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# Anticonvulsant and anxiolytic assessment of leaves from *Artemisia vulgaris* L. in mice

**Edvaldo Rodrigues de Almeida[1]*, Aluízio Roberto da Silva[1], Adelmo Cavalcanti Aragão-Neto[2], Pedro Henrique dos Santos Soares[1], Luiz Lucio Soares da Silva[1], Rômulo Pessoa e Silva[1], Dafne Carolina Alves Quixabeira[1], Taciane Leal Botelho[1] and Laís Alves Lins Marinho[1]**

[1]Departamento de Antibióticos, Universidade Federal de Pernambuco. CEP 50670901 Recife Pernambuco, Brasil.
[2]Departamento de Odontologia, Universidade Federal de Pernambuco. CEP 50670901 Recife Pernambuco, Brasil.

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**The present study had as an objective evaluation of the anticonvulsant and anxiolytic effect of *Artemisia vulgaris* through the methanol extract of its leaves. Swiss albino mice were subjected to dosages of 50, 100 and 300 mg/kg methanol extract for the study of the anticonvulsant action. The animals had suffered the response from the following convulsants: pentilenotetrazol 100 mg/kg, picrotoxin 10 mg/kg, strychnine 2 mg/kg, pilocarpine 600 mg/kg and lidocaine 200 mg/kg. In the evaluation of the anxiolytic action of the plant, the mice have been submitted to the elevated plus-maze and the Marble-Burying test (EPM and MBT). As standard drug, diazepam 2 mg/kg was used in all the experimental models. The methanol extracts presented excellent anticonvulsant activity, increasing the time of latency for the beginning of the first seizure in all the tests. The ideal dose from the methanol extract was 100 and 300 mg/kg. The anxiolytic activity was also observed using the EPM and MBT.**

**Key words:** *Artemisia vulgaris*, methanolic extract, presence of essential oils, model of convulsion and anxiety, mice.

## INTRODUCTION

*Artemisia vulgaris* L (Asteraceae) (AV) is one of the several species of the genus *Artemisia*, also known as fleur-de-St. John, wormwood. It is natural to the temperate regions of Europe, Asia and North Africa. It is widespread throughout the world. It is an aromatic, herbaceous perennial plant with woody roots. It is an aromatic perennial shrub of 2 to 8 feet elevation and mostly found in Himalayas, India and Pakistan (Kapoor, 1990). It is known to contain adenine, amyrin, artemisiketone, borneol, cadinenol, coumarin, fernenol, esculin, esculetin, inulin, muurolol, myrcene, nerol, molybdenum, quercetin, scopoletin, β-sitosterol, spathulenol, stigmasterol, tauremisin, tetracosanol, thujone, vulgarin, vulgarol, vulgarole and umbelliferone (Geissman, 1970; Duke, 1992). *A. vulgaris* is cited for possessing various medicinal properties according to the literature such as antiallergic (Rodrigues-Alves et al., 2008), antibacterial (Chen et al., 1989), antihypertensive (Tigno et al., 2000), antinociceptive (Pires et al., 2009), antiplasmodial (Tran et al., 2003) and hepatoprotective (Gilani et al., 2005) properties. It improves blood circulation in the ischemic rat mesentery (Tigno and Gumila, 2000). The plant has folklore reputation as analgesic, anthelminthic, antipyretic, antispasmodic, antiseptic, diaphoretic, diuretic, emmenagogue, expectorant, hypoglycaemic, tonic and is considered useful in the treatment of asthma, cancer, colic, cough, diarrhea, dyspepsia, depression, epilepsy, headache, haemorrhage, hepatosis, inflammation and rheumatism (Kapoor, 1990). According to popular usage and the data

*Corresponding author. E-mail: edvaldo.ra@gmail.com. Tel/Fax: +5581 21267346.

obtained in the literature, anticonvulsant identification was performed using the following convulsants: pentilenotetrazol (PTZ 100 mg/kg), picrotoxin (Picrot 10 mg/kg), strychnine (Stry 2 mg/kg), pilocarpine (Piloc 600 mg/kg) and lidocaine (Lid 200 mg/kg).

## Plant

The plant was identified by University Prof. Marlene Carvalho de Alencar Barbosa and vouched for specimen, which was deposited in the Geraldo Mariz Herbarium (UFPE) under Botanical Department N° 29048. In the obtention of the methanol (ME), 500 g of dry material were used, which was previously ground in a forage machine. The extraction was performed by using solvents of increasing polarity (hexane, ethyl acetate and methanol). First, the hexane at 40°C for 10 min was used and the solid residue was removed by filtration. The ethyl acetate was added to the resulting solid, after 24 h of rest; the material was heated and filtered again. To the new solid separated material, the last solvent, methanol was added after 24 h, and the methanol was filtered and removed on Rota-vapor. Then lyophilization was carried out, resulting in a yield of 25 g of a dark, viscous material, which was carefully sealed and stored at -20°C until the time of testing.

## Extraction and identification of compounds

Fifteen grams of previously ground leaves were inserted in a cellulose cartridge on which a piece of glass wool was placed (enough to fill the cartridge). After preparing the sample, the cartridge was inserted into the Soxhlet apparatus, utilizing as the solvent, methanol, at approximately 92°C for 4 h. The extract obtained was concentrated in a rotary-evaporator under a reduced pressure, at the temperature of 45°C until dryness and submitted to evaluation. The identification of some constituents of the essential oils was achieved based on their retention indexes (determined in relation to a series of normal alkalis), and by comparison of their mass spectral fragmentation patterns (NIST database/Chemical data system), and literature data (Harbone, 1998; List and Schmidt, 1998; Geissman, 1970).

## MATERIALS AND METHODS

Swiss albino mice (*Mus muscullus*) weighing on average 20 to 25 g were kept in the vivarium of the Department of Antibiotics of UFPE, Pernambuco, Brazil, at a temperature of 23 ± 2°C with a light and dark cycle of 12 h (10:00 am and 4:00 pm). The animals were given tap water *ad libitum* and a standard diet (LABINA Purina®). All experiments were conducted between 09:00 am and 04:00 pm. The drugs were administered intraperitoneally (i.p). The Ethics Committee approved this study for Animal Experimentation of the Center for Biological Sciences (EAEC-Federal University of Pernambuco, Brazil).

### Drugs and treatments

Diazepam (DPZ; Merck, Brazil) was dissolved in saline solution and used as a positive control (2 mg/kg). It was administered intraperitoneally (i.p.) at a volume of 0.1 ml/10 g of body weight. Negative control groups consisted of inhaled 0.2% Tween or intraperitoneally saline solution. DPZ (2.5 mg/kg, i.p) was used as the standard anxiolytic drug, and PTZ (100 mg/kg, i.p), Picrot (10 mg/kg, i.p), Stry (2 mg/kg, i.p), Piloc (600 mg/kg, i.p) and Lid (200 mg/kg, i.p) as convulsants drugs. All drugs were obtained from Sigma Aldrich, Mo, USA, and Tween 80 (Merck®) and Lidocaine (Lidoston®). After the experiments, the animals were killed with an overdose of urethane and sent for incineration.

### Penbarbital sleeping time

The test was performed in six groups of mice (n=10). Six groups received the ME at doses of 100, 300 and 500 mg/kg, i.p., while the control group received normal saline (0.1 ml/10 g). The 2nd group was given PBS (55 mg/kg, i.p), the 3rd group was given DPZ (2 mg/kg, i.p), 4th group was given ME 100, 5th group 300, and 6th 500 mg/kg i.p. Thirty minutes later, pentobarbital sodium (55 mg/kg, i.p) was administered to each mouse to induce sleep. The between loss and recovery of righting reflex was used as an index of hypnotic effect (Speroni and Minghetti, 1988).

### Anticonvulsant tests

The mice were divided into six groups (10 animals/group/convulsants). The 1st group received PTZ (100 mg/kg, i.p), the 2nd group received Picrot (10 mg/kg, i.p), the 3rd group received Picrot (10 mg/kg, i.p), the 4th group received Stry (2 mg/kg, i.p), the 5th group received Piloc (600 mg/kg, i.p) and the 6th group Lid (200 mg/kg, i.p) and DPZ (2 mg/kg, i.p) was served as control positive. The test group received the ME at doses of 100, 300, and 500 mg/kg, i.p. After 1 h, each convulsant was administered to the animals in their respective group. The number of mice which exhibited convulsion, the lethal time, and the latency to first convulsion was recorded (André et al., 2009; De Toledo, 2000; Freitas et al., 2006; Sayyah et al., 2002; Jensen and Petersen, 1983).

### Elevated plus-maze (EPM) test

A device consisting of two open arms (30 × 5 × 0.25 cm) and two closed arms (30 × 5 × 15 cm) of a common central platform (5 × 5) evaluated the EPM. Two pairs of identical arms were opposite each other. The entire apparatus was elevated to a height of 40 cm from the floor. At the beginning of the session, the mouse was placed in the center of the maze, its head facing an open arm and allowed to explore the labyrinth for 5 min. The following parameters were observed: the time spent on each arm. The apparatus was thoroughly cleaned with a wet towel with 70% alcohol after each animal. The mice were divided into 5 groups. DPZ (2 mg/kg, i.p) was used as positive and the ME doses of 100, 300, and 500 mg/kg i.p. All experiment was conducted between 10:00 am and 04:00 pm. After each test, the equipment was cleaned with 70% ethanol, with a paper towel (Lister, 1987).

### Marble-Burying test (MBT)

Twenty-five spheres of glass beads (20 mm diameter) were used for each individual test. Polyethylene cages (30 × 36 × 13 cm) constructed with a vinyl roof with holes for air-intake, with a 5 cm

layer of sawdust on the floor of the cage. Each mouse in each group was placed in individual cages with sawdust for 15 min (habituation) and then the animal was returned to its cage of origin. Twenty-five glass beads were placed randomly spaced 3 cm from each other, with a layer of 5 inches of sawdust in the habituation cage. The animals were reintroduced (one animal at a time) into the cage (in each test the mice were returned to the same cage in which it had been accustomed to). Ten animals were used in each group. The test group received the ME at doses of 100, 300, and 500 mg/kg i.p. After 15 min, the test was terminated by the withdrawal of the mouse and the number of balls that were more than two-thirds covered with sawdust was counted. After each test, the sawdust was replaced and the glass beads were washed with water and with 70% alcohol/water (v/v) (Njung'e and handley, 1991).

#### Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) with post hoc Duncan's test. $p<0.05$ was considered significant. All data are expressed as mean ± standard deviation (SD) using the program Graph Pad Prism, version 5.00.

## RESULTS

### Sodium pentobarbital-induced sleep time

Saline, DPZ, and ME were give 30-min prior to pentobarbital injection (i.p) and latency to loss of righting reflex was measured. In the saline treated control animals, the righting reflex was lost after 77±03 s of pentobarbital injection (Figure 1). Injection of ME at dose 300 and 500 mg/kg, significantly suppressed the latency to sleep *p<0.01 similar to DPZ (2 mg/kg, i.p).

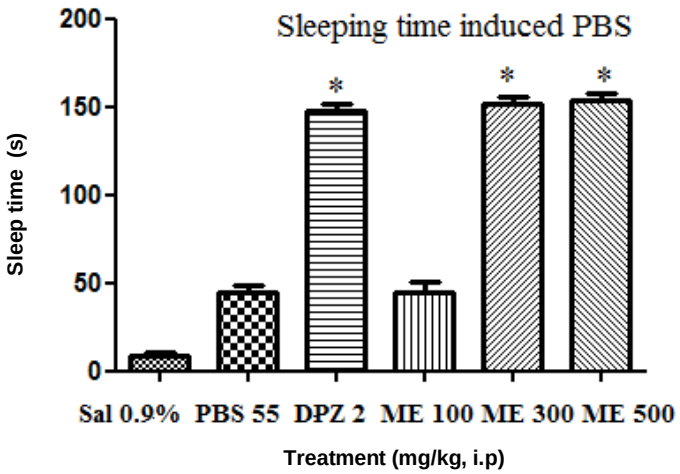


**Figure 1.** Sodium Pentobarbital-induced sleep time.

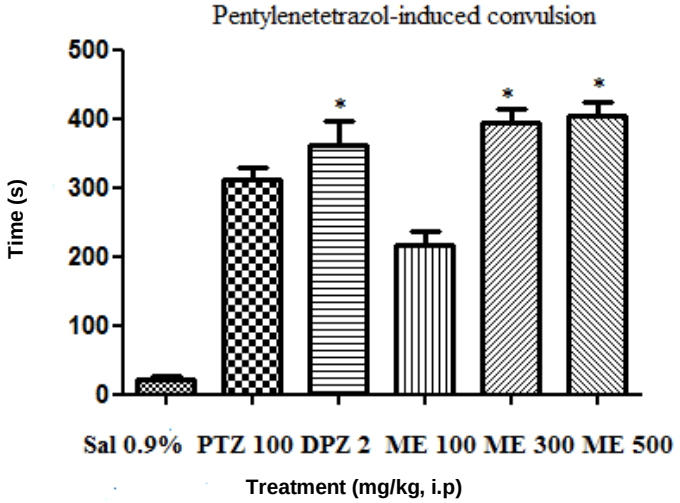


**Figure 2.** Effect of ME Pentilenotetrazol (PTZ) induced Convulsion

### Effect of ME PTZ-induced convulsion

The ME inhibited generalized clonic-tonic convulsions induced by PTZ (100 mg/kg, i.p) at doses of 300 and 500 mg/kg (*p<0.5) (Figure 2), as in accordance with statistical analysis, using analysis of variance one-way ANOVA and followed by a post hoc Duncan's test.

### Effect of ME Picrot-induced convulsion

The ME inhibited generalized clonic-tonic convulsions induced by Picrot (10 mg/kg, i.p) at doses of 300 and 500 mg/kg (*p<0.05) (Figure 2), as in accordance with statistical analysis, using analysis of variance one-way ANOVA and followed by a post hoc Duncan's test.

### Effect of ME Stry-induced convulsion

The ME inhibited generalized convulsions induced by Stry (2 mg/kg, i.p) at doses of 300 and 500 mg/kg (*p<0.05) (Figure 2), as in accordance with statistical analysis, using analysis of variance one-way ANOVA followed by a post hoc Duncan's test.

### Effect of ME Piloc-induced convulsion

The ME inhibited generalized convulsions induced by Piloc (600 mg/kg, i.p) at doses of 300 and 500 mg/kg (*p<0.5) (Figure 2), as in accordance with statistical analysis, using ANOVA followed by a post hoc Duncan's test (*p<0.05).

### Effect of ME Lid-induced convulsion

The ME inhibited generalized convulsions induced by Lid (200 mg/kg, i.p) at doses of 300 and 500 mg/kg (*p<0.5) (Figure 2), as in accordance with statistical analysis, using one-way ANOVA followed by a post hoc Duncan's test.

## MBT

To examine this premise, we studied the effect of the representative of ME on burying behavior. As expected, DPZ (2 mg/kg) exhibited significant increase in the marble burying. These data were evaluated using the one-way ANOVA followed by a post hoc Duncan's tests ($p>0.5$).

## Elevated plus-maze (EPM)

Effect of DZP 2 mg/kg, i.p, and ME 100, 300 and 500 mg/kg (i.p) on the values of time spent in the open arms during 5 min in the EPM. The data from this figure show that doses of ME were not statistically significant.

## DISCUSSION

In this study with *A. vulgaris*, we initially used the classic animal model of seizures induced by PTZ, since it is known that blockade of seizures in this type of mice is a characteristic of some drugs that belong to the anticonvulsant class and which depress the central nervous system (CNS) (Duveau et al., 2011). Considering the excellent results found in this study by the action of ME when submitted to PBS (Figure 1, *$p<0.05$), particularly at doses of 300 and 500 mg/kg. Which yielded similar results DPZ 2 mg/kg; one may suggest that the ME of *A. vulgaris* seems to act by promoting a CNS depressant effect at doses of 300 to 500 mg/kg i.p. Considering the result found in this study by the action of ME when submitted to PTZ, the enhancement of synaptic inhibition mediated by GABAA, reducing neuronal excitability and increasing the seizure threshold (Figure 2, *$p<0.05$). The Picrot is a model of recurring focal epilepsy applicant to petit mal or absence seizures. The Picrot is a powerful stimulant of the CNS being used in the chemical induction of seizures by antagonism to GABAA (Freitas et al., 2006; Meldrum and Rogawski, 2007; Khongsombat et al., 2008). Its mechanism action is similar to PTZ, promoting chloride channel opening at the GABAA receptor (Figure 3, $p<0.05$). The seizures induced by systemic administration of Stry only consist of tonic extensions. Stry is potent convulsants and acts mainly as a selective agonist of postsynaptic inhibition mediated by glycine. Its main action is to increase the excitability of the spinal reflex (Berg et al., 2003). When animals were subjected to Stry, the ME analyzed in this study showed anticonvulsant activity at doses tested, with doses of 300 and 500 mg similar to Diazepam. Thus, it can be suggested that the ME effectively reduces the blockade of glycine receptors in the spine (Figure 4, *$p<0.05$). The seizures induced by Piloc may also be useful to characterize the mechanisms of the action of anticonvulsant drugs for the treatment of epilepsy. The seizure model induced by Piloc in animals is widely used

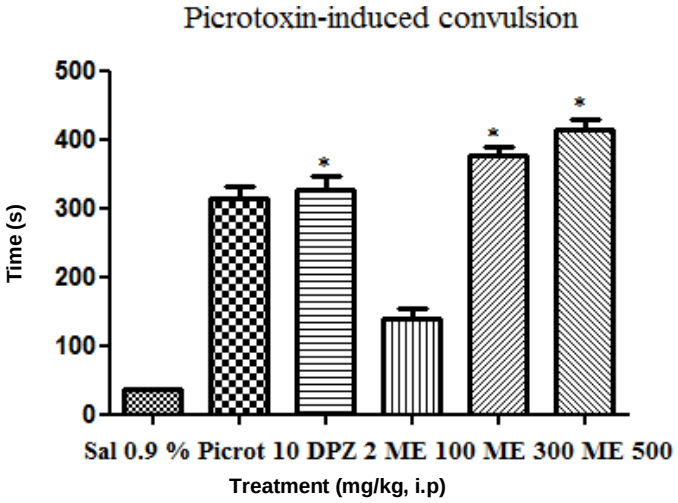


**Figure 3.** Effect of ME Picrotoxin (Picrot) induced Convulsion.

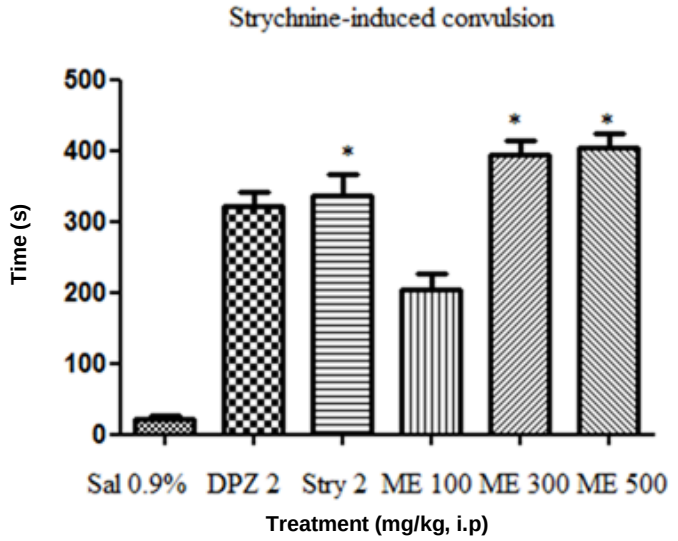


**Figure 4.** Effect of ME Strychnine-induced convulsion.

to study the pathophysiology of the convulsive process. Since, it reproduces the behavioural and electroencephalography (EEG) changes that are similar to temporal lobe epilepsy in humans (André et al., 2009; Cavazos et al., 2003; Leite et al., 2002; Millan et al., 1993). The doses of the ME tested in this study had positive results, increasing the latency to the onset of the first seizure, and doses of 300 and 500 mg/kg had similar results to DPZ 2 mg/kg (Figure 5, *$p<0.05$). Therefore, it is clear that the extract has an effective mechanism of action in partial seizures, which are located in the temporal lobe. The stimulatory effect of local anesthetics on the Central Nervous System is the indirect result of a depression of inhibitory canters in the brain. If serum levels of Lid reach 7.5 to 10 mcg/ml, generalized tonic-clonic seizures occur (Kragh et al., 1993; De Toledo,

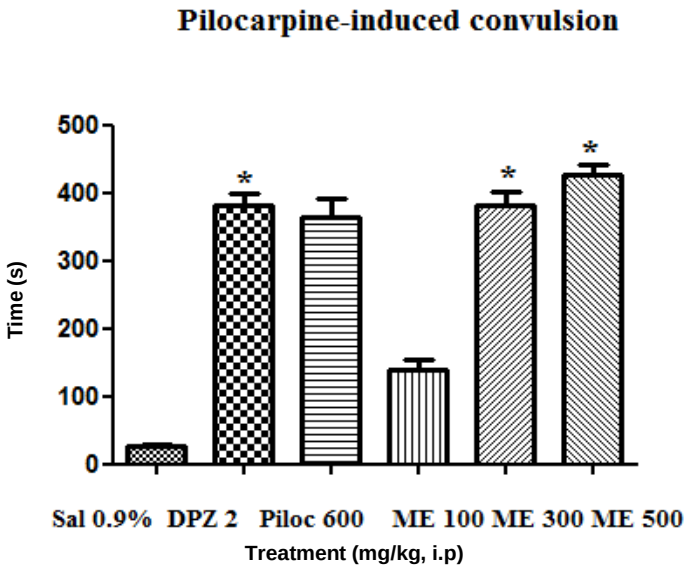


**Figure 5.** Effect of ME Pilocarpine-induced convulsion.

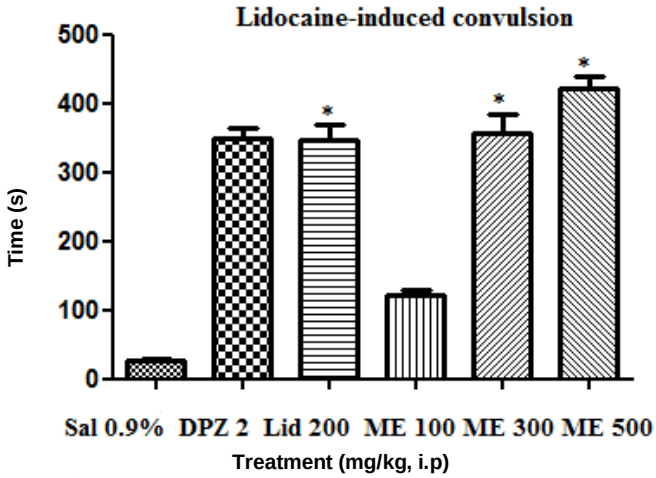


**Figure 6.** Effect of ME Lidocaine-induced convulsion.

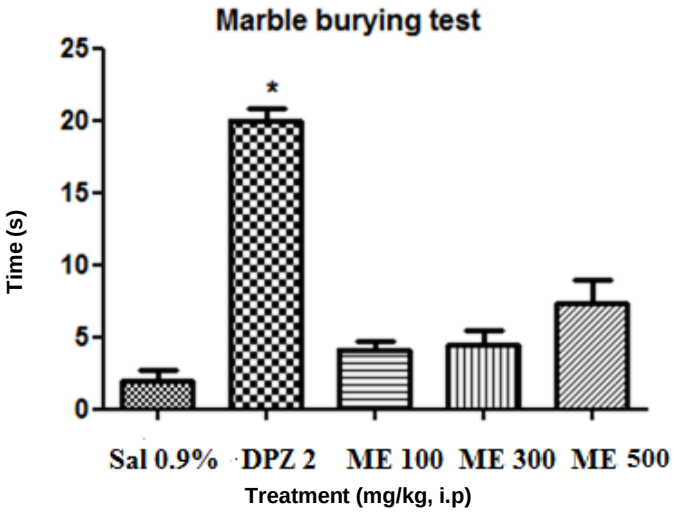


**Figure 7.** Marble-Burying test.

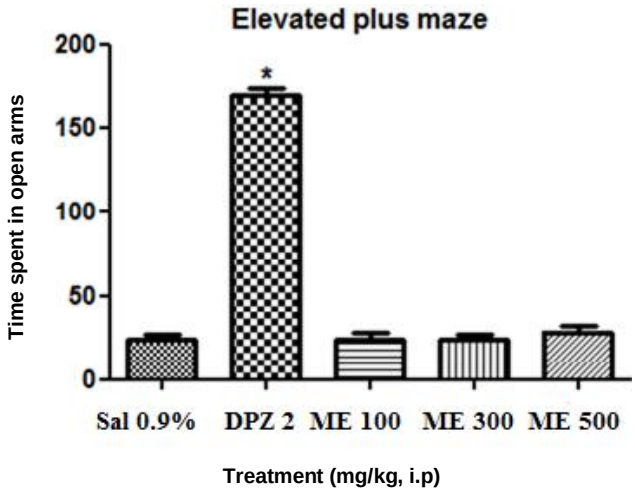


**Figure 8.** Elevated plus-maze (EPM)

2000; D'eramo et al., 2003; Chiu et al., 2004). Seizures are typically of short duration and self-limiting. However, respiratory arrest is common because of the depression of the respiratory center. The progression of symptoms with hypoxia, cyanosis and cardiac arrest is facilitated by a noxious combination of increased consumption of oxygen, caused by the convulsive movements and decreased supply (respiratory depression). In this study, we observed an increase in latency to the onset of tonic-clonic seizures at all doses of ME, at doses of 300 and 500 mg/kg when the animals were submitted to Lid 200 mg/kg i.p. The dose of 300 and 500 mg/kg of ME was similar to diazepam (Figure 6, *p<0.05). Although we identified the anticonvulsant action, we did not find any anxiolytic action in the models tested. Also in the MBT, animals treated with the ME did not show the anxiolytic action (Figure 8, p>0.5), just the standard group (DPZ 2 mg/kg, i.p) was responsible for behavioral changes that do support an anxiolytic profile, demonstrated by a number of spheres hidden (Figure 7, p>0.5). The administration of DPZ reduced the number of glass beads (p<0.01) (Aguirre-Hernández et al., 2007; Barbosa et al., 2008). The validity of the plus maze test for the evaluation of anxiolytic or anxiogenic drugs is well-documented (Pellow and File, 1986; Lister, 1987). In this study, treatment with DPZ (2 mg/kg, i.p), a benzodiazepine drug, led to a significant increase in time spent in open arms (Dhawan et al., 2002; Mora et al., 2005). The administration of the ME, whereas resulted in behavioural similar to animals that received saline (Figure 8, p>0.5). The results of this study indicate that the anticonvulsant activity of *A. vulgaris* was shown to be effective in various mechanisms of induction of seizures

tested, which may suggest that the extract works by promoting the enhancement of synaptic inhibition mediated by GABAA, reducing the blockage of glycine receptors in the spine, in addition to performing effectively in partial seizure located in the temporal lobe. The dose of 300 and 500 mg/kg of ME may be suggested as ideal (*$p<0.05$) due to its anticonvulsant action in various models, but it did not show an anxiolytic action in the models tested (Figures 7 and 8, $p>0.5$). In previous studies, the action of essential oils Linalool and Pinene in the treatment of convulsions induced by PTZ was demonstrated (Linck et al., 2009; Ilhan et al., 2006; Sayyah et al., 2002). The effects of ME observed may be related to the presence of essential oils (Linalool and Pinene), identified in the ME of *A. vulgaris*, and associated with the presence of flavonoids (Wolfman et al., 1998; Linck et al., 2009; Sayyah et al., 2002; Carvalho et al., 2011; Choudhary et al., 2011).

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