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# Antiepileptogenic, antioxidant and genotoxic evaluation of rosmarinic acid and its metabolite caffeic acid in mice

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## Abstract

### Aims

Antioxidant compounds have been extensively investigated as a pharmacological alternatives to prevent epileptogenesis. Rosmarinic acid (RA) and caffeic acid (CA) are compounds with antioxidant properties, and RA has been shown to inhibit GABA transaminase activity (*in vitro*). Our aim was to evaluate the effect of RA and CA on seizures induced by pentylenetetrazole (PTZ) using the kindling model in mice.

### Main methods

Male CF-1 mice were treated once every three days during 16 days with RA (1, 2 or 4 mg/kg; i.p.), or CA (1, 4 or 8 mg/kg; i.p.), or positive controls diazepam (1 mg/kg; i.p.) or vigabatrin (600 mg/kg; p.o.), 30 min before PTZ administration (50 mg/kg; s.c.). After the last treatment, animals were sacrificed and the cortex was collected to evaluate free radicals (determined by 2',7'-dichlorofluorescein diacetate probe), superoxide dismutase (SOD) and genotoxic activity (Alkaline Comet Assay).

### Key findings

Rosmarinic acid 2 mg/kg increased latency and decreased percentage of seizures, only on the 4th day of observation. The other tested doses of RA and CA did not show any effect. Rosmarinic acid 1 mg/kg, CA 4 mg/kg and CA 8 mg/kg decreased free radicals, but no dose altered the levels of enzyme SOD. In the comet assay, RA 4 mg/kg and CA 4 mg/kg reduced the DNA damage index.

### Significance

Some doses of rosmarinic acid and CA tested showed neuroprotective action against oxidative and DNA damage produced in the kindling epilepsy model, although they did not produce antiepileptogenic effect *in vivo*.

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## Introduction

Epilepsy is characterized by unprovoked episodes of aberrant synchronous excitation of brain regions that disrupt normal functioning and cause successive seizures [7], [47]. According to the World Health Organization (WHO), about 50 million people are affected worldwide, and approximately 70–80% of patients with new-onset epilepsy enter remission when they are treated with antiepileptic drugs currently prescribed. Antiepileptic drugs, now commonly referred to as antiseizure drugs (ASDs), provide symptomatic benefits by preventing the occurrence of seizures in patients. In spite of these benefits, ASDs fail to control seizures in 20–30% of patients, or present troubling side effects [14], [20], [27]. The development of therapeutic strategies to prevent the recurrent seizures and the establishment of *status epilepticus* has been the main goal of the contemporary epilepsy research [20], [43].

The mechanisms underlying seizures are complex, and vary across the numerous seizure types that have been characterized. A failure is believed to occur in the ability to maintain the balance between brain excitation and inhibition process. Thus, neurotransmitters involved in neuronal inhibition, such as gamma aminobutyric acid (GABA), or neuronal excitation such as glutamate and aspartate, have attracted the interest of researchers aiming to elucidate the mechanisms involved in epilepsy pathogenesis [7], [33]. Furthermore, it is known that neural tissues are especially sensitive to oxygen levels, and oxidative stress is thought to be involved in epileptogenesis. Levels of reactive oxygen species (ROS) increase in response to sustained neuronal electrical activity and seizures. Therefore, antioxidants have been suggested as therapeutic design strategies for the treatment and modulation of epilepsy [43].

Rosmarinic acid ( $\alpha$ -*O*-caffeoyl-3,4-dihydroxyphenyl lactic acid; RA) and its major metabolite caffeic acid (CA) are compounds that occur in many plants, and present several biological activities ([3], [18], [35], [36], [37], [53]), among which antioxidative activity [12], [21], [28], [37], [39], [55]. Furthermore, RA was able to inhibit the enzyme GABA transaminase *in vitro* [4], which would increase the levels of GABA *in vivo*. This finding makes these compounds interesting targets in investigations about the treatment of epilepsy.

The aim of this study was to evaluate the possible antiepileptogenic activity of the RA and CA using the chemical kindling induced by pentylenetetrazole (PTZ) in mice. We also investigated the effects of RA and CA on the production of free radicals, on the activity of antioxidant enzyme superoxide dismutase (SOD), and on deoxyribonucleic acid (DNA) damage in total cortex of mice after the kindling model.

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## Section snippets

### Animals

Male CF1 mice (2–3 months of age, 30–40 g) were obtained from State Foundation for Health Research and Production (FEPPS). The animals were divided into ten groups: 9 groups were used in the kindling experiment (N = 8–11) and one group Sal/Sal (N = 7) was used as negative control in other measurements, totaling 82 animals. Mice were housed in plastic cages (5 per cage), with water and food *ad libitum*, under a 12-h light/dark cycle (lights on at 8:00 AM), and at a constant temperature of  $23 \pm 2$  °C. All...

## Results

This work aimed to evaluate the effect of RA and CA on seizures induced by PTZ. In the Sal-PTZ group (negative control) only 45.45% of the animals presented clonic forelimb seizures as long as or longer than 3 s on the first day of treatment. Nevertheless, after the last treatment, 100% of animals in this group showed seizure behavior ( $p = 0.0124$ ). Moreover, on the first day of treatment, Sal-PTZ group took around 21.34 min to present clonic forelimb seizures of at least 3 s, while on the last day...

## Discussion

The main activities of antiepileptic drugs in synapse include GABAergic inhibitory neurotransmission enhancement, a decrease in glutamatergic excitatory neurotransmission and interference with intracellular signaling pathways. The ability of PTZ to elicit convulsions and to induce a state of kindling begins with its influence in the release and postsynaptic action of GABA [52].

This study evaluated the effect of RA and CA on seizures induced by repeated subconvulsive doses of PTZ. Here, the...

## Conclusions

The results of this study demonstrated that, although RA and CA are not able to prevent the establishment of “kindling state”, both compounds exhibit potential to reduce free radicals and the genotoxic damage caused by the PTZ-kindling model, in some doses.

These findings suggest that these compounds have neuroprotective activity, which can be important in the prevention and management of various neurological disorders, such as epilepsy. These results point to the need to carry out new...

## Conflict of interest statement

The authors declare that there is no conflict of interest....

## Acknowledgments

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...Studies on the neuroprotective, antioxidant and antiepileptic effects of RA and CA are available in different animal models, but certain points still remain unclear. For example, it has been reported that some doses of RA and CA display neuroprotective effects against the oxidative stress generated in epilepsy even though they do not show any antiepileptogenic effects in the kindling epilepsy model [46]. Another study showed that RA dose-dependently increased latency in PTZ induced clonic and generalized seizures as well as latency in pilocarpine induced myoclonic jerks....

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