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Short Communication

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Anticonvulsant Effect of *Alternanthera brasiliana* Extract on Pentylenetetrazole-induced Seizures in Rats

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Abstract

Epilepsy is a disorder that affects 1-2% of the population and a significant percentage of these patients do not respond to anticonvulsant drugs available in the market suggesting the need to investigate new pharmacological treatments. Numerous substances have been tested for potential anticonvulsant activity, but only a few generated anticonvulsant drugs. In this study, the potential anticonvulsant effect of Alternanthera brasiliana extract was investigated using an animal model of acute epilepsy induced by pentylenetetrazol (PTZ). The animals received injections of A. brasiliana extract (20, 100 or 500 mg/kg) or vehicle; 30 minutes later, they received an injection of PTZ, and were then observed for 30 minutes. Seizure latency and duration were recorded. The administration of 20 mg/kg of A. brasiliana extract had an anticonvulsant effect when compared with the control group. Thus, further

studies using other seizure models as well as the investigation of isolated fractions of the extract are needed to elucidate the mechanisms of action of *A. brasiliana*.

Keywords

Epilepsy; Plant extracts; *Alternanthera brasiliana*; Amaranthaceae

Abbreviations: GABA- g-amino butyric acid; SPSS: Statistical Package for the Social Sciences

Introduction

Epilepsy is a disorder of the central nervous system characterized by recurrent seizures in which excessive abnormal synchronous electrical discharges affect a focal or generalized neuron population in the brain [1-3]. Almost all diseases that affect gray matter, many of those that affect white matter (metabolic diseases) and several systemic diseases may cause epileptic seizures. Symptoms range from brief episodes of absence to generalized convulsive seizures that may last up to several minutes depending on the function of the brain region that is affected. The observation of events during a seizure allows the classification of epileptic seizure. Of the types of epilepsy, temporal lobe is the most frequently resistant to pharmacologic treatment among the adult population, and accounts for at least 40% of all cases of refractory epilepsy. Epilepsy is estimated to affect 50 million people in the world, 40 million of them in developing countries [1,4-8]. Annual epilepsy incidence rates range from 40 to 70/100,000, and are as high as 122 to 190/100,000 in developing countries [6]. Socioeconomic, etiologic and sociocultural factors may explain this difference as the high incidence of epilepsy in developing countries is assigned to parasitic causes, such as neurocysticercosis [9] intracranial infections [10], cerebrovascular accidents, perinatal trauma and genetic factors [6,11].

The normal functioning of the central nervous system (CNS) depends on the balance of inhibitory and excitatory effects. If excitation exceeds inhibition, and if the imbalance is sufficiently serious, a seizure occurs. It is believed that epilepsy results from the poor functioning of GABA, the main inhibitory neurotransmitter in the mammalian brain [12-16], and its poor functioning generates hyperpolarization of neurons. A study of neurons in areas close to epileptogenic lesions showed that these cells generate prolonged postsynaptic excitatory action potentials without any inhibitory activity, even when the intensity of stimuli is very high [12]. In animal models, increased GABAergic inhibition has antiepileptic activity, whereas the administration of drugs that decrease GABAergic inhibition produces seizures [3,12].

Currently available drugs were designed to achieve satisfactory control of epileptic seizures and to reduce the risk of physical lesions, psychological problems and irreversible brain damage. Antiepileptic medication efficiently controls epileptic seizures in only 63% of the patients and 37% are refractory to treatment [17]. The quality of life of patients with epilepsy may be poor because, in addition to the fact that their condition requires life-long drug therapy, pharmacologic drugs have substantial side effects.

In recent years, the anticonvulsant properties of numerous plant extracts have been studied using animal models of epilepsy. Several studies of plant extracts using animal models have reported on their efficacy in the control of acute epileptic seizures induced by pentylenetetrazol (PTZ) [18-25]. *Alternanthera brasiliana* L. Kuntze (family: Amaranthaceae; order: Centrospermae) is an herb found in grassland ecosystems widely distributed in American countries [26]. It is a medicinal plant widely used in Brazilian popular medicine for the treatment of certain ailments, such as inflammation, pain and infection [27], cough and diarrhea [28]. *In vivo* studies suggest that *A. brasiliana* has analgesic properties against intense pain. Its extract has a more powerful analgesic activity than that of standard analgesic drugs [27]. Many plant components have medicinal properties and six flavonoids have been identified in *A. brasiliana* [28].

Methodology

Extract preparation

This study used leaves of *A. brasiliana* collected in the Lomba Grande district in Novo Hamburgo, Brazil. The plant material, dried at 36°C, underwent extraction with ethanol. Extracts were concentrated

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and stored at -20°C until their use in the study [29]. Phytochemical analysis conducted according to Costa [30] identified secondary metabolites of the alkaloid class, phenolic compounds and flavonoids. A steroid nucleus and sugars were also identified.

Animals

Forty male 3- and 4-month-old Wistar rats weighing 238-333 mg were obtained from the animal laboratory of Feevale. The animals received water and food *ad libitum* and were exposed to 12-h light-dark cycles at a temperature of 23 ± 1°C. The study was conducted between 10 am and 3 pm. Procedures for the care and use of animals followed the rules issued by the Brazilian Society of Neuroscience and Behavior [31].

Treatment

The doses of *A. brasiliana* extract used in the study were 20, 100 and 500 mg/kg. Thirty minutes after intraperitoneal (ip) administration of extract or vehicle, the animals (10 in each group) were administered a single ip dose of the seizure-inducing PTZ (60 mg/kg). The animals were observed for 30 minutes. Seizure latency and duration were recorded. This study protocol was approved by the Ethics and Research Committee of Centro Universitário Feevale.

Statistical analysis

All values are presented as mean and standard error (S.E.M). All data were subjected to analysis of variance (ANOVA) and Tukey's post-hoc test. Results were considered significant with $p \leq 0.05$. Data were evaluated using a SPSS 18.0 software package (SPSS Inc., Chicago, IL).

Results and discussion

Figures 1 and 2 showed the effect of different doses of extract on seizure latency and duration. Figure 1 shows that seizure latency did not differ significantly between groups administered different extract doses (20mg/kg, 100 mg/kg and 500 mg/kg) and the control group ($p>0.05$, ANOVA followed by Tukey test). However, the analysis of seizure duration showed that the 20 mg/kg dose had a significant anticonvulsant effect in comparison with the vehicle control group ($p<0.05$, ANOVA followed by Tukey test). No differences were found in the analysis of mortality between groups (data not shown).

Over 30% of the areas of rain forest in the planet are in Brazil, and these areas house an incalculable ecologic diversity. So far, less than 1% of the species found in the Brazilian forests have been identified. Therefore, the systematic study of these great natural resources may identify substances of great usefulness in human health. Plants are an important source of biologically active natural products and are models for the synthesis of a large number of drugs. Some examples of drugs derived from natural products that were shown to have an anticonvulsant action in animal models of epilepsy are the extracts of *Hypericum perforatum, Ginseng, Nigella sativa, Centella asiatica and Crinum jagus* [2,32-35]. Several studies have reported on the biological properties of *Alternanthera brasiliana* [26-28,36]. Our study demonstrated the anticonvulsant property of the hydroalcoholic extract of *A. brasiliana* at a dose of 20 mg/kg. Larger doses did not have the same effect; therefore, future studies should investigate whether smaller doses are also active.

Some studies showed that the pharmacologic effect of PTZ is mediated by interactions with the ion channel of the GABA-A receptor. The injection of moderate doses of PTZ (30 mg/kg) induce

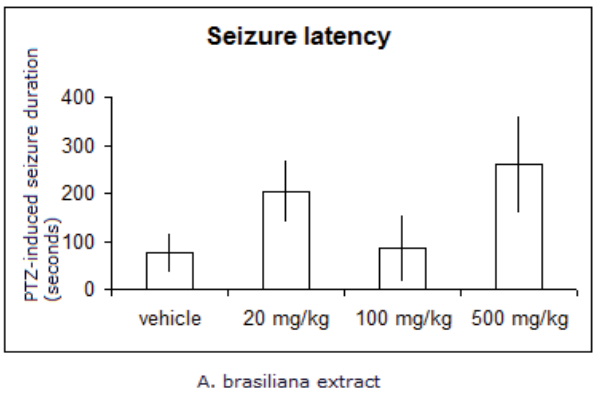

Figure 1: Effect of different doses of *A. brasiliana* on PTZ-induced seizure latency. Data expressed as mean ± standard deviation ($p>0.05$; ANOVA followed by Tukey test).

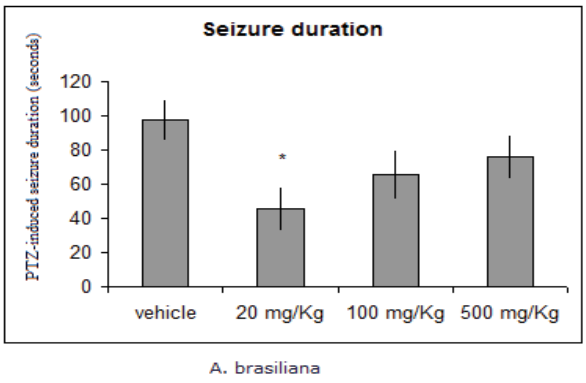

Figure 2: Effect of different doses of *A. brasiliana* on PTZ-induced seizure duration. Data expressed as mean ± standard deviation (*$p<0.05$; ANOVA followed by Tukey test).

generalized seizures with severe neurochemical effects, such as the decrease of GABA levels, and the consequent decrease of inhibitory responses, a combination that exacerbates excitation [37-39]. Therefore, the anticonvulsant effect observed in this study may be associated with the GABAergic system because PTZ acts by inhibition of this system. Flavonoids are abundantly found in all plants and have a considerable chemical diversity, with over 5000 different flavonoids described so far. Many flavonoids are polyphenolic and powerful antioxidant agents. They also have a variety of biological properties, and the activity of these flavonoids is associated with the GABA-A receptor [40]. Six flavonoids were identified in *A. brasiliana*: kampferol-3-*O*-robinoside-7-*O*-α-L-rhamnopyranoside or robinetin, quercetin 3-*O*-robinobioside-7-*O*-α-L-rhamnopyranoside or clovene, quercetin -3-*O*-robinoside, kampferol-3-*O*-robinoside, kampferol-3-*O*-rutinoside-7-*O*-α-L-rhamnopyranoside and kampferol 3-*O*-rutinoside [28]. We concluded that these flavonoids could be associated with the anticonvulsant effect of the *A. brasiliana* extract. However, future studies should investigate its mechanisms of anticonvulsant action and isolate its pharmacologically active substances.

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