Neurochem Res (2009) 34:1372–1379
DOI 10.1007/s11064-009-9917-8

ORIGINAL PAPER

In vitro Antioxidant Activity of *Valeriana officinalis* Against Different Neurotoxic Agents

Jéssie Haigert Sudati · Roselei Fachinetto · Romaiana Picada Pereira · Aline Augusti Boligon · Margareth Linde Athayde · Felix Antunes Soares · Nilda Berenice de Vargas Barbosa · João Batista Teixeira Rocha

Accepted: 17 January 2009 / Published online: 4 February 2009
© Springer Science+Business Media, LLC 2009

Abstract *Valeriana officinalis* L. (Valerian) is widely used as a traditional medicine to improve the quality of sleep. Although *V. officinalis* have been well documented as promising pharmacological agent; the exact mechanisms by which this plant act is still unknown. Limited literature data have indicated that *V. officinalis* extracts can exhibit antioxidant properties against iron in hippocampal neurons in vitro. However, there is no data available about the possible antioxidant effect of *V. officinalis* against other pro-oxidants in brain. In the present study, the protective effect of *V. officinalis* on lipid peroxidation (LPO) induced by different pro-oxidant agents with neuropathological importance was examined. Ethanolic extract of valerian (0–60 μg/ml) was tested against quinolinic acid (QA); 3-nitropropionic acid; sodium nitroprusside; iron sulfate ($FeSO_4$) and Fe^{2+}/EDTA induced LPO in rat brain homogenates. The effect of *V. officinalis* in deoxyribose degradation and reactive oxygen species (ROS) production was also investigated. In brain homogenates, *V. officinalis* inhibited thiobarbituric acid reactive substances induced by all pro-oxidants tested in a concentration dependent manner. Similarly, *V. officinalis* caused a significant decrease on the LPO in cerebral cortex and in deoxyribose degradation. QA-induced ROS production in cortical slices was also significantly reduced by *V. officinalis*. Our results suggest that *V. officinalis* extract was effective in modulating LPO induced by different pro-oxidant agents. These data may imply that *V. officinalis* extract, functioning as antioxidant agent, can be beneficial for reducing insomnia complications linked to oxidative stress.

Keywords *Valeriana officinalis* · Ethanolic extract · Pro-oxidant agents · TBARS · Deoxyribose degradation · Oxidative stress

J. H. Sudati · R. Fachinetto · R. P. Pereira ·
F. A. Soares · N. B. de Vargas Barbosa · J. B. T. Rocha (✉)
Departamento de Química, Centro de Ciências Naturais e Exatas, Programa de Pós-Graduação em Bioquímica Toxicológica, Universidade Federal de Santa Maria (UFSM), Campus Universitário, Camobi, Santa Maria, RS 97105-900, Brazil
e-mail: jbtrocha@yahoo.com.br

A. A. Boligon · M. L. Athayde
Departamento de Farmácia Industrial, Universidade Federal de Santa Maria, Campus Universitário, Camobi, Santa Maria, RS 97100-900, Brazil

Introduction

Insomnia is considered the most frequent sleep illness and affects nearly all populations throughout the world, particularly the elderly [1]. Numerous surveys conducted in countries around the world report that ~30–40% of adults have problems on initiating or maintaining sleep [2–4]. Insomnia symptoms are particularly relevant, given the prevalence of mood and anxiety disorders among individuals with chronic insomnia and the risk that insomnia poses for the subsequent development of psychiatric disorders [5, 6]. The precise cellular and molecular mechanism(s) which underlie(s) the etiology and progression of insomnia are still not fully understood. However, oxidative stress has been implicated as a major cause of cellular injures in a vast variety of clinical abnormalities particularly in the central nervous system [7–9]. In fact, it has been reported that sleep deprivation impairs antioxidant defense, leading to oxidative damage by causing imbalance between oxidants and antioxidant defenses [10, 11].

Extracts from the roots of *Valeriana officinalis* (*V. officinalis* L., Valerianaceae) have long been used in

alternative medicine for the treatment of insomnia and are the most well recognized herbal sedatives worldwide, mainly in United States and Europe [12, 13]. Most of the evidence from randomized clinical trials, with repeated administration, suggests that valerian extract produces mild sleep-inducing effects, without causing alteration of the sleep architecture or significant residual effects [14–16].

Many potential mechanisms for the pharmacological action of *V. officinalis* have been proposed based on their agonistic effects via GABA, adenosine, barbiturate and benzodiazepine receptors [17–19]. Indeed, the pharmacological properties of some components of *V. officinalis* are believed to be associated with their antioxidant activities. Recent literature data have supported a cytoprotective effect for *V. officinalis* extracts in a in vitro model of Parkinson's disease [20]. Furthermore, alcoholic extracts of valerian root have been considered promising pharmacological agent against lipid peroxidation (LPO), however this antioxidant activity was analyzed only against iron/ascorbate-induced LPO [21].

Thus, considering that insomnia can be associated with an increase in oxidative stress and that *V. officinalis* is widely used as sleep-promoting agent; the present study was planned to investigate the protective effects of ethanolic extract of *V. officinalis* (as well as its possible action mechanism) against LPO induced by different pro-oxidant agents. As the antioxidant activity of different class of compounds can vary greatly depending on the pro-oxidant used, we determined the effect of *V. officinalis* extract against neurotoxic agents such as quinolic acid and nitropropionic acid. Subsequently, it was analyzed whether the protective action of *V. officinalis* could involve Fenton reaction and/or reactive oxygen species production.

Experimental Procedure

Chemicals

Tris–HCl, QA, thiobarbituric acid, malonaldehyde bis-(dimethyl acetal; MDA) gallic acid and valeric acid (minimum 99%) were obtained from Sigma (St Louis, MO, USA). Sodium nitroprusside was obtained from Merck (Darmstadt, Germany). Ferrous sulphate, ethylenediamintetracetic (EDTA), chloridric acid and acetic acid were obtained from Merck (Rio de Janeiro, RJ, Brazil).

Plant Material

A standard tincture of *V. officinalis* (10 g of valerian roots per 100 ml of ethanol) was obtained from Bio extracts (São Paulo, Brazil) and tested at concentrations of 0–60 µg/ml.

Pro-oxidant Agents

The 3-nitropropionic acid (3-NPA), quinolinic acid (QA), sodium nitroprusside (SNP), iron sulfate ($FeSO_4$), EDTA and H_2O_2 were used as pro-oxidant agents in in vitro assays.

Animals

Male wistar rats (±3 months old), weighing between 270 and 320 g, from our own breeding colony (Animal House-holding, UFSM, Brazil) were kept in cages with free access to foods and water in a room with controlled temperature (22°C ± 3) and in 12-h light/dark cycle with lights on at 7:00 a.m. The animals were maintained and used in accordance to the guidelines of the Brazilian association for laboratory animal science (COBEA).

Preparation of Brain Homogenates

On the day of the experiments the rats were sacrificed by decapitation and the encephalic tissue (whole brain) was rapidly dissected, placed on ice and weighed. Tissues were immediately homogenized in cold 10 mM Tris–HCl, pH 7.5 (1/10, w/v). The homogenate was centrifuged for 10 min at 4,000*g* to yield a pellet that was discarded and a low-speed supernatant (S1) that was used in the experiments.

Preparation of Cortical Slices

Rats were decapitated and the left cerebral hemisphere was used for preparation of cortical slices. Cortexes were dissected and coronal slices (0.4 mm thickness) were obtained from the parietal area using a McIlwain tissue chopper.

Thiobarbituric Acid Reactive Substances

Thiobarbituric acid reactive substances (TBARS) production was determined as described by Ohkawa et al. [22] and Puntel et al. [23]. Aliquots of the homogenate (200 µl) from tissues were incubated at 37°C in a water bath in the presence of different concentrations of ethanolic extract of *V. officinalis* (0–60 µg/ml) and with the respective pro-oxidant agents. Color reaction was developed by adding 200 µl 8.1% SDS (sodium dodecyl sulphate) to the reaction mixture containing S1 from rat brain. This was subsequently followed by the addition of 500 µl of acetic acid/HCl (pH 3.4) mixture and 500 µl 0.6% thiobarbituric acid (TBA). This mixture was incubated at 100°C for 1 h. TBARS produced were measured at 532 nm and the absorbance was compared with the standard curve using malondialdehyde (MDA).

Deoxyribose Degradation

Deoxyribose degradation was determined by method of Halliwell et al. [24]. Deoxyribose is degraded by hydroxyl radicals with the release of thiobarbituric acid (TBA) reactive substances. Deoxyribose (3 mM) was incubated at 37°C for 30 min with 50 mM potassium phosphate (pH 7.5) plus iron sulfate ($FeSo_4$) (0.1 mM) and/or H_2O_2 (1 mM) to induce deoxyribose degradation and *V. officinalis* at a concentration of 0–12 µg/ml. After incubation, 0.4 ml of TBA 0.8% and 0.8 ml of TCA 2.8% were added, and the tubes were heated for 20 min at 100°C and spectrophotometric measured at 532 nm.

FOX Assay

Lipid peroxidation (LPO) was determined in cortex of rat's brain according to Monserrat et al. [25]. The method is based on the oxidation of Fe^{2+} by lipid hydroperoxides (FOX reactive substances) at acid pH in the presence of the Fe^{3+} complexing dye, xylenol orange (Sigma). Samples were homogenized (1:20 w/v) in 100% cold (4°C) methanol. The homogenate was then centrifuged at 1,000*g*, for 10 min at 4°C. The supernatant was collected and used for LPO determination (580 nm). Cumene hydroperoxide (CHP; Sigma) was employed as standard.

ROS Measurement

Slices from cortex were incubated during 2 h at 37°C in a buffer containing artificial cerebrospinal fluid (ACSF; in mM: NaCl, 120; KCl, 0.5; $NaHCO_3$, 35; $CaCl_2$, 1.5; $MgCl_2$, 1.3; NaH_2PO_4, 1.25; glucose, 10) bubbled with 95% O_2 + 5% CO_2 and *V. officinalis* (10, 20, 40 µg/ml) on the presence or absence of QA (1 mM), in a final volume of 2 ml. At the end of incubation, slices were homogenized and an aliquot of 1 ml was collected in order to read the ROS production. About 10 µM of 2′,7′-dichlorofluorescein diacetate (DCHF-DA) was added to supernatants and samples were read after 1 h. The DCF fluorescence intensity emission was recorded at 520 nm (with 480 nm excitation) as described by Pérez-Severiano et al. [26].

Phytochemical Analysis of *Valeriana officinalis* Extracts

Silica gel 60 F254-precoated TLC plates (Merck, Germany) were used. Dichloromethane (DCM) extracts were dissolved in methanol and 10–30 µl of this solution was used for chromatography. As a reference solution 10 mg of vanillin and 10 µl of anisaldehyde were dissolved in 10 ml of methanol. Alternatively, the reference solution was composed of reference valepotriates (1 mg/ml). The plate was developed twice over a 10 cm path using hexane-methyl ethyl ketone 8:2 as the mobile phase. The spots were visualized under UV light (254 nm) after spraying with a solution of 0.1% 2,4-dinitrophenylhydrazine (DNPH) in hydrochloric acid (25%)–glacial acetic acid 1:1, subsequently heated at 110°C for 10 min. It was confirmed the presence of valtrate (Rf 0.7), dihidrovaltrate (Rf 0.65) and acetoxivaltrate (Rf 0.55) in commercial extract used in this study (data not shown).

Quantification of Valeric Acid and Identification of Gallic Acid by HPLC Analysis

High performance liquid chromatography (HPLC-DAD) was performed with the HPLC system (Shimadzu, Kyoto, Japan), Prominence Auto-Sampler (SIL-20A), equipped with Shimadzu LC-20 AT reciprocating pumps connected to the degasser DGU 20A5 with integrator CBM 20A, UV-VIS detector DAD SPD-M20A and Software LC solution 1.22 SP1. Reverse phase chromatographic analyzes were carried out in isocratic conditions using C-18 column (4.6 mm × 250 mm) packed with 5 µm diameter particles, the mobile phase was methanol: water (80:20 v/v), 0.5% H_3PO_4; pH 2. The mobile phase was filtered through a 0.45 µm membrane filter and then degassed by an ultrasonic bath prior to use. Stock solutions of valeric acid standard reference were prepared in the HPLC mobile phase at a concentration range of 3.12–50.0 mg/ml. All solutions and samples were first filtered through a 0.45 µm membrane filter (Millipore). Quantification was carried out by the integration of the peaks using external standard method at 220 nm. The flow rate was 1.5 ml/min and the injection volume was 10 µl. The chromatographic peaks were confirmed by comparing their retention time and DAD-UV spectra with those of the reference standards and by spiking the isolated compounds in the plant sample. The presence of gallic acid in the plant was confirmed by the HPLC (290 nm; injection volume = 5 µl; flow rate = 1 ml/min; column = C18; mobile fase = methanol; H_2O and 0.4% acetic acid) in comparison with a standard reference of gallic acid (data not shown). All chromatographic operations were carried out at ambient temperature and in triplicate.

Statistical Analysis

Values were expressed as mean ± SEM. Statistical analysis were performed by one-way ANOVA, followed by Duncan's multiple range tests when appropriated. When data did not present homogeneity of variance, they were log transformed. The results were considered statistically significant for $P < 0.05$.

Results

HPLC Analyzes

According to obtained cromatograms, it was found a peak with a retention time (r.t.) at 2.57 min corresponding to valeric acid ($y = 668.6x + 3{,}361$, $r = 0.9960$; Fig. 1a). Valeric acid concentration was 6.11 mg/ml in the analyzed sample (10 mg/ml). Additionally, a peak (r.t. = 2.74 min) can be attributed to the presence of gallic acid in the tincture of *V. officinalis* used in this work (data not shown).

TBARS Production

3-NPA × V. officinalis

3-NPA caused a significant increase on TBARS production in brain homogenates (S1) when compared to the control ($P < 0.05$) and its pro-oxidant effect was abolished by *V. officinalis* in a concentration dependent-manner (Fig. 2).

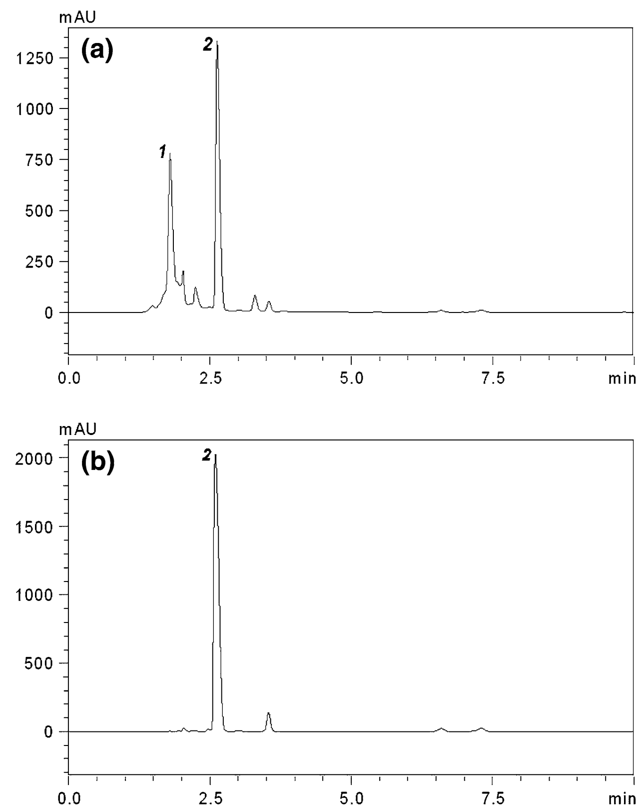


Fig. 1 **a** High performance liquid chromatography of *V. officinalis* tincture. ***1*** Represents an unknown peak; ***2*** corresponds to valeric acid peak. **b** Represents a high performance liquid chromatography of valeric acid (peak ***2***) used as standard reference. Chromatographic conditions are described in the experimental section

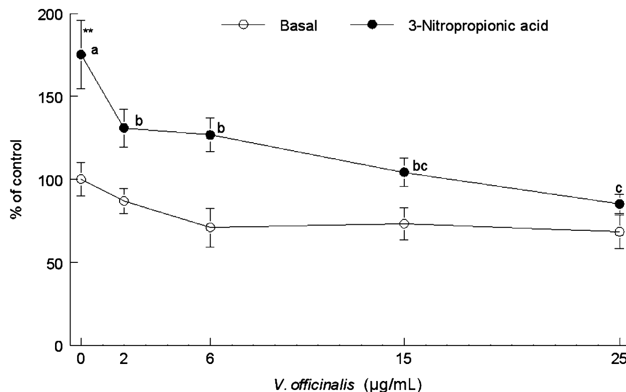


Fig. 2 Effects of *V. officinalis* on 3-NPA (2 mM)-induced TBARS production in brain homogenates. Values are expressed as mean ± SEM of 3–4 independent experiments performed in duplicate or triplicate. *a*, *b*, *c* Represent differences in relation to 3-NPA-induced TBARS production when compared to those induced by 3-NPA and treated with *V. officinalis*. **Significant difference between 3-NPA-induced TBARS and basal values

QA × V. officinalis

QA produced a significant increase on TBARS formation in brain homogenates that was blocked by *V. officinalis* ($P < 0.05$); however there was no significant differences among the extract concentrations (Fig. 3). Under basal conditions, *V. officinalis* also caused a significant reduction on TBARS production in relation to control ($P < 0.05$).

SNP × V. officinalis

SNP induced a significant increase on TBARS formation in brain homogenates ($P < 0.05$) that was reduced in a concentration dependent-manner to basal levels by *V. officinalis* extract ($P < 0.05$; Fig. 4).

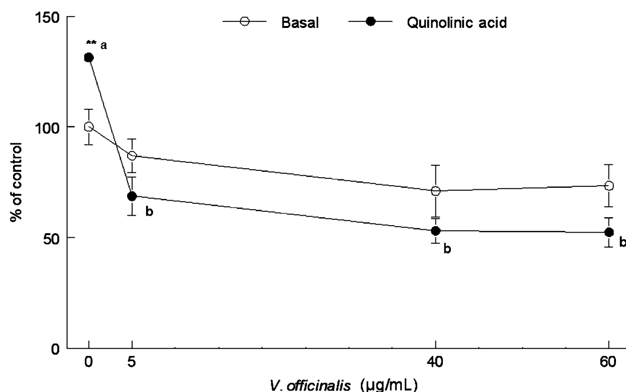


Fig. 3 Effects of *V. officinalis* on QA (2 mM)-induced TBARS production in brain homogenates. Values are expressed as mean ± SEM of 3–4 independent experiments performed in duplicate or triplicate. *a*, *b* Represent differences in relation to QA-induced TBARS levels when compared to those induced by QA and treated with *V. officinalis*. **Significant difference between QA-induced TBARS and basal values

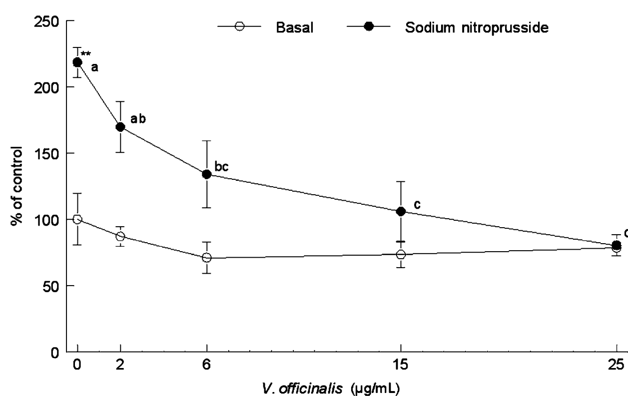


Fig. 4 Effects of *V. officinalis* on SNP (5 µM)-induced TBARS production in brain homogenates. Values are expressed as mean ± SEM from 3 to 4 independent experiments performed in duplicate or triplicate. *a*, *b*, *c* Represent differences in relation to SNP-induced TBARS values when compared to those induced by SNP and treated with *V. officinalis*. **Significant difference between SNP-induced TBARS and basal values

Fe^{2+} × *V. officinalis*

Statistical analyzes revealed that Fe^{2+} induced a significant stimulation in brain TBARS levels ($P < 0.05$), which were partially reduced by *V. officinalis* extract in a concentration-dependent manner ($P < 0.05$; Fig. 5).

Fe^{2+}/EDTA × *V. officinalis*

Fe^{2+}/EDTA caused a significant increase in TBARS levels ($P < 0.05$; Fig. 6) that were reduced by *V. officinalis* ($P < 0.05$). The presence of BHT did not modify TBARS production during color development (Fig. 6b) when compared to assay without BHT (Fig. 6a). The lack of effect of BHT can be attributed to the presence of SDS,

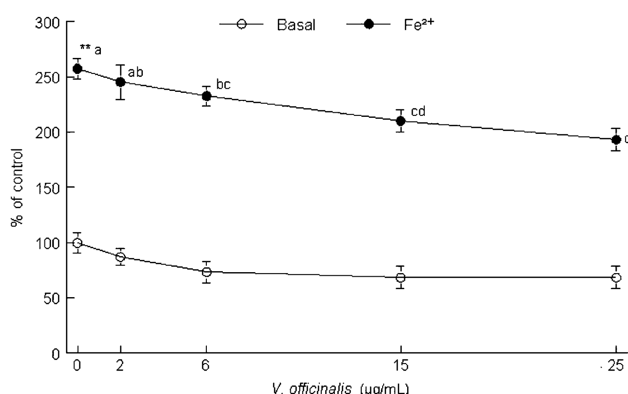
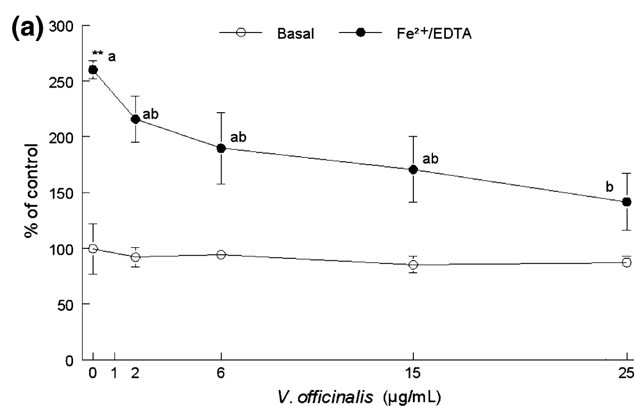


Fig. 5 Effects of *V. officinalis* on Fe^{2+} (10 µM)-induced TBARS production in brain homogenates. Values are expressed as mean ± SEM from 3 to 4 independent experiments performed in duplicate or triplicate. *a*, *b*, *c*, *d* Represent differences between Fe^{2+}-induced TBARS when compared to those induced by Fe^{2+} and treated with *V. officinalis*. **Significant difference between Fe^{2+}-induced TBARS and basal values



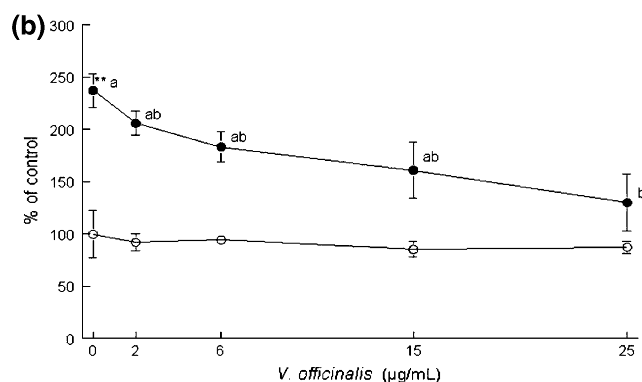


Fig. 6 Effects of *V. officinalis* on Fe^{2+}/EDTA (100 µM)-induced TBARS production without BHT (**a**) and with BHT (**b**) in brain homogenates. Values are expressed as mean ± SEM from 3 to 4 independent experiments performed in duplicate or triplicate. *a*, *b*, *c* Represent differences in relation Fe^{2+}/EDTA-induced TBARs when compared to those induced by Fe^{2+}/EDTA and treated with *V. officinalis*. **Significant difference between Fe^{2+}/EDTA-induced TBARS and basal values

which reduces considerably the production of TBARS during color development. In a previous study of our laboratory, TBARS production was about 25–35% higher in tubes that were boiled in the absence of SDS (SDS added after color development) [23].

Deoxyribose Degradation × *V. officinalis*

Deoxyribose degradation was stimulated 2.5 times by H_2O_2 and 3.5 times by Fe^{2+} plus H_2O_2. *V. officinalis* caused a significant decrease in deoxyribose degradation induced by H_2O_2 (about 20%, $P < 0.05$) and by Fe^{2+} plus H_2O_2 (about 35%, $P < 0.05$; Fig. 7).

Fox Assay

Fe^{2+}/EDTA produced an increase on cortical lipid oxidation when compared with basal condition ($P < 0.05$) and *V. officinalis* diminished the Fe^{2+}/EDTA pro-oxidant effect ($P < 0.05$; Fig. 8).

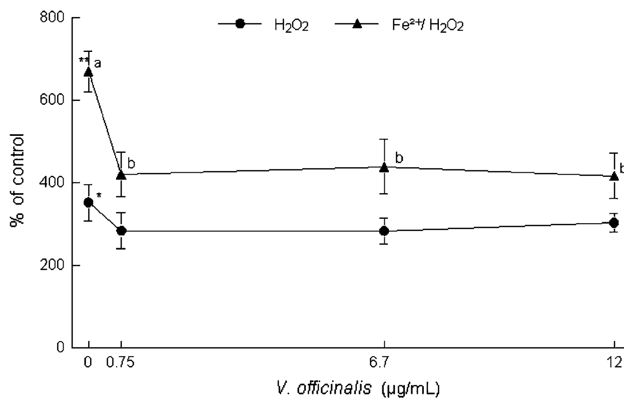


Fig. 7 Effects of *V. officinalis* on H_2O_2 (1 mM) and/or Fe^{2+}/H_2O_2-induced deoxyribose degradation. The deoxyribose was incubated for 20 min with H_2O_2 or Fe^{2+}/H_2O_2 in the presence or absence of *V. officinalis*. Values are expressed as mean ± SEM from 3 to 4 independent experiments performed in duplicate or triplicate. *, **Represent differences in relation Fe^{2+}plus H_2O_2, H_2O_2-induced deoxyribose degradation when compared to basal values. *a*, *b* Represent differences in relation Fe^{2+}/H_2O_2-induced deoxyribose degradation when compared to those induced by Fe^{2+}/H_2O_2 and treated with *V. officinalis*

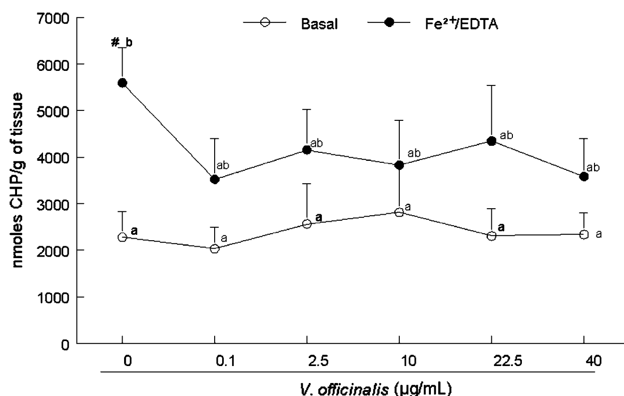


Fig. 8 Effect of *V. officinalis* on Fe^{2+}/EDTA (100 µM) induced LPO. Values are expressed as mean ± SEM of 3–4 independent experiments performed in quadruplicate. *a*, *b* Represent differences among all groups. #Significant difference in relation to Fe^{2+}/EDTA-induced LPO when compared to the basal values (without Fe^{2+}/EDTA and *V. officinalis*)

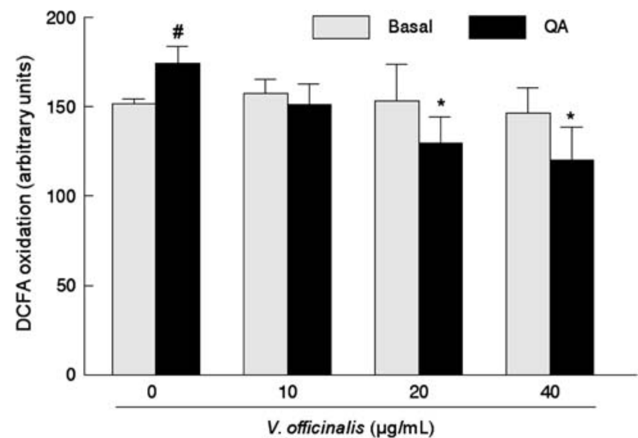


Fig. 9 Effect of QA (1 mM)-induced ROS production in rat brain cortical slices. Data of ROS levels are presented as fluorescence intensity emission. Data are expressed as mean ± SEM of three different experiments. #Significant differences between QA-induced ROS production and basal values. *Significant differences between QA-induced ROS values when compared to those induced by QA and treated with *V. officinalis*

ROS Production

The incubation of brain cortical slices with QA (1 mM) caused an increase in ROS production when compared to basal conditions ($P < 0.05$) and *V. officinalis* blocked the pro-oxidant effect of QA ($P < 0.05$; Fig. 9).

Discussion

Insomnia is a condition that affects a large percentage of population around the world causing impairment in normal life style [1]. The neuropathophysiologic basis of insomnia remains unclear, but several lines of evidence suggest that this disorder is associated with changes in the homeostasis of neurotransmitters and can be associated with an increase in oxidative stress [9–11]. In this way, an over production of reactive oxygen species can promotes a deregulation in physiological cellular performance and increase the susceptibility to disease.

In folkloric medicine the pharmacological agent *V. officinalis* is widely suggested as a possible alternative to benzodiazepine treatment against insomnia [27, 28]. Besides, a recent study from our laboratory demonstrated that *V. officinalis* seems not produce any oxidative damage to CNS after chronic in vivo administration [29]. Of potential pharmacological significance, the results presented here have indicated that tincture of *V. officinalis* prevents oxidative damage in brain preparations induced by different pro-oxidants QA, SNP, NPA, Fe(II) and Fe(II)/EDTA complex. The data obtained with iron are somewhat similar to that published recently by Malva et al. [21], where they have shown that *V. officinalis* extract protected cultured hippocampal neurons from iron/ascorbate-induced LPO and neurotoxicity in a model of Parkinson diseases [20]. Brain is extremely sensitive to iron overload and intracerebral injection of Fe(II) causes neurotoxic effects [30]. Here we have expanded the findings of Malva et al. [21] to other neurotoxic pro-oxidants. The efficacy and the antioxidant potency of some extracts varied depending on the pro-oxidant used [31–33], tincture of *V. officinalis* showed to be in accordance with it. In fact, we have observed that it was more effective against NPA, QA and SNP than Fe(II) or Fe(II)/EDTA complex, which may

indicate that *V. officinalis* extract can have a more wide-ranging antioxidant activity in the central nervous system. In line with this, *V. officinalis* also blocked the ROS production in brain cortical slices induced by QA.

Many studies have tested the effects of medicinal plants in in vivo models of different pathologies and preclinical tests frequently confirm the folk use of the plant extracts [34]. However, for the majority of plants, including *V. officinalis*, the exact mechanisms by which the extracts act are unknown. There are many studies showing that *V. officinalis* modulates anxiety and insomnia [35] possibly by interacting with different neurotransmitter systems [17–19, 35]. However, the occurrence of oxidative stress in insomnia and anxiety [1] can indicate that *V. officinalis* could exert its pharmacological effects, at least in part, via modulation of oxidative stress. In this vein, here we have demonstrated that *V. officinalis* is a potent antioxidant against neurotoxins that act via distinct mechanisms [30, 36–42]. We have also observed that *V. officinalis* modulates the pro-oxidant effects of QA in brain homogenates and in cortical slices where the cells are more preserved. In fact, literature has indicated that *V. officinalis* can inhibit MK-801 binding to brain membranes [19]; suggesting an interaction between *V. officinalis* and NMDA receptors [40, 43–46].

In summary, our findings suggest that *V. officinalis* extract was an effective modulator of LPO induced by different neurotoxic pro-oxidant agents. Indeed, these data may imply that *V. officinalis* extract, by functioning as antioxidant agent, may be beneficial for reducing insomnia complications linked to oxidative stress.

Acknowledgments The financial support by CAPES/SAUX/PRO-AP, VITAE Fundation, CNPq, FAPERGS, ICTP and FINEP research grant ''Rede Instituto Brasileiro de Neurociência (IBN-Net)'' # 01.06.0842-00 is gratefully acknowledged.

References

1. World Health Organization (1992) The ICD-10 classification of mental and behavioral disorders. World Health Organization, Geneva
2. Ancoli-Israel S, Roth T (1999) Characteristics of insomnia in the United States: results of the 1991 National Sleep Foundation Survey. I Sleep 22:S347–S353
3. Sateia MJ, Doghramji K, Hauri PJ, Morin CM (2000) Evaluation of chronic insomnia. An American academy of sleep medicine review. Sleep 23:243–308
4. Ohayon M (2002) Epidemiology of insomnia: what we know and what we still need to learn. Sleep Med Rev 6:97–111. doi:10.1053/smrv.2002.0186
5. Markesbery WR (1997) Oxidative stress hypothesis in Alzheimer's disease. Free Radic Biol Med 23:134–147. doi:10.1016/S0891-5849(96)00629-6
6. Riemann D, Voderholzer U (2003) Primary insomnia: a risk factor to develop depression? J Affect Disord 76:255–259. doi:10.1016/S0165-0327(02)00072-1
7. Halliwell B, Gutteridge JHC, Cross CE (1992) Free radicals, antioxidants and human disease: where we are now? J Lab Clin Med 119:598–620
8. Halliwell B, Gutteridge JMC (eds) (1999) Free radicals in biology and medicine, 3rd edn. Oxford University Press, Oxford, p 936
9. Olanow CW (1993) A radical hypothesis for neurodegeneration. Trends Neurosci 16:439–444. doi:10.1016/0166-2236(93)90070-3
10. Cirelli C (2006) Cellular consequences of sleep deprivation in the brain. Sleep Med Rev 10:307–321. doi:10.1016/j.smrv.2006.04.001
11. D'Almeida V, Lobo LL, Hipolide DC, De Oliveira AC, Nobrega JN, Tufik S (1998) Sleep deprivation induces brain region-specific decreases in glutathione levels. NeuroReport 9(12):2853–2856
12. Houghton PJ (1999) The scientific basis for the reputed activity of valerian. J Pharmacol 51:505–512. doi:10.1211/0022357991772772
13. Richman A, Witkowski JP (1998) 4th Annual herbal products sales survey. Whole Foods 21:19–26
14. Gyllenhaal C, Merritt SL, Peterson SD, Block KI, Gochenour T (2000) Efficacy and safety of herbal stimulants and sedatives in sleep disorders. Sleep Med Rev 4:229–251. doi:10.1053/smrv.1999.0093
15. Meoli AL, Rosen C, Kristo D, Kohrman M, Gooneratne N, Aguillard RN, Fayle R, Troell R, Touwnsend D, Claman D, Hoban T, Mahowald M (2005) Clinical practice review committee of the American academy of sleep medicine. Oral nonprescription treatment for insomnia: an evaluation of products with limited evidence. J Clin Sleep Med 2:173–187
16. Hallam KT, Olver JS, McGrath C, Norman TR (2003) Comparative cognitive and psychomotor effects of single doses of *Valeriana officinalis* and triazolam in healthy volunteers. Hum Psychopharmacol 18:619–625. doi:10.1002/hup.542
17. Mueller CE, Brattstrom A, Abourashed EA, Koetter U (2002) Interactions of valerian extracts and a fixed valerian-hop extract combination with adenosine receptors. Life Sci 71:1939–1949. doi:10.1016/S0024-3205(02)01964-1
18. Schumacher BSS, Hoelzl J, Khudeir N, Hess S, Mueller CE (2002) Lignans isolated from valerian: identification and characterization of a new olive oil derivative with partial agonistic activity at A1 adenosine receptors. J Nat Prod 65:1479–1485. doi:10.1021/np010464q
19. Ortiz JG, Rassi N, Maldonado PM, González-Cabrera S, Ramos I (2006) Commercial valerian interactions with [^{3}H]-Flunitrazepam and [^{3}H]MK-801 binding to rat synaptic membranes. Phytother Res 20(9):794–798. doi:10.1002/ptr.1960
20. Oliveira DM, Barreto G, De Andrade DEV, Saraceno E, Aon-Bertolino L, Capani F, El Bacha RS, Giraldez LD (2009) Cytoprotective effect of *Valeriana officinalis* extract on an in vitro experimental model of Parkinson disease. Neurochem Res 34:215–220. doi:10.1007/s11064-008-9749-y
21. Malva JO, Santos S, Macedo T (2004) Neuroprotective properties of *Valeriana officinalis* extracts. Neurotox Res 6(2):131–140
22. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–358. doi:10.1016/0003-2697(79)90738-3
23. Puntel RL, Nogueira CW, Rocha JBT (2005) *N*-Methyl-D-aspartate receptors are involved in the quinolinic acid, but not in the malonate pro-oxidative activity in vitro. Neurochem Res 30:417–424. doi:10.1007/s11064-005-2617-0
24. Halliwell B, Gutteridge JMC, Aruoma OI (1987) The deoxyribose method: a simple "Test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. Anal Biochem 165:215–219. doi:10.1016/0003-2697(87)90222-3
25. Monserrat JM, Geracitano LA, Pinho GLL, Vinagre TM, Faleiros M, Alciati JC, Bianchini A (2003) Determination of lipid

peroxides in invertebrates tissues using the Fe(III). Xylenol orange complex formation. Arch Environ Contam Toxicol 45:177–183. doi:10.1007/s00244-003-0073-x

26. Pérez-Severiano P, Rodríguez-Pérez M, Pedraza-Chaverrí J, Maldonado PD, Medina Campos ON, Ortíz-Plata A, Sánchez-García A, Villeda-Hernández J, Galván-Arzate S, Aguilera P, Santamaría A (2004) S-allylcysteine, a garlic-derived antioxidant, ameliorates quinolinic acid-induced neurotoxicity and oxidative damage in rats. Neurochem Int 45:1175–1183. doi:10.1016/j.neuint.2004.06.008
27. Kupfer DJ, Reynolds CFIII (1997) Current concepts: management of insomnia. N Engl J Med 336:341–346. doi:10.1056/NEJM199701303360506
28. Holz J, Godau P (1989) Receptor binding studies with *Valeriana officinalis* on the benzodiazepine receptor. Planta Med 55:642. doi:10.1055/s-2006-96221
29. Fachinetto R, Villarinho JG, Wagner C, Pereira RP, Ávila DS, Burger ME, Calixto JB, Rocha JBT, Ferreira J (2007) *Valeriana officinalis* does not alter the orofacial dyskinesia induced by haloperidol in rats: role of dopamine transporter. Prog Neuro-psychopharmacol Biol Psychiatry 31:1478–1486. doi:10.1016/j.pnpbp.2007.06.028
30. Bostanci MO, Bağirici F (2008) Neuroprotective effect of aminoguanidine on iron-induced neurotoxicity. Brain Res Bull 76:57–62. doi:10.1016/j.brainresbull.2007.11.011
31. Oboh G, Puntel RL, Rocha JBT (2007) Hot pepper (Capsicum annuum, Tepin and Capsicum chinese, Habanero) prevents Fe2+-induced lipid peroxidation in brain—in vitro. Food Chem 102:178–185. doi:10.1016/j.foodchem.2006.05.048
32. Pereira RP, Fachinetto R, Prestes AS, Puntel RL, Silva GNS, Heinzmann BM, Boschetti TK, Athayde ML, Bürger ME, Morel AF, Morsh VM, Rocha JBT (2009) Antioxidant effects of different extracts from *Melissa officinalis*, *Matricaria recutita* and *Cymbopogon citrates*. Neurochem Res (in press). doi:10.1007/s11064-008-9861-z
33. Wagner C, Fachinetto R, Dalla Corte CL, Brito VB, Severo D, Dias GOC, Morel AF, Nogueira CW, Rocha JBTR (2006) Quercitrin, a glycoside form of quercetin, prevents lipid peroxidation in vitro. Brain Res 1107:192–198. doi:10.1016/j.brainres.2006.05.084
34. Rates SMK (2001) Plants as source of drugs. Toxicon 39:603–613. doi:10.1016/S0041-0101(00)00154-9
35. Abourashed EA, Koetter U, Brattstrom A (2004) In vitro binding experiments with a valerian, hops, and their fixed combination extract (Ze91019) to selected central nervous system receptors. Phytomedicine 11:633–638. doi:10.1016/j.phymed.2004.03.005
36. Brouillet E, Hantraye P, Ferrante RJ, Dolan R, Leroy-Willig A, Kowall NW, Beal MF (1995) Chronic mitochondrial energy impairment produces selective striatal degeneration and abnormal choreiform movements in primates. Proc Natl Acad Sci USA 92:7105–7109. doi:10.1073/pnas.92.15.7105
37. Tŭnez I, Montilla P, Muñoz MC, Feijoo M, Salcedo M (2004) Protective effect of melatonin on 3-nitropropionic acid-induced oxidative stress in synaptosomes in an animal model of Huntington's disease. J Pineal Res 37:252–256. doi:10.1111/j.1600-079X.2004.00163.x
38. Bates JN, Baker MT, Guerra R, Harrison DG (1990) Nitric oxide generation from nitroprusside by vascular tissue. Biochem Pharmacol 42:157–165. doi:10.1016/0006-2952(91)90406-U
39. Chen J, Chang B, Williams M, Murad F (1991) Sodium nitroprusside degenerates cultured rat striatal neurons. NeuroReport 2:121–123. doi:10.1097/00001756-199103000-00002
40. Loiacono RE, Beart PM (1992) Hippocampal-lesions induced by microinjection of the nitric-oxide donor nitroprusside. Eur J Pharmacol 216:331–333. doi:10.1016/0014-2999(92)90381-D
41. Bellé NAV, Dalmolin GD, Fonini G, Rubin MA, Rocha JBT (2004) Polyamines reduces lipid peroxidation induced by different pro-oxidant agents. Brain Res 1008:245–251. doi:10.1016/j.brainres.2004.02.036
42. Santamaría A, Ríos C (1993) MK-801, an *N*-Methyl-D-aspartate receptor antagonist, blocks quinolinic acid-induced lipid peroxidation in rat corpus striatum. Neurosci Lett 159:51–54. doi:10.1016/0304-3940(93)90796-N
43. Goda K, Kishimoto R, Shimizu S, Hamane Y, Ueda M (1996) Quinolinic acid and active oxygens. Possible contribution of active oxygens during cell death in the brain. Adv Exp Med Biol 398:247–254
44. Graf E, Mahoney JR, Bryant RG, Eaton JW (1984) Iron catalyzed hydroxyl radical formation: stringent requirement for free iron coordination site. J Biol Chem 259:3620–3624
45. Gogvadze V, Walter PB, Ames BN (2003) The role of Fe^{2+}-induced lipid peroxidation in the initiation of the mitochondrial permeability transition. Arch Biochem Biophys 414:255–260
46. Zago MP, Verstraeten SV, Oteiza PI (2000) Zinc in the prevention of Fe^{2+} initiated lipid and protein oxidation. Biol Res 33(2):143–150

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.