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Antioxidant properties of antiepileptic drugs levetiracetam and clonazepam in mice brain after *in vitro*-induced oxidative stress

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This study aimed to investigate the *in vitro* antioxidant activity of the novel anticonvulsant levetiracetam and benzodiazepine clonazepam. To do this, the mice brain homogenates were incubated with levetiracetam (50, 100 or 200 μg/ml) or clonazepam (50, 100 or 200 μg/ml), and then, submitted to heating at 37°C for 1 h. Ascorbic acid (vitamin C, 200 μg/ml) was used as reference antioxidant drug. The markers of oxidative stress, such as lipid peroxidation, nitrite-nitrate content, catalase activity, and reduced glutathione (GSH) levels, were measured in brain homogenates. The group submitted to the heating-induced oxidative stress showed an increase in lipid peroxidation, nitrite-nitrate content, and catalase activity. Previous incubation with levetiracetam and clonazepam, mainly at lower doses (50 and 100 μg/ml), and similarly to vitamin C, prevented these pro-oxidative changes, reducing the lipid peroxidation, nitrite-nitrate contents and catalase activity, and increasing GSH levels. These findings demonstrate antioxidant properties of levetiracetam and clonazepam, and help to elucidate the role of protection against oxidative stress in the neuroprotective mechanism of antiepileptic drugs.

Key words: Levetiracetam, clonazepam, oxidative stress, vitamin C, antiepileptic drugs.

INTRODUCTION

The role of free radical-mediated reactions in human neuropathology continues to attract interest. The production of free radicals is associated with injury to cell structures and the pathogenesis of many neurological disorders, such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis and epilepsy (Li et al., 2013; Kong and Lin, 2010). Over-accumulation of reactive oxygen species (ROS) caused by imbalance between the generation and elimination of these entities frequently results in severe harmful effects to cells, a

phenomenon known as oxidative stress (OS) (Gutteridge and Halliwel, 2010; Kong and Li, 2010). Furthermore, in sufficiently high concentrations, nitrogenous compounds, mainly nitric oxide (NO), can also damage cellular structures in a process known as nitrosative stress (NS). This occurs through nitrosation of amines and thiols, which also results in an excess of oxidative species and subsequent molecular damage (Aguiar et al., 2012).

In this context, OS and NS are possible mechanisms implicated in the pathogenesis of epilepsy (Chang and Yu, 2010). Studies have already verified that status epilepticus changes redox potential and decreases the level of ATP, which can lead to a collapse in brain energy production and supply (Aguiar et al., 2012; Wasterlain et al., 1993). Moreover, it was demonstrated that an increase in mitochondrial OS and NS occurs after persistent seizures and promotes subsequent dysfunction of this organelle and cell death (Freitas et al., 2010; Ueda et al., 1997). Therefore, since mitochondrion is the main source of ATP for neurons and has an important role in the homeostasis of intracellular calcium, injury to it may strongly affect neuronal excitability and synaptic transmission (Chang and Yu, 2010). Further support for the role of free radicals in seizures comes from the successful use of exogenously administered antioxidants, as vitamin C and vitamin E, to protect the brain against seizure-induced damage (Barros et al., 2007; Murashima 1998; Santos et al., 2009; Ueda et al., 1997).

The second-generation antiepileptic drug levetiracetam (LEV) is a new molecule that is clearly differentiated from conventional antiepileptic drugs by its pharmacologic properties and mechanisms of action (Gibbs et al., 2006; Lyseng-Williamson 2011; Ueda et al., 2009). Although the exact molecular mechanism of action of LEV remains uncharacterized, it is known that this drug binds to synaptic vesicle protein 2A. By binding to this vesicle, this drug appears to act as a modulator of synaptic vesicle exocytosis, leading to direct inhibition of pre-synaptic neurotransmitter release (Rigo et al., 2002). Moreover, LEV also has been previously demonstrated to protect against oxidative stress-induced neurotoxicity in several models of seizures (Oliveira et al., 2007; Ueda et al., 2009; Zona et al., 2001).

Benzodiazepines, such as clonazepam (CNZ), are established and important agents for the treatment of seizures and epilepsy (Talarek and Fidecka, 2003). Studies have suggested the antioxidant properties of some benzodiazepines. CNZ has been shown to have a protective effect against free radical mediated brain damage in models of diabetic and depressive-like behavior in mice, which involve an oxidative stress process (Haeser et al., 2007; Wayhs et al., 2013[a]; Wayhs et al., 2013[b]). Moreover, nitric oxide synthase (NOS) inhibitors can potentiate the anticonvulsant action of benzodiazepines, which suggests the participation of NO system, and possibly of the NS, in the neuroprotective effect of these drugs (Borowicz et al., 2000; Talarek and

Fidecka, 2003).

However, although the literature suggests that the anticonvulsant effects of antiepileptic drugs (AEDs) could be related to their antioxidant properties, the basis of these properties is still unclear. Therefore, this work aimed to investigate the antioxidant activity of the novel anticonvulsant levetiracetam and benzodiazepine anticonvulsant clonazepam. The study also aimed to investigate how these AEDs could counteract oxidative stress in brain tissues. The investigation of the antioxidant activity of these AEDs and their possible neuroprotective effect may provide stronger evidences for the hypothesis of the involvement of oxidative damage in the pathophysiology of epilepsy.

MATERIALS AND METHODS

Drugs

Levetiracetam (Keppra®) was obtained from UCB Pharmaceutical Sector (Chemin du Foriest, Belgium); clonazepam (Rivotril®) was purchased from A F Hoffmann-La Roche AG (Brazil); ascorbic acid (vitamin C) was obtained from Sigma-Aldrich® (St. Louis, MO, USA). All other chemicals were purchased from Sigma-Aldrich® (St. Louis, MO, USA).

Animals

Experimentally naive, male Swiss mice Mus musculus, from the Animal House of the Federal University of Ceará, weighing 25 to 30 g, were used. The animals were maintained at a controlled temperature (24±2°C) with a 12-h dark/light cycle and food and water *ad libitum*. Mice were caged in groups of 8 in a 41 × 34 × 16 cm cage.

The animals were used according to the NIH Guide for the Care and Use of Laboratory Animals. The experiments were performed after approval of the protocol by the Ethics Committee on Animal Research of the Federal University of Ceará (with protocol number 59/07), in accordance with the Ethical Principles in Animal Research adopted by the Brazilian College of Animal Experimentation (COBEA). Moreover, the experiments were performed to minimize the number of rats and their suffering, following the ethical doctrine of the three "R"s: Reduction, Refinement and Replacement.

Assessment of antioxidant capacity *in vitro*

Antioxidant activity of the compounds was evaluated by measuring the production of thiobarbituric acid (TBARS), as an indicator of lipid peroxidation, reduced glutathione (GSH) levels and catalase activity. In order to assess the effects of these drugs on nitric oxide production, nitrite-nitrate levels were determined. Mice were decapitated and the brains were removed rapidly under standard conditions at 4°C. The whole brain, apart from the cerebellum, was homogenized in 50 mM potassium phosphate buffer (pH 7.4) and the concentration adjusted to 1 g wet weight of brain per 60 ml. Then, 250 μl of the brain homogenates was maintained in the absence or presence of LEV (50, 100 or 200 μg/ml), CNZ (50, 100 or 200 μg/ml) or vitamin C (VIT C) (200 μg/ml) for 24 h at 10°C. Following this, oxidative stress was induced by incubation of the brain homogenates for 1 h at 37°C with samples without oxidative stress acting as controls (Auddy et al., 2003; Mattei et al., 1998; Stocks et al., 1974).

Measurement of TBARS

The homogenates were incubated in a water bath for 1 h at 37°C. After incubation, assessment of the levels of thiobarbituric acid concentration was performed according to the method described by Houng et al. (1998), with absorbance measured at a wavelength of 532 nm and expressed as micromol of malondialdehyde (MDA)/mg of protein. The protein concentration was determined by using Lowry assay (Lowry et al., 1951).

Nitrite-nitrate determination

For the assessment of nitrite-nitrate, derived from nitric oxide (NO), 100 µl of Griess reagent (1% sulfanilamide dissolved in 5% H_3PO_4, 0.1% N-(1-naphthyl)-ethylenediamine dihydrochloride and distilled water, 1:1:1:1) was added to 100 µl of brain homogenate or to 100 µl of $NaNO_2$ at concentrations ranging from 0.75 to 100 µM (standard curve) or for the blanks to 100 µl of the buffer used in the homogenates. The absorbance was measured with a reader plate at 560 nm (Green et al., 1981).

Measurement of catalase activity

This was measured by using hydrogen peroxide to generate H_2O and O_2. The substrate mixture contained 0.3 ml of hydrogen peroxide in 50 ml of 0.05 M phosphate buffer, pH 7.0. The sample aliquot (20 µl) diluted with phosphate buffer was added to 980 µl of the substrate mixture. Absorbances were read after 1 and 6 min at 230 nm. A standard curve was established using purified catalase (Sigma MO, USA) and the results were expressed as µM/min/mg protein (Chance and Maehly, 1955). Protein measurement was performed with the Lowry assay (Lowry et al., 1951).

Determination of glutathione (GSH) levels

GSH levels were evaluated to estimate endogenous defenses against oxidative stress. The method was based on the reaction of Ellman's reagent (DTNB) with free thiol groups. The measurement of this parameter was performed according to the test steps of Sedlak and Lindsay (1998). GSH concentration was determined by the absorbance at 412 nm and was expressed as µg of GSH/µg of protein. The protein concentration was determined with the Lowry assay (Lowry et al., 1951).

Statistical analysis

Data were expressed as mean ± standard error of mean (SEM). Analysis of variance (ANOVA) was used for the statistical analysis followed by Student–Newman–Keuls test to identify differences between experimental groups. Values of $p < 0.05$ were considered significant. The results of VIT C group was compared with internal groups for each antiepileptic drug, LEV or CNZ, studied in this work.

RESULTS

According to *in vitro* antioxidant assays, brain homogenates exposed to oxidative stress, without pre-incubation with CNZ, LEV or VIT C, (stress control) showed an increase in the lipid peroxidation and nitrite-nitrate contents, when compared with brains not exposed

to oxidative stress (control) ($p<0.0001$). In comparison, previous incubation with LEV ($p<0.001$) or CNZ ($p<0.05$), at all tested concentrations, and with VIT C ($p<0.0001$) diminished the TBARS levels when compared with stress control. Regarding nitrite-nitrate concentrations, LEV, only at concentrations of 50 and 100 µg/ml ($p<0.0001$), CNZ, at all tested concentrations ($p<0.05$), and VIT C ($p<0.0001$) were able to significantly reduce nitrite-nitrate concentration when compared with stress control. Results of TBARS measurements: LEV (Control: 0.87 + 0.08; Stress control: 5.01 + 0.44; LEV200: 1.21 + 0.11; LEV100: 1.17 + 0.15; LEV50: 0.94 + 0.09; VIT C: 0.89 + 0.6) (Figure 1); CNZ (Control: 0.79 + 0.05; Stress control: 6.57 + 0.15; CNZ200: 2.76 + 0.27; CNZ100: 3.00 + 0.27; CNZ50: 2.15 + 0.23; VIT C: 0.89 + 0.6) (Figure 2), results were expressed in µmol of malondialdehyde (MDA)/mg of protein. Results of nitrite-nitrate levels: LEV (Control: 4.25 + 0.42; Stress control: 57.73 + 5.96; LEV200: 47.53 + 2.59; LEV100: 27.76 + 1.22; LEV50: 34.61 + 1.69; VIT C:12.35 + 0.8) (Figure 3); CNZ (Control: 4.81 + 0.39; Stress control: 58.56 + 5.26; CNZ200: 33.18 + 1.38; CNZ100: 27.76 + 1.22; CNZ50: 27.57 + 1.21; VIT C: 12.35 + 0.8) (Figure 4), results were expressed in µM.

A significant increase in catalase activity was observed in the group of homogenates submitted to oxidative stress (stress control) as compared to the control group ($p<0.0001$). In the samples pre-incubated with both test drugs, LEV ($p<0.0001$) and CNZ ($p<0.05$), at all concentrations, the catalase activity was reduced compared to the stress control, showing that LEV and CNZ pre-incubation was able to keep catalase activity at normal levels. Pre-incubation with VIT C also demonstrated a significant reduction of catalase activity compared to the stress control ($p<0.0001$). Results of catalase activity: LEV (Control: 8.24 + 0.80; Stress control: 42.37 + 4.05; LEV200: 4.99 + 1.31; LEV100: 7.09 + 1.61; LEV50: 7.34 + 1.35; VIT C: 6.68 + 0.38) (Figure 5); CNZ (Control: 7.40 + 0.60; Stress control: 38.15 + 4.15; CNZ200: 1059 + 1.03; CNZ100: 13.42 + 1.93; CNZ50: 10.06 + 0.96; VIT C: 6.68 + 0.38) (Figure 6), results were expressed in µM/min/mg protein.

No alteration in GSH levels was observed in the samples after the *in vitro* induced oxidative stress. An increase in GSH level was shown at the lowest concentration of CNZ (50 µg/ml) compared to the control group ($p < 0.05$). An increase in GSH was also demonstrated with the two lowest tested concentrations of LEV (50 and 100 µg/ml) as compared to the control ($p < 0.0001$) and to the stress control ($p<0.001$). An increase in GSH levels also occurred in samples with VIT C as compared to the control ($p < 0.001$) and to the stress control ($p<0.001$). Results of GSH levels: LEV (Control: 34.23+ 3.14; Stress control: 38.76+ 3.89; LEV200: 50.26 + 5.17; LEV100: 79.59 + 7.63; LEV50: 69.03 + 5.89; VIT C:57.4 + 4.31) (Figure 7); CNZ (Control: 27.96 + 1.12; Stress control: 28.87 + 2.54;
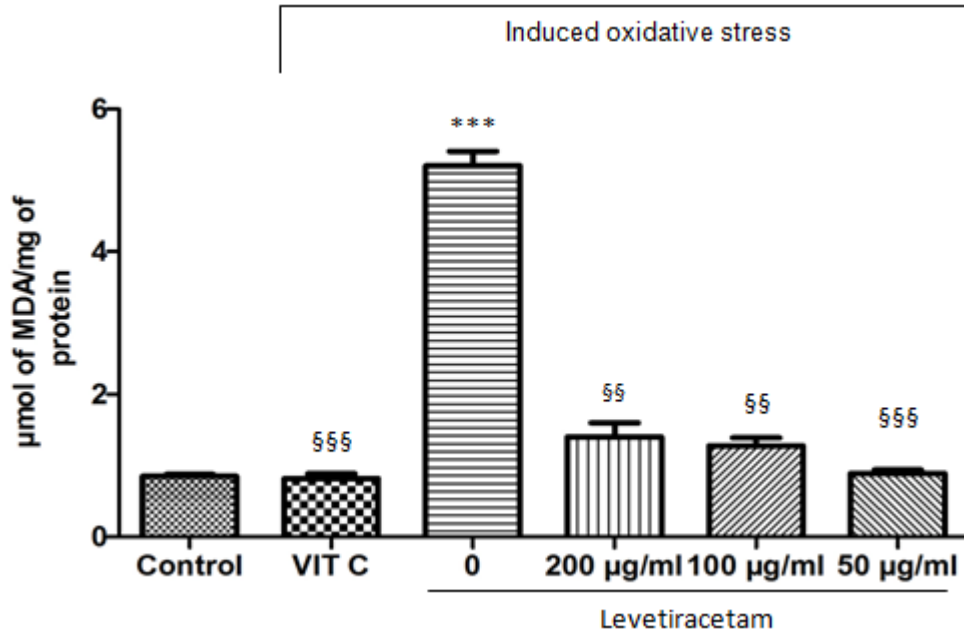
Figure 1. Lipid peroxidation was decreased in samples treated with either LEV as with VIT C. Levels of TBARS (lipid peroxidation level) in mice brain homogenates submitted or not (control) to oxidative stress in the absence (stress control) or presence of different concentrations of levetiracetam (50, 100 or 200 µg/ml) or VIT C (200 µg/ml), after 1 h of incubation of brain homogenate at 37ºC. Each bar represents the mean ± SEM, n=8, ***$p<0,0001$ vs. control group; §§ = $p<0.001$, §§§$p<0.0001$ vs. stress control group (one-way ANOVA followed by post-hoc Student–Newman–Keuls test).
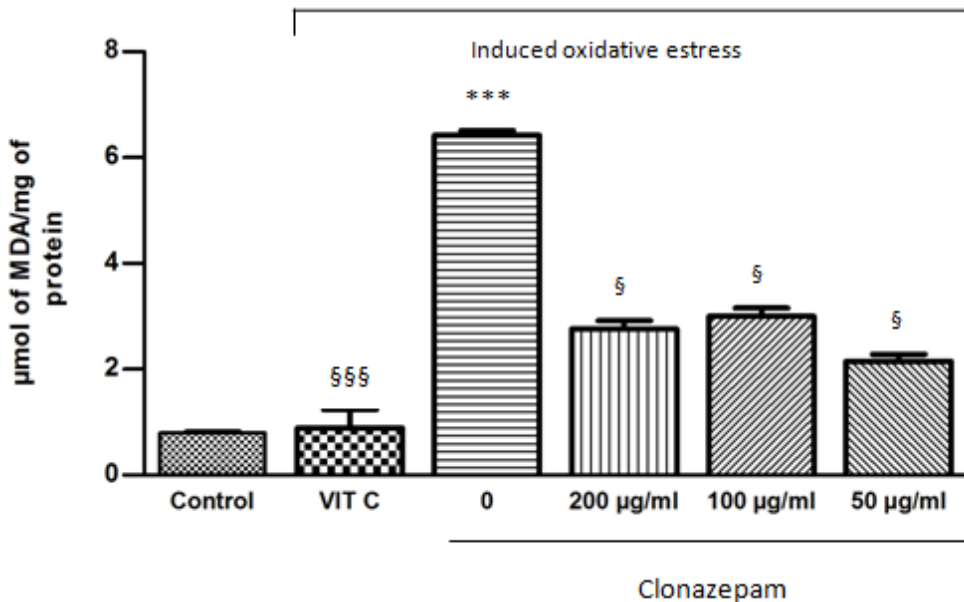

Figure 2. Lipid peroxidation was decreased in samples treated with either CNZ as with VIT C. Levels of TBARS (lipid peroxidation level) in mice brain homogenates submitted or not (control) to oxidative stress in the absence (stress control) or presence of different concentrations of clonazepam (50, 100 or 200 µg/ml) or VIT C (200 µg/ml), after 1 h of incubation of brain homogenate at 37ºC. Each bar represents the mean ± SEM, n=8, ***$p<0.0001$ vs. control group; § = $p<0.05$, §§§$p<0.0001$ vs. stress control group (one-way ANOVA followed by post-hoc Student–Newman–Keuls test).
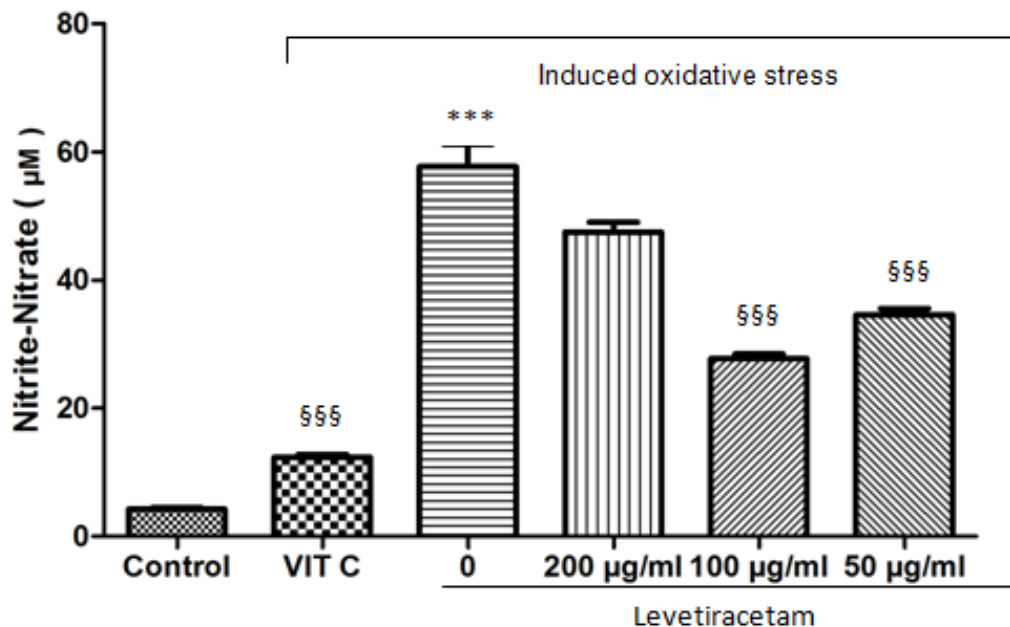
Figure 3. Reduction of nitrite-nitrate content in the samples treated with either LEV as with VIT C. Nitrite-nitrate level in mice brain homogenates submitted or not (control) to oxidative stress in the absence (stress control) or presence of different concentrations of levetiracetam (50, 100 or 200 μg/ml) or VIT C (200 μg/ml), after 1 h of incubation of brain homogenate at 37ºC. Each bar represents the mean ± SEM, n=8, ***$p<0.0001$ vs. control group; §§§$p<0.0001$ vs. stress control group (one-way ANOVA followed by post-hoc Student–Newman–Keuls test).
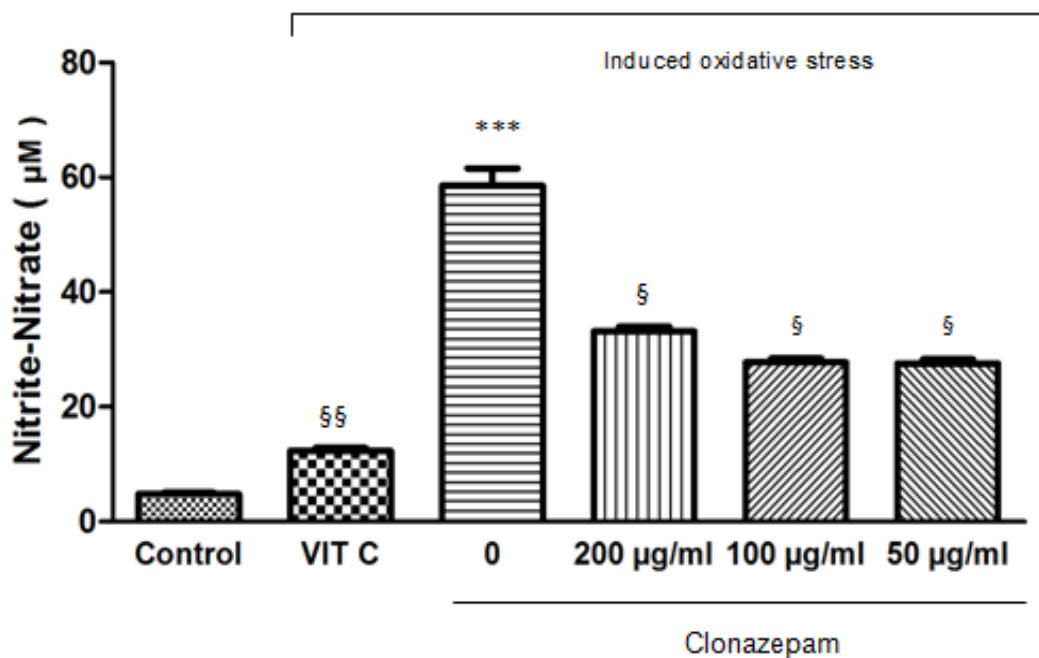

Figure 4. Reduction of nitrite-nitrate content in the samples treated with either CNZ as with VIT C. Nitrite-nitrate level in mice brain homogenates submitted or not (control) to oxidative stress in the absence (stress control) or presence of different concentrations of clonazepam (50, 100 or 200 μg/ml) or VIT C (200 μg/ml), after 1 h of incubation of brain homogenate at 37ºC. Each bar represents the mean ± SEM, n=8, ***$p<0.0001$ vs. control group; §$p<0.05$, §§$p<0.001$ vs. stress control group (one-way ANOVA followed by post-hoc Student–Newman–Keuls test).
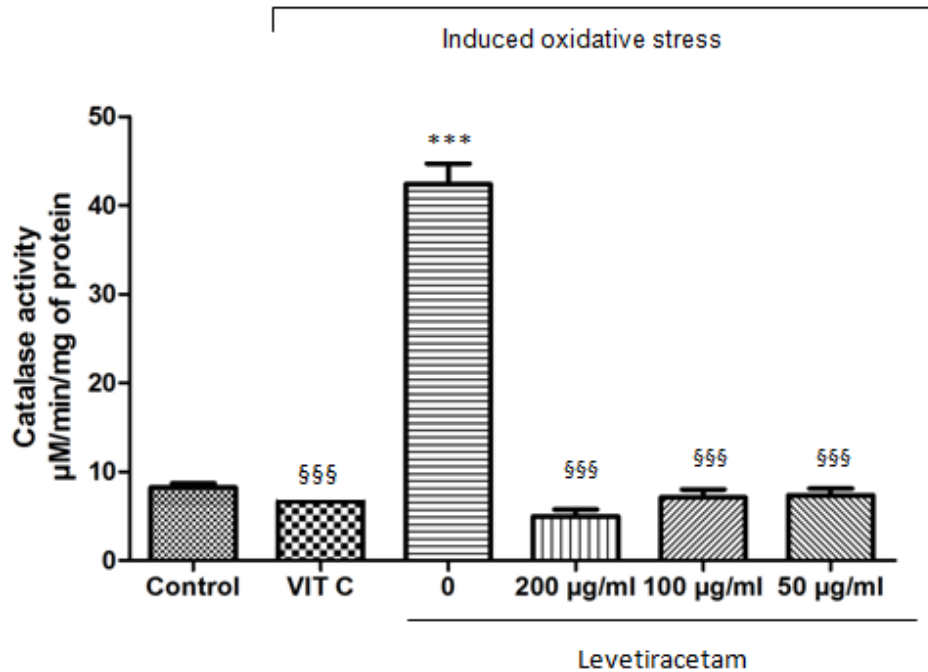
Figure 5. LEV and VIT C were able to approximate the catalase activity of normal levels. Catalase activity in mice brain homogenates submitted or not (control) to oxidative stress in the absence (stress control) or presence of different concentrations of levetiracetam (50, 100 or 200 μg/ml) or VIT C (200 μg/ml), after 1 h of incubation of brain homogenate at 37ºC. Each bar represents the mean ± SEM, n=8, ***$p<0.0001$ vs. control group; §§§$p<0.0001$ vs. stress control group (one-way ANOVA followed by post-hoc Student–Newman–Keuls test).
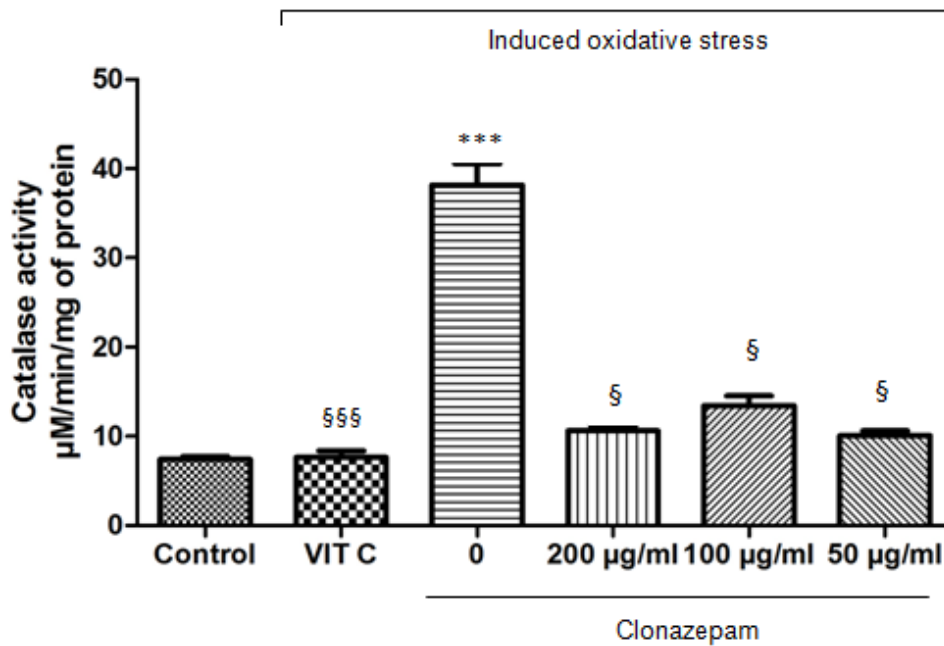

Figure 6. CNZ and VIT C were able to approximate the catalase activity of normal levels. Catalase activity in mice brain homogenates submitted or not (control) to oxidative stress in the absence (stress control) or presence of different concentrations of clonazepam (50, 100 or 200 μg/ml) or VIT C (200 μg/ml), after 1 h of incubation of brain homogenate at 37ºC. Each bar represents the mean ± SEM, n=8, ***$p<0.0001$ vs. control group; §$p<0.05$, §§§$p<0.0001$ vs. stress control group (one-way ANOVA followed by post-hoc Student–Newman–Keuls test).
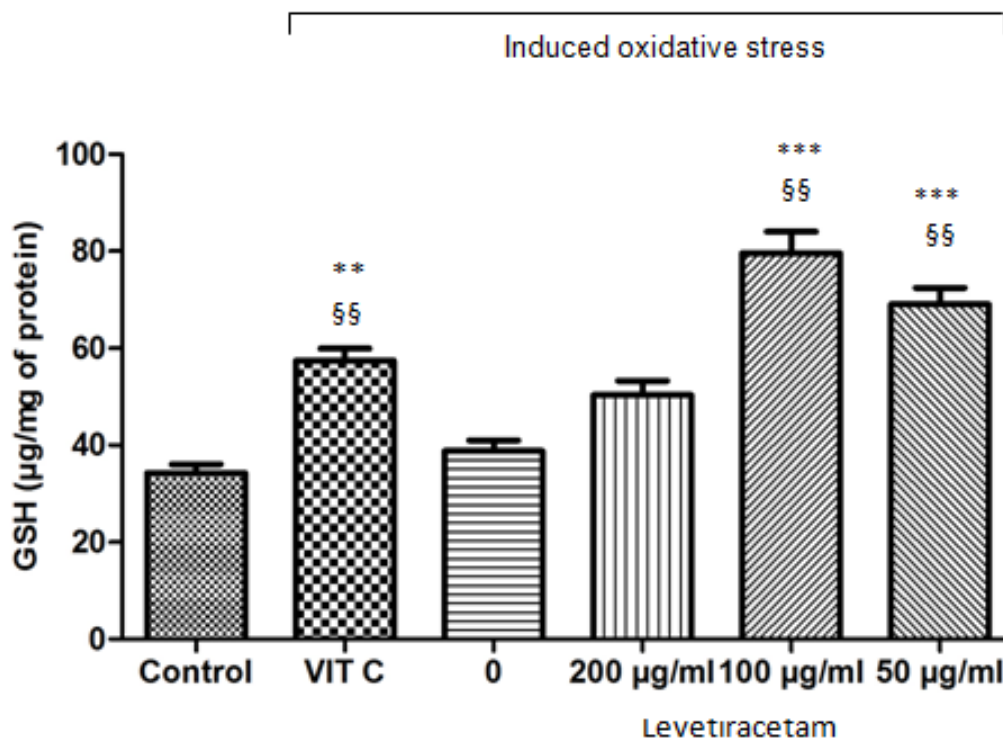
Figure 7. GSH increased especially with the treatment with the two lower concentrations tested of LEV and with VIT C. GSH level in mice brain homogenates submitted or not (control) to oxidative stress in the absence of different concentrations of levetiracetam (50, 100 or 200 µg/ml) or VIT C (200 µg/ml), after 1 h of incubation of brain homogenate at 37°C. Each bar represents the mean ± SEM, n=8, **p<0.001, ***p<0.0001 vs. control group; [§§]p<0.001 vs. stress control group (one-way ANOVA followed by post-hoc Student–Newman–Keuls test).

CNZ200: 26.86 + 0.49; CNZ100: 28.44 + 0.06; CNZ50: 33.89+ 1.54; VIT C: 57.4 + 4.31) (Figure 8), results were expressed in µg/µg of protein.

DISCUSSION

In this study, to further investigate the antioxidant properties of levetiracetam and clonazepam, these drugs were tested in an established and specific *in vitro* model of oxidative stress, proposed by Stocks et al. (1974) and modified by Mattei et al. (1998) and Huong et al. (1998). This model is based on the fact that brain homogenates autoxidize spontaneously and reproducibly when heated to 37°C for 1 h, and this preparation may be used to assay the antioxidant effect of several agents in neuronal substrate (Huong et al., 1998; Mattei et al., 1998; Stocks et al., 1974). Moreover, to verify the potential antioxidant capacity of these AEDs in this model, the results obtained were compared with these drugs with the powerful water-soluble antioxidant vitamin C (VIT C). Previous studies have demonstrated not only the well-recognized antioxidant effects of VIT C, but also its anticonvulsant action, suggesting that this drug behaves as a potential neuronal protective agent, capable of attenuating the behavioral and pro-oxidative changes involved in development of seizures (Santos et al., 2009; Xavier et al., 2007).

Our results showed, as expected, that samples submitted to this *in vitro* stress model showed an increase in MDA and nitrite-nitrate concentrations and higher levels of catalase activity when compared with control samples not submitted to heating. These findings clearly indicate that an oxidative process occurred in brain homogenates submitted to this model. Furthermore, the elevation in free radical formation can be accompanied by a rapid compensatory increase in the activity of free radical scavenging enzymes (Ferreira and Matsubara, 1997; Bellissimo et al., 2001). The high level of catalase activity observed in our results suggests the development of an adaptive mechanism against oxidative stress and further supports that this process occurs in this model.

In a similar fashion to VIT C, treatment with LEV and CNZ, at all doses tested, was able to prevent the elevation of MDA levels induced by *in vitro* stress model, with values of MDA similar to those observed in the control group. Regarding nitrite-nitrate concentrations,
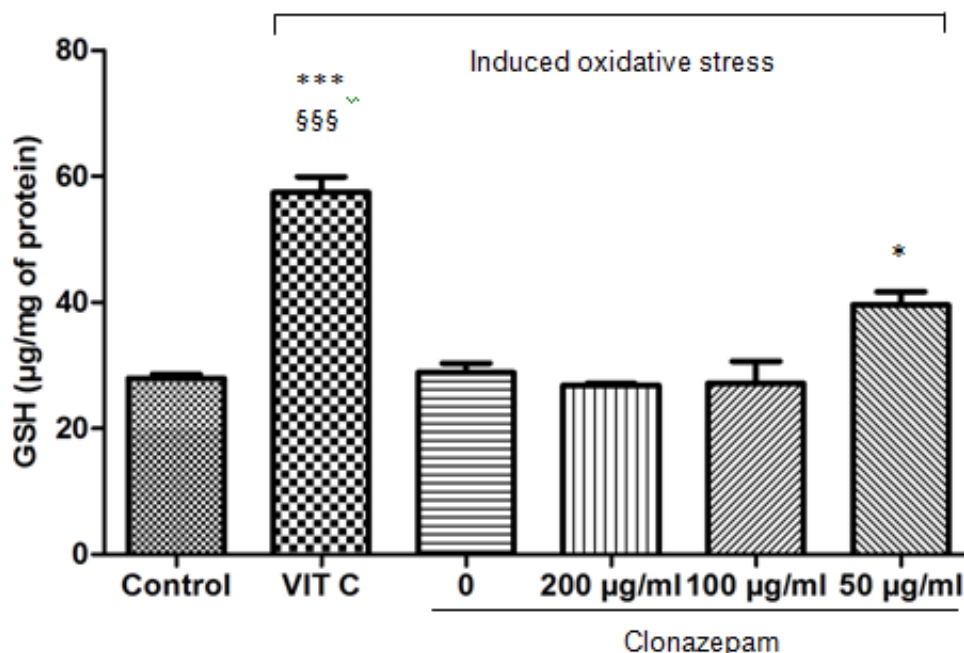
Figure 8. GSH increased especially with treatment with the lower concentration tested of CNZ and with VIT C. GSH level in mice brain homogenates submitted or not (control) to oxidative stress in the absence of different concentrations of clonazepam (50, 100 or 200 µg/ml) or VIT C (200 µg/ml), after 1 h of incubation of brain homogenate at 37°C. Each bar represents the mean ± SEM, n=8, *p<0.05,***p<0.0001 vs. control group; §§§p<0.0001 vs. stress control group (one-way ANOVA followed by post-hoc Student–Newman–Keuls test).

both AEDs demonstrated the capacity to reduce the changes induced by heating, mainly at the lowest doses. VIT C was also able to reduce considerably the nitrite-nitrate concentrations in brain homogenates. Catalase activity was restored to control levels with pre-incubation with LEV and CNZ, and the same effect was observed in samples incubated with VIT C. Finally, the GSH amount was increased by the incubation with both AEDs, especially in lowest doses, and also by incubation with VIT C, when compared with groups submitted to stress procedure.

The degree of lipid peroxidation in tissues is commonly measured by determining the amount of mean product of this process, MDA, and this analysis may relate directly to the level of ROS-mediated injury to a tissue (Kunz et al., 2008; Schihiri, 2014). Free radical-mediated lipid peroxidation proceeds by a chain mechanism, that is, once it was initiated, free radicals can oxidize both lipid molecules in biological membranes and low density lipoproteins. This phenomenon has been shown to induce disturbance to membrane organization and functional loss/modification of proteins and DNA (Frantseva et al., 2000; Schihiri, 2014).

The generation of oxidative damage appears to result from excitotoxicity mechanisms already recognized for participating in several pathological conditions that lead to the development of seizures (Dubinsky et al., 1995;

Duchen, 2000). In this context, overactivation of the glutamate receptors, especially the NMDA receptors, would lead to an increase in intracellular calcium, resulting to mitochondrial dysfunction and generation of ROS and pro-apoptotic factors, fundamental for the damage and cell death (Patel, 2004).

In this context, it has been found that NO is produced in response to N-methyl-D-aspartate (NMDA) receptor stimulation and may be involved in the modulation of neuronal damage (Ferrer et al., 2000; Mülsch et al., 1994). Moreover, the literature has suggested that seizures induce alterations in NO metabolism, increasing the production of its reactive metabolites (nitrite and nitrate). These metabolites, in turn, can interact with NMDA glutamatergic receptors and potentiate its excitotoxicity action on the CNS (Santos et al., 2009). Therefore, elevated nitrite-nitrate levels may be implicated in lipid peroxidation and others oxidative damages on brain tissues submitted to injury situations, as seizures.

Interestingly, according to our findings, LEV and CNZ, similarly to VIT C, was able to reduce the oxidative stress in brain homogenates by decreases of both TBARS and nitrite-nitrate concentrations. Together, these results reinforces the participation of nitrergic pathway, mainly through NO, in oxidative damage in brain tissues and the ability of these antiepileptic drugs, at least in part, through

their antioxidant properties to protect the neurons of oxidative damage.

Furthermore, there is an endogenous ROS scavenging enzyme system, involving the cooperative action of superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px) enzymes. The oxidative stress involved, for example, in status epilepticus and seizures can alter the activity of these enzymes, such catalase, promoting an adaptive cellular response to increased free radicals milieu (Ferreira and Matsubara, 1997; Freitas et al., 2004; Bellissimo et al., 2001). In this context, pre-incubation with LEV and CNZ, preserved the catalase activity in control levels. VIT C was also able to preserve the catalase activity at levels similar to controls samples. These results indicated a possible reduction in toxic reactive substrates in the samples incubated with both AEDs and suggest that these drugs, similarly to a powerful exogenous antioxidant, could help the brain cells to counteract the stress-induced reactive oxygen species overproduction and the oxidative damage.

In the brain, GSH is thought to play a central role in defense against reactive oxygen species. This substance can either directly detoxify reactive oxygen species as can act as a substrate for several peroxidases (Dean et al., 2011). Under conditions of overproduction of free radicals or a deficiency of antioxidant systems, GSH is consumed and its levels fall (Wang and Cynader, 2000). In the present work, no alterations in GSH concentrations were observed in the group of samples submitted to oxidative stress compared to control. However, an increase in this biomarker was induced by pre-incubation with the two lowest tested concentrations of LEV and by the lowest tested concentration of CNZ. An important augment in GSH amount also occurred in samples treated with VIT C. This effect may be probably attributed the capacity of these compounds to avoid the depletion of endogenous antioxidants reserves by the reactive species. Additionally, some of these drugs may potentiate the biosynthesis of brain antioxidants, such as GSH, increasing their amounts. In this context, according to Al-Shorbagy et al. (2013), LEV would be able to up-regulate the action of cystine/glutamate exchanger and to increase the cysteine concentrations in glial cells, the limiting substrate to production of GSH (Al-Shorbagy et al., 2013; Arakawa and Ito, 2007; Kau et al., 2008).

Our findings demonstrate no concentration dependent effects of both LEV and CNZ on the parameters investigated. In fact, in general, the lowest concentrations tested present the highest antioxidant capacity and this property became distinctly smaller with the higher concentration of LEV and CNZ. These results suggest that high concentrations of these drugs could inhibit part of the endogenous antioxidant defense. In accordance with this, previous reports pointed to pro-oxidative effects related to long-term therapy or to administration of high doses of AEDs. In this context, increase in TBARS and decrease in the levels of thiols were detected in blood samples from patients under long-term AED therapy (Alshafei et al., 2013; Devi et al., 2008; Tan et al., 2009).

Therefore, this study reinforces that the pro-oxidant/antioxidant balance in brain is modulated by antiepileptic drugs. In addition, the ability of these compounds to reduce brain damage caused by seizures and their biochemical changes (that is., markers of oxidative stress) further supports the involvement of free radicals in seizure generation and highlights the possible role for antioxidants as adjuncts to antiepileptic drugs for better seizure control (Devi et al., 2008).

Conclusion

In the present work, it was demonstrated, by an *in vitro* model, the antioxidant ability of two antiepileptic drugs, with different mechanism of action, and that this property is mediated, at least in part, by reduction of lipid peroxidation and nitrite-nitrate contents, preservation of catalase activity at control parameters and increase of GSH levels. These results allow us to infer the participation of this effect in the neuroprotective mechanism of these drugs, and reinforce, even indirectly, the hypothesis of oxidative damage in the pathophysiology of epilepsy.

Additionally, our findings also suggest a potential therapeutic use of antioxidant compounds as alternative or complementary tools to the conventional treatment for this disease. However, more studies need to be performed to elucidate the relation between antioxidant activity and protection against seizure development and epilepsy.

Conflict of Interests

The authors have not declared any conflict of interests.

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