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Valeriana officinalis ameliorates vacuous chewing movements induced by reserpine in rats

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Abstract Oral movements are associated with important neuropathologies as Parkinson's disease and tardive dyskinesia. However, until this time, there has been no known efficacious treatment, without side effects, for these disorders. Thus, the aim of the present study was to investigate the possible preventive effects of *V. officinalis,* a phytotherapic that has GABAergic and antioxidant properties, in vacuous chewing movements (VCMs) induced by reserpine in rats. Adult male rats were treated with reserpine (1 mg/kg, s.c.) and/or with *V. officinalis* (in the drinking water, starting 15 days before the administration of the reserpine). VCMs, locomotor activity and oxidative stress measurements were evaluated. Furthermore, we carried out the identification of valeric acid and gallic acid by HPLC in the *V. officinalis* tincture. Our findings demonstrated that reserpine caused a marked increase on VCMs and the co-treatment with *V. officinalis* was able to reduce the intensity of VCM. Reserpine did not induce oxidative stress in cerebral structures (cortex, hippocampus, striatum and *substantia nigra*). However, a significant positive correlation between DCF-oxidation (an estimation of oxidative stress) in the cortex and VCMs ($p < 0.05$) was observed. Moreover, a negative correlation between $Na^{+}K^{+}$-ATPase activity in *substantia nigra* and the number of VCMs was observed ($p < 0.05$). In conclusion, *V. officinalis* had behavioral protective effect against reserpine-induced VCMs in rats; however, the exact mechanisms that contributed to this effect have not been completely understood.

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Keywords Oral dyskinesia · Parkinson's disease · Reserpine · Vacuous chewing movements · *Valeriana officinalis*

Abbreviations

Tardive dyskinesia	TD
Parkinson's disease	PD
Vacuous chewing movements	VCMs
Orofacial dyskinesia	OD
Monoaminoxidase	MAO
Valeriana officinalis	*V. officinalis*
Reactive oxygen species	ROS
2′,7′-Dichlorodihydrofluorescein diacetate	DCFH-DA
Thiobarbituric acid reactive substances	TBARS

Introduction

Oral movements are important symptoms associated with neuropathological and pharmacological conditions such as tardive dyskinesia (TD) and Parkinson's disease (PD) (for review see Andreassen and Jorgensen 2000; Thomas and Beal 2007). These syndromes are particularly important due to their high prevalence in humans (Donaire and Gil-Saladie 2001; Jicha and Salomone, 1991; Llorca et al. 2002; Paille et al. 2004a; Smythies 1999). Of note, PD is the second most prevalent age-related neurodegenerative disorder with over one million cases in the USA alone (Bove et al. 2005). Several works have focused on the mechanisms involved in the pathogenesis of this syndrome. However, the mechanisms involved in the development of this syndrome remain still unclear.

In this respect, literature data have proposed different animal models to study oral movement disturbances, because they can be associated with brain disorders observed in Parkinson diseases and tardive dyskinesia (TD). Consequently, oral dyskinesia can be used as potential predictors of neurodegenerative diseases, in particular vacuous chewing movements (VCMs), which have been used as potential surrogate models of orofacial dyskinesia (OD) (Abílio et al. 2002; 2003; 2004; Carvalho et al. 2003; Faria et al. 2005; Neisewander et al. 1991; Neisewander et al. 1994; Raghavendra et al. 2001; Thaakur and Himabindhu 2009; Waddington 1990) and/or parkinsonism-like symptoms (Baskin and Salamone 1993; Salamone and Baskin 1996; Paille et al. 2004b). Regarding the development of VCMs, literature data have paid special attention to the role of oxidative stress (Abílio et al. 2003; Burger et al. 2003; Naidu et al. 2004). However, changes in the balance between GABAergic, dopaminergic and glutamatergic neurotransmission can be considered more important factors in the installation of VCMs that could be followed by oxidative stress (Andreassen and Jorgensen 2000; Burger et al. 2005a; Dekeyser 1991; Fibiger and Lloyd 1984).

One model that has been extensively used in the literature to induce VCMs involves acute treatment with reserpine. Reserpine causes depletion of vesicular dopamine stores, which can increase dopamine levels and, consequently, its metabolism via monoaminoxidase (MAO). In this scenario, exacerbation of dopamine metabolism can lead to overproduction of free radicals, particularly in basal ganglia (Abílio et al. 2003a; Bilska and Dubiel 2007; Burger et al. 2003; Naidu et al. 2004).

Another important hypothesis has discussed the involvement of pallidal and nigral GABAegic pathways in the development of VCMs in rats. This hypothesis is supported by studies in rats, where GABA agonists inhibited the development of reserpine and neuroleptic-induced VCMs (Gao et al. 1994; Kaneda et al. 1992; Peixoto et al. 2003). Importantly, there are also some human studies showing that GABA agonists can improve symptoms of TD (Morselli et al. 1985; Tamminga et al. 1979; 1983). However, literature data have also indicated the lack of effect of GABA agonist in human TD (Egan et al. 1997). Thus, the study of preparations, which could modulate both oxidative stress and GABAergic neurotransmission, can be considered of interest in the treatment of movement disorders.

Valerian root (*Valeriana officinalis* L., Valerianaceae) has been used for centuries as a calming and sleep-promoting herb (McCabe 2002; Morazzoni and Bombardelli 1995) and it is among the most widely used medicinal herbs (Fugh-Berman and Cott 1999). The mechanisms involved in the pharmacological and therapeutic activities of *V. officinalis* have not yet been completely clarified. Literature data have indicated that an increase in GABAergic transmission could dictate the therapeutic properties of this plant extract (Abourashed et al. 2004; Cavadas et al. 1995; Houghton 1999; Mennini et al. 1993). Recently, literature data have indicated that *V. officinalis* extracts exhibit antioxidant activity in different in vitro models (Malva et al. 2004; Sudati et al. 2009) and presented cytoprotective effect on an in vitro experimental model of PD (Oliveira et al. 2009). Furthermore, no toxicity of its use has been reported in humans or in rodents after chronic treatment with *V. officinalis* extracts (Fachinetto et al. 2007b; Tabach et al. 2009).

In this context, considering that *V. officinalis* has antioxidant properties and that the development of VCMs seems to involve oxidative stress participation, we have tested the effect of *V. officinalis* in a reserpine-induced VCM models in rats. Furthermore, we investigate the presence of a phenolic compound that has potential antioxidant properties (Ban et al. 2008; Pereira et al. 2009; Wu et al. 2009) and of valeric acid, which can be one of the components responsible for the pharmacological activities of *V. officinalis*.

Materials and methods

Animals

Male Wistar rats weighing 270–320 g and 3–3.5 months of age, from our own breeding colony, were kept in cages with three or four animals in each and with continuous access to foods and *V. officinalis* or its vehicle (ethanol 1%) in a room with controlled temperature (22 ± 3°C) and on a 12-h light/dark cycle with lights on at 7:00 a.m. The animals were maintained and used in accordance with the guidelines of the Brazilian Society of Association for Laboratory Animal Science (SBCAL).

Drugs

Reserpine (methyl reserpate 3,4,5-trimethoxybenzoic acid ester), gallic acid and valeric acid (minimum 99%) were obtained from Sigma (St. Louis, MO, USA). A standard tincture of *V. officinalis* (10 g of valerian roots per 100 mL of ethanol) was obtained from Bio extracts (São Paulo, Brazil).

In vitro assays

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 analyses were carried out in isocratic conditions using C-18 column (4.6 × 250 mm) packed with 5 μm-diameter particles, and the mobile phase was methanol:water (80:20 v/v) and 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 peak 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 HPLC (290 nm; injection volume = 5 μL; flow rate = 1 mL/min; column = C18; mobile phase = methanol: H_2O and 0.4% acetic acid) in comparison with a standard reference of gallic acid. All chromatographic operations were carried out at ambient temperature and in triplicate.

Preparation of cortical slices for in vitro assay

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

In vitro cell viability by tetrazolium salt method (MTT assay)

The viability assay was performed by the colorimetric 3(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method. Slices from the cortex of rats were pre-incubated at 37°C for 1 h in cerebral spinal fluid buffer (CSF) (pH 7.4) (1:10 (w:v)) in the presence or absence of *V. officinalis* (0–32 μg/mL) and of iron sulfate (10 μM). Immediately after preincubation, 0.5 mg/ml of MTT was added to the medium containing the slices, followed by incubation at 37°C for1 h. The formazan product generated during the incubation was solubilized in dimethyl sulfoxide (DMSO) and measured at 570 and 630 nm. Only viable slices are able to reduce MTT (Mosmann, 1983).

Ex vivo assays

Treatments

The rats were divided into four groups: the control group received acetic acid 0.1% (that was the reserpine vehicle, s.c.) and ethanol 1% (in the drinking water, it was *V. officinalis* vehicle); the *V. officinalis* group received acetic acid 0.1% (s.c.) and *V. officinalis* 1% (in the drinking water); the reserpine group received reserpine 1 mg/kg (s.c.) and ethanol 1% (in the drinking water); and the reserpine plus *V. officinalis* group received reserpine (s.c.) and *V. officinalis* 1% (in the drinking water). The number of animals in each group that received treatment was 6, 6, 8 and 7 for control, *V. officinalis*, reserpine and reserpine plus *V. officinalis* groups, respectively. *V. officinalis* treatment started 15 days before the administration of the reserpine. After 15 days of treatment with *V. officinalis*, two doses of reserpine or its vehicle were administered subcutaneously (s.c.) every other day (1 mg/kg, s.c.) as previously described by Burger et al. (2003; 2005a). *V. officinalis* was administered in the drinking water in a proportion of 1% (final concentration of 100 mg/mL). The dosage was calculated every week by the amount of water drunk assuming equal drinking among the four animals. Thus, each animal received *V. officinalis* extract in a dosage of about 200–250 mg/kg per day.

V. officinalis and its vehicle were given daily before the beginning of the dark cycle. A reduction in liquid intake among the groups was not observed (data not shown).

Behavioral analysis

Quantification of VCMs

Behavior measurement of VCMs was assessed before the treatment with reserpine or its vehicle (basal evaluation and

habituation section; data not shown). The effect of drugs on behavior was examined at the beginning of *V. officinalis* administration (before *V. officinalis* and reserpine treatment), after 15 days of *V. officinalis* (to detect any potential effect of *V. officinalis* intake on VCM) and after the two administrations of reserpine (after reserpine treatment). To quantify the occurrence of VCMs, rats were placed individually in cages (20 × 20 × 19 cm) and hand-operated counters were employed to quantify the frequency of VCMs. VCMs are defined as single mouth openings in the vertical plane not directed toward physical material. If VCMs occurred during a period of grooming, they were not taken into account. The behavioral parameters were measured continuously for 6 min after a period of 6 min of adaptation. During the observation sessions, mirrors were placed under the floor of the experimental cage to permit observation when the animal was faced away from the observer. Experimenters were always blind with regard to the treatment conditions.

Open-field test

To analyze possible changes in spontaneous locomotor activity caused by treatment with reserpine and/or *V. officinalis*, the animals were placed individually in the center of an open-field arena (40 × 40 × 30 cm) with black plywood walls and a white floor divided into nine equal squares, as previously described (Broadhurst 1960). The effect of drugs on behavior was examined at the beginning of *V. officinalis* administration (basal evaluation; data not shown), before reserpine administration (and after 15 days of *V. officinalis treatment*) and after reserpine treatment. The number of rearing, number of line crossings and the time of immobility were measured over 2 min and taken as an indicator of locomotor activity, as previously described (Fachinetto et al. 2007b). Sections of locomotor activity were evaluated immediately before the quantification of VCMs.

Tissue preparations

Rats were killed about 24 h after the last session of behavioral quantification (on the 4th day after the first administration of reserpine). The brains were immediately excised and placed on ice. The cortex, hippocampus, striatum and the region containing the substantia nigra were separated, weighed and homogenized in ten volumes (w/v) of 10 mM Tris–HCl, pH 7.4.

Oxidative stress parameters

To evaluate the levels of reactive oxygen species (ROS), the homogenates were centrifuged for 10 min at 1,500×*g*. Immediately after centrifugation, an aliquot of supernatant was used for 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) oxidation. DCFH-DA-oxidation was determined spectrofluorimetrically using 7 µM of DCFH-DA. Fluorescence was determined at 488 nm for excitation and 520 nm for emission. A standard curve was carried out using increasing concentrations of 2′,7′-dichlorofluorescein (DCF) incubated in parallel (Pérez-Severiano et al. 2004).

To assess lipid peroxidation, we quantified thiobarbituric acid reactive substances (TBARS). The homogenates were centrifuged for 10 min at 1,500×*g*. Immediately after centrifugation, an aliquot of 200 µl or of supernatant was incubated for 1 h at 37°C and then used for lipid peroxidation quantification as earlier described (Ohkawa et al. 1979).

To verify protein carbonyl, cortical and nigral tissues were homogenized in ten volumes (w/v) of 10 mM Tris–HCl buffer, pH 7.4. The protein carbonyl content was determined by the method described by Yan et al. (1995) with some modifications. Briefly, homogenates were diluted 1:8 in 10 mM Tris–HCl buffer, pH 7.4, and 1 ml of aliquots were mixed with 0.2 ml of 2,4-dinitrophenylhydrazine (10 mM DNPH) or 0.2 ml HCl (2 M). After incubation at room temperature for 1 h in dark, 0.5 ml of denaturing buffer (150 mM sodium phosphate buffer, pH 6.8, containing 3% SDS), 2 ml of heptane (99.5%) and 2 ml of ethanol (99.8%) were added sequentially, mixed with vortex agitation for 40 s and centrifuged for 15 min. After that, the protein isolated from the interface was washed two times with 1 ml of ethyl acetate/ethanol 1:1 (v/v) and suspended in 1 ml of denaturing buffer. Each DNPH sample was read at 370 nm against the corresponding HCl sample (blank), and total carbonylation calculated using a molar extinction coefficient of 22,000 M^{-1} cm^{-1} according to Levine et al. (1990).

The ATPase activity from brain regions was measured spectrophotometrically by determining the inorganic phosphate (Pi) released (Fiske and Subbarow 1925). Na^+/K^+-ATPase activity was calculated as the difference between the total Mg^{2+}-ATPase activity (samples without ouabain) and Mg^{2+}-ATPase activity determined in the presence 0.5 mmol/L of ouabain. Both activities were determined in the presence of 125 mmol/L NaCl and 20 mmol/L KCl.

Statistical analysis

Data from behavioral parameters were analyzed by one-way or two-way ANOVA or paired *t* test. Data from TBARS, ROS quantification, carbonyl content and cell viability were analyzed by one-way ANOVA, followed by Tukey post hoc test when appropriate. A possible relationship between oxidative stress parameters and VCM

were also determined using linear regression analysis. Results were considered significant when $p < 0.05$.

Results

HPLC analyses

HPLC analysis of *V. officinalis* extract revealed a peak with a retention time of 2.57 min, which corresponds to valeric acid (Fig. 1a,b). 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 (Fig. 1c,d).

Effect of *V. officinalis* on Fe(II)-induced cerebral cortex slices toxicity (cell viability)

Iron sulfate (10 µM) caused a significant decrease in cell viability relative to control and *V. officinalis,* at all tested concentrations (2, 8, 16 and 32 µg/mL). It was able to cause a concentration-dependent protection against cell toxicity provoked by Fe(II) in cortical brain slices (Fig. 2).

Effects of *V. officinalis* on reserpine-induced VCMs

Reserpine caused a marked increase on VCMs when compared with its vehicle ($p < 0.001$; Fig. 3). Furthermore, paired comparisons of VCM before and after reserpine within the same group revealed also a significant increase in VCM frequency after reserpine ($p < 0.05$; Fig. 3). Consumption of *V. officinalis* tincture had no effect on the frequency of VCM (after 15 days, which is indicated in the "before" panel in Fig. 3) or after the two doses of acetic acid solution (the vehicle of reserpine, which is indicated in the panel "after" in Fig. 3). Paired comparisons also did not indicate significant changes in the number of VCMs in the *Valeriana officinalis* group. In addition, *V. officinalis* prevented the increase in the incidence of VCM caused by reserpine. This result was also confirmed by paired comparisons within the group, i.e., the VCM scores after reserpine injection (panel "after" in Fig. 3) were similar to that obtained before reserpine (panel "before" in Fig. 3). Rats that received the two vehicles (control group) did not show any change in the VCM scores in the two measurements presented in Fig. 3 (paired comparison).

Effects of long-term treatment with *V. officinalis* and reserpine on locomotor activity in rats

Reserpine did not change locomotor activity, as assessed by the number of rearing, crossings and immobility in the

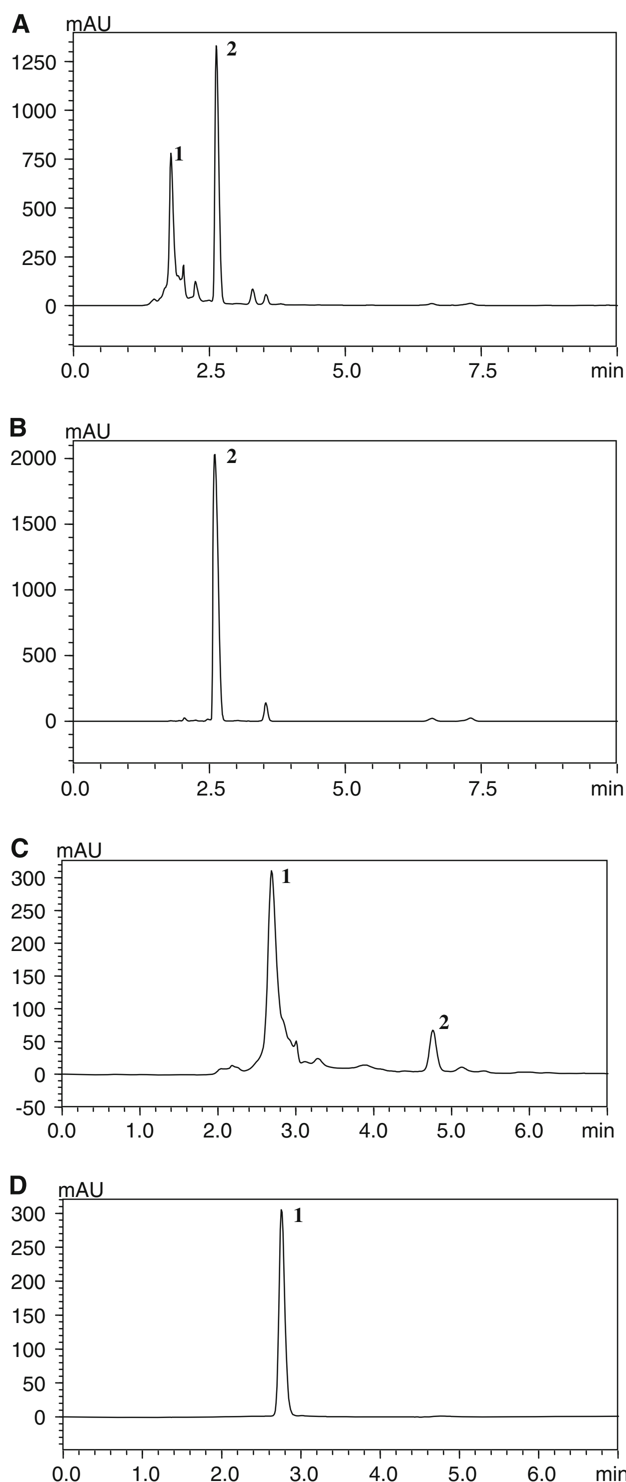

Fig. 1 **a** High performance liquid chromatography of *V. officinalis* tincture. 1 Represents an unknown peak; 2 corresponds to valeric acid peak. **b** Represents a high performance liquid chromatography of valeric acid (peak 2) used as standard reference. **c** Represents a high performance liquid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

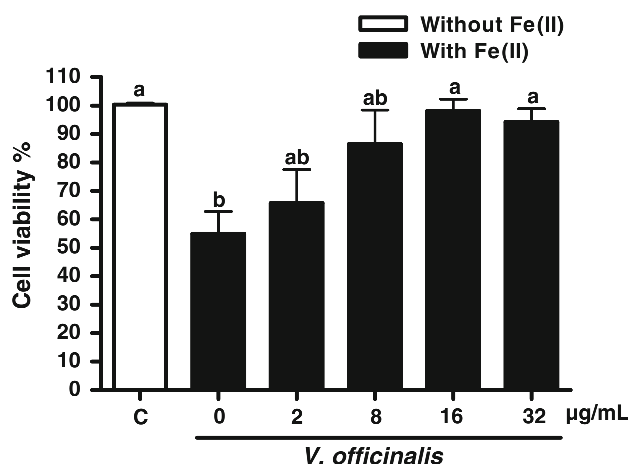

Fig. 2 Effect of *V. officinalis* (0–32 µg/mL) on iron sulfate (10 µM) induced a decrease in cell viability in slices from the cortex of rats. Values are presented as means ± SEM. One-way ANOVA followed by Tukey's post hoc test. Different symbols represent statistically significant differences

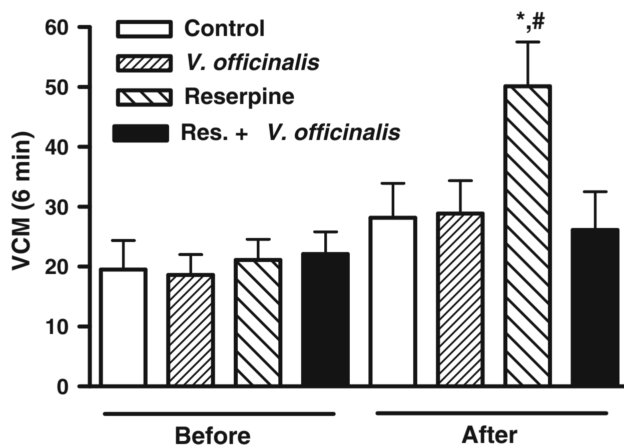

Fig. 3 Effects of *V. officinalis* on orofacial dyskinesia before and after treatment with reserpine. Number of vacuous chewing movements (VCM) during 6 min. Values are presented as means ± SEM (control, $n = 6$; *V. officinalis*, $n = 6$; reserpine, $n = 8$; reserpine + *V. officinalis*, $n = 7$). *Significant differences from control group (one-way ANOVA followed by Tukey's post hoc tests) and # represents significant differences in the same group of rats before treatment with reserpine (paired *t* test)

open-field test, when compared with the group treated with the two vehicles (control group) (Table 1), which is in accordance with literature data (Aguiar et al. 2009).

V. officinalis also did not cause change in locomotor activity alone or when administered concomitantly with reserpine, when compared with control and the reserpine group (Table 1).

Effects of reserpine and *V. officinalis* on oxidative stress parameters

Treatment with *V. officinalis* did not modify cortical, hippocampal, striatal or nigral TBARS levels, DCFH-DA oxidation or protein carbonyl levels. The activity of Na^+/K^+-ATPase was not modified by *V. officinalis* treatment. Similarly, reserpine treatment (alone or with *V. officinalis*) did not change the oxidative stress parameters (cortical, hippocampal, striatal or nigral TBARS levels, DCFH-DA oxidation and protein carbonyl levels) Na^+/K^+-ATPase activity was also not altered in the reserpine or *V. officinalis* + reserpine groups (Table 2).

However, a positive correlation between DCF production in the cortex and the number of VCMs ($r = 0.42$ and $p = 0.04$; Fig. 4a), and also a significant negative correlation between Na^+/K^+-ATPase activity in the *substantia nigra* and intensity of VCMs ($r = 0.41$ and $p = 0.05$; Fig. 4b) were observed for all the groups analyzed. The other correlations were not significant (data not shown).

Discussion

In the present study, we investigated the possible preventive effects of *V. officinalis*, a medicinal plant widely used to improve disturbances in sleep, against vacuous chewing movements (VCM) in rats. Considering this objective, we used an animal model of oral movement disorder induced by reserpine (Abílio et al. 2003; Bilska and Dubiel 2007; Castro et al. 2006; Naidu et al. 2004; Peixoto et al. 2005). However, this model is contradictory, because some authors consider reserpine model of VCMs as a model of OD (Abílio et al. 2002; 2003; 2004; Carvalho et al. 2003; Faria et al. 2005; Neisewander et al. 1991, 1994; Raghavendra et al. 2001), while others consider reserpine as a model of parkinsonism (Aguiar et al. 2009; Baskin and Salamone 1993; Paille et al. 2004b; Salamone and Baskin 1996;).

Here, we observed that reserpine treatment caused an increase in VCMs, which is in accordance with literature data (Burger et al. 2003; Castro et al. 2006; Busanello et al. 2011). Furthermore, we have demonstrated for the first time that co-treatment with *V. officinalis* can protect against VCMs in rats. These results contrast with previous data of our group where *V. officinalis* could not protect OD induced by haloperidol treatment (Fachinetto et al. 2007b). The discrepancy in both results reinforces the idea that reserpine treatment could represent a model of parkinsonism better than OD in rats. However, we cannot disregard that there were similar events between both models of neurotoxicity, which could explain the protective effect of

Table 1 Effects of reserpine and *V. officinalis* treatment on locomotor activity (Mean ± SEM)

Treatment time	Treatment	Locomotor activity		
		Rearing	Crossings	Immobility
Before	Control ($n = 14$)	6.78 ± 0.69	15.71 ± 2.14	47.71 ± 6.38
	V. officinalis ($n = 13$)	9.76 ± 1.50	17.92 ± 2.41	42.38 ± 6.62
After	Control ($n = 6$)	4.66 ± 1.96	9.83 ± 4.28	71.17 ± 15.99
	V. officinalis ($n = 6$)	4.83 ± 1.05	8.00 ± 1.06	71.67 ± 7.98
	Reserpine ($n = 8$)	6.37 ± 1.10	12.00 ± 2.39	44.38 ± 9.37
	Res. + *V. officinalis* ($n = 7$)	7.29 ± 1.49	18.71 ± 5.87	51.86 ± 8.95
Basal	Control ($n = 14$)	12.64 ± 1.08	20.50 ± 1.83	38.71 ± 4.92
	V. officinalis ($n = 13$)	12.31 ± 0.90	23.62 ± 1.68	33.77 ± 4.59
15 days	Control ($n = 14$)	6.78 ± 0.69	15.71 ± 2.14	47.71 ± 6.38
	V. officinalis ($n = 13$)	9.76 ± 1.50	17.92 ± 2.41	42.38 ± 6.62
18 days	Control ($n = 6$)	4.66 ± 1.96	9.83 ± 4.28	71.17 ± 15.99
	V. officinalis ($n = 6$)	4.83 ± 1.05	8.00 ± 1.06	71.67 ± 7.98
	Reserpine ($n = 8$)	6.37 ± 1.10	12.00 ± 2.39	44.38 ± 9.37
	Res. + *V. officinalis* ($n = 7$)	7.29 ± 1.49	18.71 ± 5.87	51.86 ± 8.95

Table 2 Effects of reserpine and *V. officinalis* treatment on oxidative stress parameters in the brain regions of rats (Mean ± SEM)

Brain regions	TBARS (nmol of MDA/g tissue)	H_2-DCF-oxidation (nmol of DCF/g tissue)	Protein carbonyl (nmol carbonyl/g tissue)	Na^+/K^+ ATPase activity (nmol Pi/g tissue)
Cortex				
Control	126.50 ± 25.90	74.55 ± 25.21	7.92 ± 0.58	28.75 ± 6.58
V. officinalis	110.70 ± 13.92	84.10 ± 17.60	11.12 ± 1.52	15.96 ± 3.29
Reserpine	124.20 ± 23.61	83.30 ± 18	7.90 ± 0.84	18.85 ± 2.72
Reserpine + *V. officinalis*	141.50 ± 26.39	67.47 ± 13.53	9.99 ± 2.04	21.58 ± 4.26
Hippocampus				
Control	79.93 ± 6.31	81.63 ± 32.27	8.11 ± 0.68	17.89 ± 3.35
V. officinalis	111.20 ± 24.48	81.75 ± 15.57	8.68 ± 1.10	12.36 ± 2.18
Reserpine	99.25 ± 22.70	72.65 ± 19.96	7.55 ± 1.54	12.38 ± 2.40
Reserpine + *V. officinalis*	99.40 ± 21.13	53.37 ± 5.88	10.28 ± 2.79	16.05 ± 3.02
Striatum				
Control	72.72 ± 7.25	62.38 ± 20.69	8.48 ± 0.75	11.75 ± 1.96
V. officinalis	118.10 ± 28.43	84.59 ± 15.61	8.66 ± 0.87	12.91 ± 2.76
Reserpine	116.9 ± 21.71	69.70 ± 13.87	7.52 ± 0.48	13.16 ± 2.50
Reserpine + *V. officinalis*	101.30 ± 18.76	58.25 ± 7.19	11.32 ± 2.43	14.2 ± 3.00
Substantia nigra				
Control	81.03 ± 4.16	89.03 ± 18.96	7.90 ± 0.98	21.06 ± 4.95
V. officinalis	104.80 ± 16.18	109.90 ± 26.95	10.13 ± 1.73	21.17 ± 4.82
Reserpine	103.10 ± 22.12	95.79 ± 18.87	8.91 ± 1.48	18.08 ± 4.53
Reserpine + *V. officinalis*	88.81 ± 13.51	76.23 ± 13.52	10.63 ± 2.06	24.85 ± 7.72

some compounds in both models (Burger et al. 2003; 2005b).

Since literature data have demonstrated that the development of VCMs is associated with oxidative stress (Abílio et al. 2003; Burger et al. 2003; Naidu et al. 2004), we tested *V. officinalis*, which has been shown to exhibit antioxidant properties in different in vitro models (Malva et al. 2004; Oliveira et al. 2009; Sudati et al. 2009). Thus, we investigated if the protective effect of *V. officinalis* was caused through oxidative stress. However, we have not found alterations in gross oxidative stress parameters after reserpine administration. The discrepancies between these results and previously published data (Burger et al. 2004; Teixeira et al. 2009) are difficult to explain, but may be

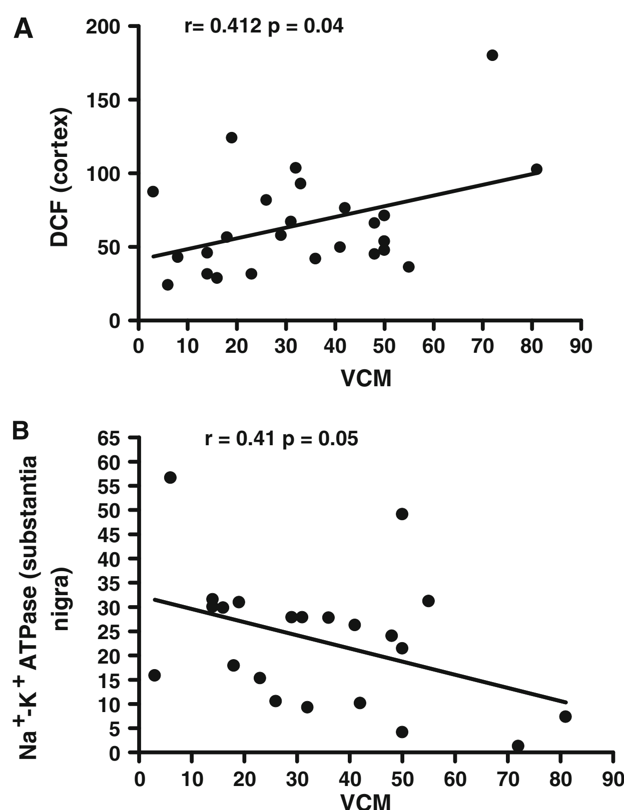

Fig. 4 **a** Linear regression analysis between ROS production in cortex and number of VCMs developed by acute treatment with reserpine. Significance was considered when $p < 0.05$. **b** Linear regression analysis between Na^+/K^+-ATPase activity in substantia nigra and intensity of VCMs developed by acute treatment with reserpine. Significance was considered when $p < 0.05$

related to the fact that oxidative stress after reserpine treatment could occur in specific areas and can spread to other brain areas depending on subtle factors that were not controlled in these studies. Furthermore, recently we have observed that fluphenazine and haloperidol, classical antipsychotic drugs that can also lead to VCMs in animal models, did not cause gross punctual changes in oxidative stress in different brain areas of rats (Fachinetto et al. 2007a, b). However, we have observed a positive correlation between cerebral DCFH-DA-oxidation and VCM frequency, indicating that ROS can play a role in OD development. We have also detected a negative correlation between VCM frequency with Na^+K^+-ATPase activity in *substantia nigra*. This SH-containing enzyme is a key component of cellular ion homeostasis and oxidative damage can cause its inactivation (Thevenod and Friedmann 1999). Since we have not observed significant changes in oxidative stress after the treatments with *V. officinalis* and/or reserpine, the negative correlation between Na^+K^+-ATPase activity and VCMs can be related to others factors not related to oxidative stress. However, we have previously observed a correlation between VCM and neurochemical parameters that can be considered as indirect markers of oxidative stress (Burger et al. 2005a).

We have observed a clear protective effect of *V. officinalis* against reserpine-induced development of VCMs. However, we did not observe significant alterations in locomotor activity as assessed by open-field test, which indicated a specific effect of *V. officinalis* on VCM.

The pharmacological activities of *V. officinalis* are attributed to its different constituents, including valepotriates (valtrate/isovaltrate and dihydrovaltrate) (Von der Hude et al. 1985), valerenic acid and valeric acid. We have confirmed the presence of some of these components, particularly valeric acid, which confirms the quality of the used tincture. Besides, we investigated one tentative mechanism by which the beneficial effects of *V. officinalis* has been linked to a potentiation of GABAergic transmission (Cavadas et al. 1995; Houghton 1999; Mennini et al. 1993; Ortiz et al. 1999; Santos et al. 1994). Of particular importance, literature data have indicated that pharmacological activation of GABAergic neurotransmission can reduce reserpine-induced development of VCMs in rodents (Araujo et al. 2005; Castro et al. 2006; Peixoto et al. 2003, 2005, White et al. 1997a, b). In this vein, *Withania somnifera* extracts can protect against reserpine-induced neurotoxic effects tentatively via a GABAergic activation (Kulkarni and Dhir 2008; Naidu et al. 2006).

Literature data have suggested that iron deposition associated with dopaminergic neurotransmission can be an important factor in the development of dyskinesias in different pathologies (Arreguin et al. 2009; Aisen et al. 1999; Qian et al. 1997; Swaiman 1991). The relatively high content of iron in basal ganglia can also contribute to worsen oxidative stress in this dopamine-rich region (Arreguin et al. 2009). Indeed, it has been postulated that iron and dopamine interaction in basal ganglia can be an important factor for the development of PD and TD syndromes (Aisen et al. 1999; Qian et al. 1997; Swaiman 1991). Thus, we have investigated the potential antioxidant effect of *V. officinalis* against Fe(II)-induced neurotoxicity in brain cortical slices and observed that Fe(II) caused a decrease in cell viability that was counteracted by *V. officinalis*, indicating an additional mechanism via which the extract could protect against the neurotoxic effect of reserpine.

The results presented here suggest that *V. officinallis* extracts can be a protective agent against reserpine-induced oral dyskinesia. In fact, *V.officinallis* extracts prevented VCMs induced by reserpine. Literature data have suggested that reserpine-induced OD can be used as a model of PD or TD (Abílio et al. 2002, 2003, 2004; Baskin and Salamone 1993; Carvalho et al. 2003; Faria et al. 2005; Neisewander et al. 1991, 1994; Paille et al. 2004b; Raghavendra et al. 2001; Salamone and Baskin 1996). In view of the fact that there are no effective treatments for

these pathologies, literature data should investigate whether the regular intake of *V.officinalis* could reduce the incidence of oral dyskinesia in patients using drugs that can increase the incidence of VCMs.

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