difference between groups. Data are expressed as mean ± S.E.M. (n = 8 per group). Statistically significant differences $^{a}p < 0.05$ comparing to Saline (Post hoc with Tukey's test).

DISCUSSION

Geraniol (GR) is an acyclic monoterpene alcohol present in essential oils of various plants, such as *Cymbopogon winterianus* Jowitt (Poaceae) (Quintans-Junior *et al.*, 2008; Silva *et al.*, 2010), *Ocimum basilicum* (Lamiaceae) (Oliveira *et al.*, 2009) and in other aromatic plant species (Silva *et al.*, 2010), and studies have reported the anticonvulsant activity of these plants. In the present work, we investigated the anticonvulsant effect of GR and of the inclusion complex GR:β-CD in PTZ and STN-induced convulsions models, because these models are commonly used in preliminary screening tests for characterizing potential anticonvulsant drugs.

We reported that GR, at the dose 200 mg/kg delayed the onset of tonic-clonic convulsions in the PTZ model (Figure 2) and reduced the percentage of animals that convulsed (Table 1). Some studies have reported that others monoterpenes such as linalool (Elisabetsky *et al.*, 1995), citronellol (Sousa *et al.*, 2006), citronellal (Melo *et al.*, 2011), limonene, beta-myrcene and citral (Viana *et al.*, 2000), also showed anticonvulsant activity in PTZ-induced convulsions. The effects of the monoterpenes can occur through several mechanisms due to their structural diversity, once they can be acyclic or cyclic, and classified as alcohols, aldehydes, ketones and others. However, some monoterpenes have similar structures and could modulate the same receptors (Passos *et al.*, 2009).

It is widely recognized that the PTZ mechanism of action is associated to the inhibition of the activity of gamma aminobutyric acid (GABA) at $GABA_A$ receptors (Fisher, 1989; De Sarro *et al.*, 1999; Fradley *et al.*, 2007). GABA is the major inhibitory neurotransmitter in the brain and impaired GABAergic inhibitory neurotransmission is involved in the pathogenesis of several types of epilepsy (Loscher & Sshimidit, 2002). The enhancement and inhibition of GABA neurotransmission will attenuate or enhance seizures, respectively (Gale, 1992). Therefore, drugs that enhance $GABA_A$ receptor-mediated activity potentially attenuate PTZ-induced convulsions, by either inhibiting seizures or increasing their latency to onset (Smith *et al.*, 2007).

GR is practically insoluble in water (Lapczynski *et al.*, 2008), a physical property which may impair its pharmacological action. Solubility is an important parameter for achieving the desired concentration of a drug in the systemic circulation so that pharmacological response can be observed (Zaheer *et al.*, 2011). Cyclodextrins (CDs) are a family of cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic central cavity. In the pharmaceutical industry they are used to enhance aqueous solubility and chemical stability of drugs (Zaheer et al., 2011). β-CD is the most used CD in pharmaceutical industry, because it is most accessible and the lowest-priced (Del Valle, 2004). The inclusion complex GR:β-CD used in our study was the same as used by Menezes *et al.* (2012), in which thermal analysis clearly indicated the formation of complex.

Our results suggest that the inclusion of geraniol within β-CD molecule improved its bioavailability. We showed that GR:β-CD enhanced its anticonvulsant efficacy in the PTZ-induced convulsion model, since the inclusion complex GR:β-CD showed anticonvulsant effect at doses 100 and 200 mg/kg compared to GR which showed a significant effect only at a higher dose (200 mg/kg). Increased solubility might result in improved bioavailability, which increases pharmacological efficacy and allows a reduction in the dose of the drug administered (Del Valle, 2004).

On the other hand, in the strychinine-induced convulsion test, GR did not prevent convulsions (Table 2) and did not alter latency to convulsion (Figure 4) when compared to controls. STN is a competitive glycine receptor antagonist, which increases postsynaptic excitability and ongoing activity in the brain stem and spinal cord (Webb & Lynch, 2007). Since GR did not prevent the convulsions induced by STN, our results suggest that involvement on glycinergic neurotransmission is unlikely.

Since GR delayed the onset of PTZ convulsion in our study, it is probable that GR may be interfering with GABAergic mechanisms to exert its anticonvulsant effect. To determine whether the anticonvulsant effect of GR occurs through the GABAergic system, the animals were pretreated with flumazenil, a clinically used competitive antagonist that binds the benzodiazepine site of the $GABA_A$ receptor (Oliveira *et al.*, 2009). Our results shown that the effect of GR was not significantly decreased by flumazenil pretreatment (Figure 5), at a dose that antagonizes the anticonvulsant effects of DZP (2 mg/kg), suggesting that GR anticonvulsant effect does not involve the benzodiazepine site of the $GABA_A$ receptor. The same results were found by others studies that evaluated the anticonvulsant effect of others monoterpenes (Almeida *et al.*, 2008; Quintans-Junior *et al.*, 2010).

In conclusion, the present data indicate an anticonvulsant activity of GR in the PTZ-induced convulsion model and the inclusion of GR and β-CD enhanced this effect. The precise mechanisms of the anticonvulsant effect of GR and the inclusion complex GR:β-CD are not clear, although our evidence points that GR anticonvulsant effect seems to be independent of benzodiazepine receptors. Nevertheless, further studies are needed for elucidating the mechanisms involved.

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