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

Jéssie Haigert Sudati · Roselei Fachinetto · Romaiana Picada Pereira · Aline Augusti Boligon · Margaret 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 (FeSO₄) and Fe²⁺/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

**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, ethylenediaminetetraacetic (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 (FeSO₄), EDTA and H₂O₂ 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/HCI (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 (FeSO₄) (0.1 mM) and/or H₂O₂ buffer containing artificial cerebrospinal fluid (ACSF; in slices from cortex were incubated during 2 h at 37°C/C176). ROS Measurement

ROS production. About 10 l 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 Fe²⁺ by lipid hydroperoxides (FOX reactive substances) at acid pH in the presence of the Fe³⁺ complexing dye, xylene 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; NaHCO₃, 35; CaCl₂, 1.5; MgCl₂, 1.3; NaH₂PO₄, 1.25; glucose, 10) bubbled with 95% O₂ and 5% CO₂ and V. officinalis at a concentration of 0–12 g/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.

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 Shimazu 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 analyses 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% H₃PO₄; 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; H₂O 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).

**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. 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.

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 (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 SNP-induced TBARS levels when compared to those induced by SNP and treated with *V. officinalis*. **Significant difference between SNP-induced TBARS and basal values.
Fe²⁺ × V. officinalis

Statistical analyzes revealed that Fe²⁺ 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).

Fe²⁺/EDTA × V. officinalis

Fe²⁺/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, 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 H₂O₂ and 3.5 times by Fe²⁺ plus H₂O₂. V. officinalis caused a significant decrease in deoxyribose degradation induced by H₂O₂ (about 20%, P < 0.05) and by Fe²⁺ plus H₂O₂ (about 35%, P < 0.05; Fig. 7).

Fox Assay

Fe²⁺/EDTA produced an increase on cortical lipid oxidation when compared with basal condition (P < 0.05) and V. officinalis diminished the Fe²⁺/EDTA pro-oxidant effect (P < 0.05; Fig. 8).
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 \((P < 0.05; \text{Fig. } 9)\).

**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]. 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 an antioxidant agent, may be beneficial for reducing insomnia complications linked to oxidative stress.

Acknowledgments The financial support by CAPES/SAUX/PROAP, VITAE Foundation, CNPq, FAPERGS, ICTP and FINEP research grant “Receita Instituto Brasileiro de Neurociência (IBN-Net)” # 01.06.0842-00 is gratefully acknowledged.

References


