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Valeriana officinalis does not alter the orofacial dyskinesia induced by haloperidol in rats: Role of dopamine transporter

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Abstract

Chronic treatment with classical neuroleptics in humans can produce a serious side effect, known as tardive dyskinesia (TD). Here, we examined the effects of *V. officinalis*, a medicinal herb widely used as calming and sleep-promoting, in an animal model of orofacial dyskinesia (OD) induced by long-term treatment with haloperidol. Adult male rats were treated during 12 weeks with haloperidol decanoate (38 mg/kg, i.m., each 28 days) and with *V. officinalis* (in the drinking water). Vacuous chewing movements (VCMs), locomotor activity and plus maze performance were evaluated. Haloperidol treatment produced VCM in 40% of the treated rats and the concomitant treatment with *V. officinalis* did not alter either prevalence or intensity of VCMs. The treatment with *V. officinalis* increased the percentage of the time spent on open arm and the number of entries into open arm in the plus maze test. Furthermore, the treatment with haloperidol and/or *V. officinalis* decreased the locomotor activity in the open field test. We did not find any difference among the groups when oxidative stress parameters were evaluated. Haloperidol treatment significantly decreased [^{3}H]-dopamine uptake in striatal slices and *V. officinalis* was not able to prevent this effect. Taken together, our data suggest a mechanism involving the reduction of dopamine transport in the maintenance of chronic VCMs in rats. Furthermore, chronic treatment with *V. officinalis* seems not produce any oxidative damage to central nervous system (CNS), but it also seems to be devoid of action to prevent VCM, at least in the dose used in this study.

Keywords: Dopamine uptake; Haloperidol; Medicinal plant; Oxidative stress; Tardive dyskinesia; *V. officinalis*

1. Introduction

Neuroleptic drugs are used in the treatment of severe psychiatric disorders, especially schizophrenia. Haloperidol is a classical or typical neuroleptic that is widely used in the treatment of schizophrenic patients. Its pharmacological action involves the blockage of dopamine D_2 receptors (Creese et al., 1976). However, chronic use of haloperidol can be associated with the development of TD in 20–25% of the patients (Kane and Smith, 1982) and its prevalence increases strongly with age (Kane and Smith, 1982; Woerner et al., 1991; Yassa and Jeste, 1992). TD is characterized by involuntary and abnormal movements of the orofacial region, and sometimes, trunk and

Abbreviations: CNS, central nervous system; CSF, cerebro spinal fluid; DCF, 2',7'-dichlorofluorescein; DCFH-DA, 2',7'-dichlorodihydrofluorescein diacetate; DNPH, 2,4-dinitrophenylhydrazine; GABA, γ-aminobutyric acid; Halo, Haloperidol; OD, orofacial dyskinesia; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TD, tardive dyskinesia; Val, *Valeriana officinalis*; VCMs, vacuous chewing movements; *V. officinalis*, *Valeriana officinalis.*

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members musculature that can appear during treatment with neuroleptic drugs or after its withdrawal (Kane, 1995). The main clinical problem is that TD is irreversible in the majority of the cases (Casey, 1985; Crane, 1973; Jeste et al., 1979).

Although some hypotheses have been postulated to explain at molecular and cellular levels this syndrome, the exact mechanisms involved in TD development remain unclear. The classical hypothesis to explain TD development is that typical neuroleptics can develop dopaminergic supersensitivity after its chronic use (Andreassen and Jorgensen, 2000; Burt et al., 1977; Klawans and Rubovits, 1972; Rubinstein et al., 1990). The chronic blockage of dopamine receptors for these drugs can produce a compensatory increase in the number and the sensitivity of dopaminergic receptors, which could culminate in a hyperdopaminergic state and clinical symptoms, such as TD (Cavallero and Smeraldi, 1995; Kane, 1995). Besides receptors, the dopamine transporter (DAT) could be also implicated in TD development since dopamine uptake by DAT is the primary pathway for the clearance of extracellular dopamine and consequently for the regulation of the magnitude and duration of dopaminergic signaling (Beckman and Quick, 1998; Kahlig and Galli, 2003). In fact, it has been demonstrated that TD patients present reduced levels of DAT (Yoder et al., 2004). Others neurochemical hypothesis has been proposed for the development of TD during the last decades. They include disturbed balance between dopaminergic and cholinergic systems, dysfunction of striatonigral GABAergic neurons, excitotoxicity promoted by glutamate and overproduction of free radicals (Andreassen and Jorgensen, 2000; Cadet et al., 1986, 1987; Lohr, 1991).

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). Although its exact mechanism of action is not well understood, studies indicate that the CNS effect of valerian might occur through interaction with GABA, melatonin, adenosine or serotonin systems in the brain (Abourashed et al., 2004). These data suggests that *V. officinalis* could be useful for TD treatment since several of its pharmacological targets are related with TD development (Araujo et al., 2005; Fibiger and Lloyd, 1984; Morselli et al., 1985; Peixoto et al., 2004; Raghavendra et al., 2001; Rosengarten et al., 2006; Tamminga et al., 1979).

Thus, the aims of the present study were to investigate the possible action of the *V. officinalis* on a chronic model of OD induced by long-term treatment with haloperidol and also to investigate the role of dopamine uptake in maintenance of OD induced by chronic treatment with haloperidol in rats.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 270–320 g and with age from 3 to 3.5 months, from our own breeding colony were kept in cages of 3 or 4 animals each, 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 am. The animals were maintained and used in accordance to the guidelines of the Brazilian Association for Laboratory Animal Science (COBEA).

2.2. Drugs

Haloperidol decanoate was a gift from Cristália (São Paulo, Brazil). A standard tincture of *V. officinalis* (10 g of valerian roots per 100 mL of ethanol) was obtained from Bio extracts (São Paulo, Brazil).

2.3. Treatments

The rats were divided into four groups: control group received soy oil (that was the haloperidol vehicle, i.m.) and ethanol 1% (in the drinking water, that was *V. officinalis* vehicle); *V. officinalis* group received soy oil (i.m.) and *V. officinalis* 1% (in the drinking water); haloperidol group received haloperidol decanoate (i.m.) and ethanol 1% (in the drinking water); and haloperidol plus *V. officinalis* group received haloperidol decanoate (i.m.) and *V. officinalis* 1% (in the drinking water). The number of animals in each group that received treatment was 12, 14, 10 and 14 for control, *V. officinalis*, haloperidol and haloperidol plus *V. officinalis* groups, respectively. Haloperidol decanoate (a slow-releasing preparation of haloperidol) or its vehicle were administered intramuscularly (i.m.) every 28 days (38 mg/Kg, i.m.) that is equivalent to 1 mg/kg/day of unconjugated haloperidol. *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 about 200–250 mg/Kg/day.

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

V. officinalis treatment started 15 days before the administration of haloperidol. The treatment with haloperidol was carried out during 12 weeks concomitantly with *V. officinalis*.

2.4. Behavioral analysis

2.4.1. Quantification of VCMs

Behavior measurement of VCMs was assessed before the treatment with haloperidol or its vehicle (basal evaluation), as previously described. The effect of drugs on behavior was examined every 15 days beginning on the 15th day after the first haloperidol injection (that occurred on same day of the basal behavior) during a period of 12 weeks. To quantify the occurrence of VCMs, rats were placed individually in cages (20×20×19 cm) and hand operated counters were employed to quantify VCMs frequency. VCMs are defined as single mouth openings in the vertical plane not directed towards physical material. If VCMs occurred during a period of grooming they were not taken into account. The behavioral parameters of OD were measured continuously for 6 min after a period of

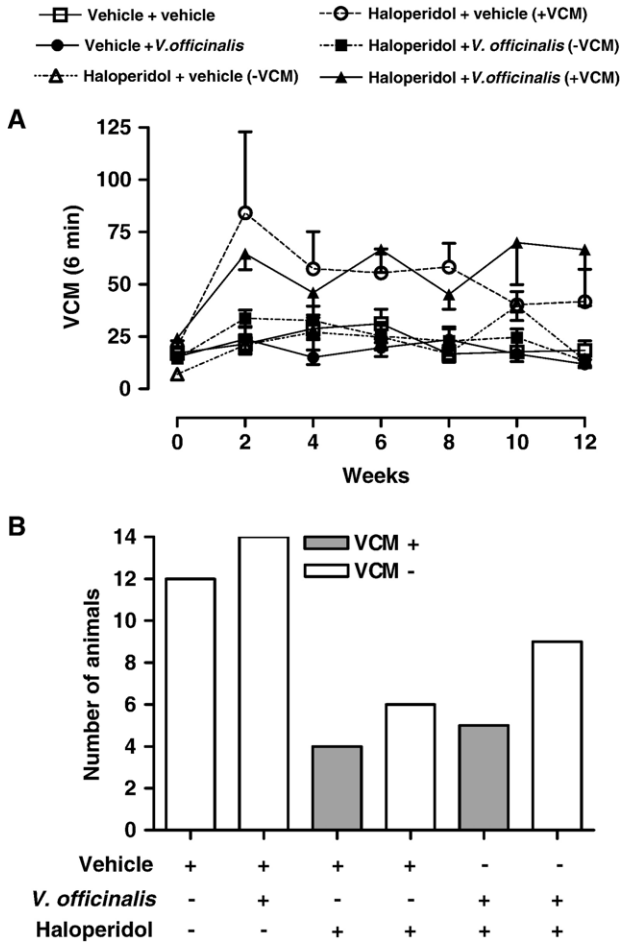

Fig. 1. Effects of *V. officinalis* on haloperidol-induced orofacial dyskinesia. A) Number of vacuous chewing movements (VCM) for 6 min during long-term treatment. Values are presented as means ± S.E.M. (Control, $n=12$; *V. officinalis*, $n=14$; haloperidol (−VCM), $n=6$; haloperidol (+VCM), $n=4$; haloperidol + *V. officinalis* (−VCM), $n=9$; haloperidol + *V. officinalis* (+VCM), $n=5$). B) Prevalence of VCMs in rats under long-term treatment with haloperidol and/or *V. officinalis*.

6 min 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 to treatments.

It was previously reported that the treatment with neuroleptic drugs does not result in the development of VCMs in all treated rats (Kane and Smith, 1982; Shirakawa and Tamminga, 1994). In the present study, we have also verified similar results about the prevalence of neuroleptic-induced VCMs. In our laboratory, control rats present maximally 40 VCMs during a period of 6 min. Thus, in this study, we analyzed the rats that developed neuroleptic-induced VCM (+VCM, more than 40 VCMs) separately from those that did not develop neuroleptic-induced VCM (−VCMs, less than 40 VCMs), as described previously (Andreassen et al., 2003; Egan et al., 1994; Shirakawa and Tamminga, 1994).

2.4.2. Open field test

To analyze changes in spontaneous locomotor activity caused by treatment with haloperidol 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 9 equal squares, as previously described (Broadhurst, 1960). The number of line crossings was measured over 2 min and taken as an indicator of locomotor activity.

2.4.3. Elevated plus maze

To evaluate the anxiety-like state caused by treatment with haloperidol and/or *V. officinalis*, animals were exposed to an elevated plus maze (Chopin et al., 1985; Pellow et al., 1985). The number of head dippings and the time spent into open or closed arms were recorded over a 2 min session. The percentage of the time spent on open arm and the percentage of the entries into the open arms were calculated, as follows: time spent or number of entries into the open arm/total time or total number of the entries into closed and open arm X 100, respectively.

2.5. Tissue preparations

Rats were killed about 24 h after the last session of behavioral quantification (on the 28th day after the last administration of haloperidol). The brains were immediately excised and put on ice. The cortex, striatum and region containing the substantia nigra were separated, weighed and homogenized in 10 volumes (w/v) of 10 mM Tris–HCl, pH 7.4. A portion of the striatum was dissected for slices used for the [^{3}H] dopamine uptake assay.

2.5.1. [^{3}H] dopamine uptake

[^{3}H] dopamine uptake was carried out as described by Holz and Coyle (1974) with some modifications. To measure [^{3}H] dopamine uptake, the striatum was cut into 400 μm slices, which were washed with a buffered solution (1) consisting of 127 mM NaCl, 1.2 mM Na_2HPO_4, 5.36 mM KCl, 0.44 mM

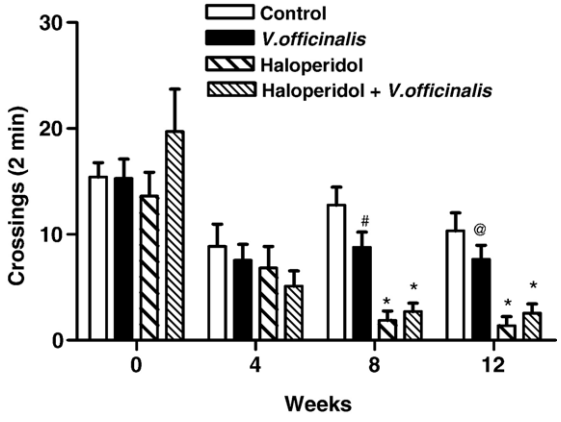

Fig. 2. Effects of *V. officinalis* on open field test in rats. Number of crossings in 2 min. Values of number of crossings are presented as means ± S.E.M. (Control, $n=12$; *V. officinalis*, $n=14$; haloperidol, $n=10$; haloperidol + *V. officinalis*, $n=14$). One way ANOVA followed by Duncan's multiple range tests. * represents significant differences from control group and # represents significant differences from control, haloperidol and haloperidol plus *V. officinalis* groups. @ represents significant differences from haloperidol and haloperidol plus *V. officinalis* groups.

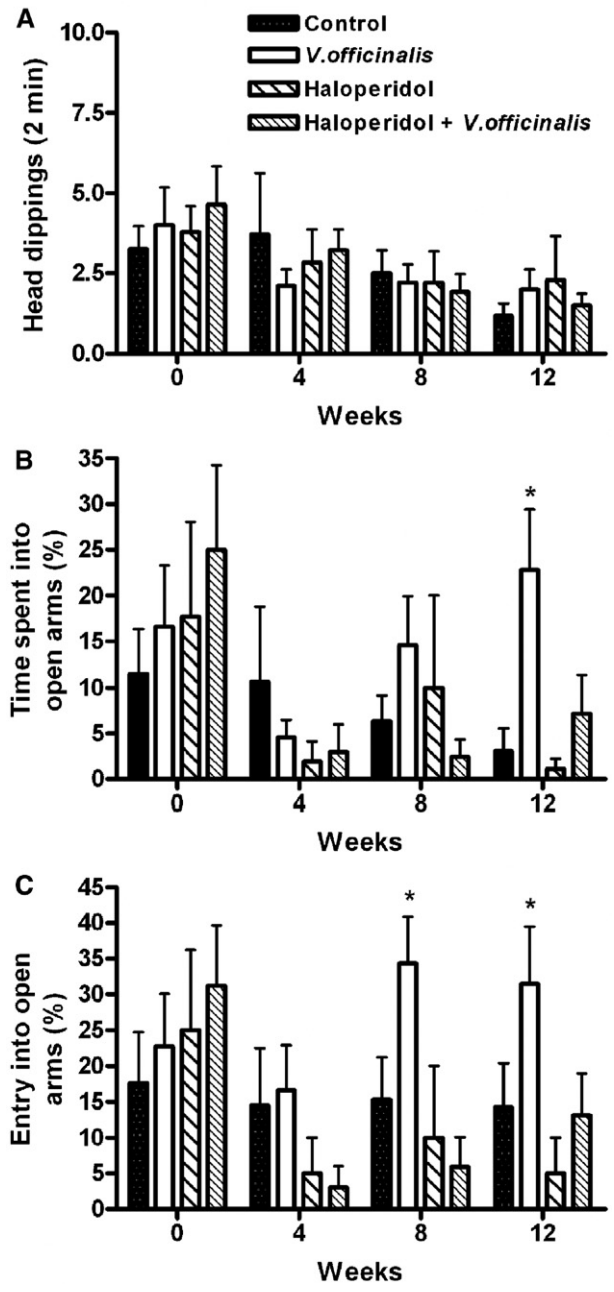

Fig. 3. Effects of *V. officinalis* and haloperidol on plus maze test in rats. A) Number of head dippings for 2 min, B) percentage of the time spent on the open arms for 2 min, C) percentage of entries into the open arms. Values are presented as means ± S.E.M. (Control, $n=12$; *V. officinalis*, $n=14$; haloperidol, $n=10$; haloperidol + *V. officinalis*, $n=14$). One way ANOVA followed by Duncan's multiple range tests. * represents significant differences among the groups into the same period of observation.

KH_2PO_4, 0.95 mM $MgCl_2$, 0.70 mM $CaCl_2$, 10 mM glucose, and 1 mM Tris–HCl, pH 7.4. Slices (0.2–0.3 mg protein) were further pre-incubated in 96 well-polycarbonate plates for 15 min at 35 °C with the buffered solution plus selegiline 1 μM. [^{3}H] dopamine was added to the incubation medium and uptake was carried out for 10 min at 35 °C, after which the reaction was stopped by five washes of 30 s each with 1 mL of iced-cold solution 1, containing 1 μM selegiline and 100 μM cocaine. Immediately after washing, 0.25 mL of 0.5 M NaOH and 0.2% sodium dodecyl sulfate (SDS) was added to the slices that were digested by 10 min incubation at 60 °C. Aliquots of the lysates were taken for protein content measurement by the Lowry et al. (1951) method. For determination of the intracellular amount of dopamine, liquid scintillation counting was used. Results were expressed as [^{3}H] dopamine uptake per mg of protein.

2.5.2. Oxidative stress parameters

To evaluate the levels of reactive oxygen species (ROS), the homogenates were centrifuged for 10 min at 1500 ×g. Just after the 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). The results were analyzed as percentage in relation to control group.

To assess lipid peroxidation, we quantified thiobarbituric acid reactive substances (TBARS). The homogenates were centrifuged for 10 min at 1500× g. Just after the 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 tissue were homogenized in 10 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 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 a dark ambient, 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, and 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).

To verify superoxide dismutase (SOD) activity, cortex, striatum or substantia nigra were adequately diluted to 40 volumes with Tris–HCl 10 mM (pH 7.5) and the assay was performed according to the method of Misra and Fridovich (1972). Briefly, epinephrine rapidly auto oxidizes at pH 10.2 producing adrenochrome, a pink colored product that can be detected at 480 nm. The addition of samples (10, 25, 50 μL) containing SOD inhibits the auto-oxidation of epinephrine. The rate of inhibition was monitored during 180 s at intervals of 30 s. The amount of enzyme required to produce 50% inhibition at 25 °C was defined as one unit of enzyme activity. The SOD activity was expressed as units/g of protein.

Table 1
Effects of haloperidol and *V. officinalis* treatments on oxidative stress parameters (mean±S.E.M; with control, $n=12$; *V. officinalis*, $n=14$; haloperidol (−VCM), $n=6$; haloperidol (+VCM), $n=4$; haloperidol+*V. officinalis* (−VCM), $n=9$; haloperidol+*V. officinalis* (+VCM), $n=9$) (Val, *V. officinalis* treatment; Halo, Haloperidol treatment; −VCM; +VCM)

Brain regions	Lipid peroxidation (nmol of MDA/g tissue)	ROS levels (% of control)	SOD activity (U/mg protein)	Protein carbonyl (nmol carbonyl/mg protein)
Cortex				
Control	95.5±5.7	112.0±16.5	377.5±39.6	26.8±3.0
Val	93.9±6.4	92.8±6.1	434.1±29.9	25.6±2.7
Halo−VCM	89.0±9.9	84.1±9.8	348.7±65.4	30.2±2.3
Halo+VCM	85.5±6.1	87.0±13.4	426.6±97.7	28.0±5.2
Val+Halo−VCM	104.5±8.2	88.0±5.5	382.0±60.8	33.0±3.6
Val+Halo+VCM	99.2±15.9	107.8±11.6	425.1±109.4	34.7±10.8
Striatum				
Control	62.2±6.7	107.5±16.7	722.7±77.1	–
Val	64.9±6.1	95.1±8.4	781.7±48.2	–
Halo−VCM	49.6±2.4	131.1±22.3	721.3±92.4	–
Halo+VCM	50.4±7.2	83.0±21.8	614.8±93.9	–
Val+Halo−VCM	55.9±7.2	103.7±8.9	653.8±92.2	–
Val+Halo+VCM	49.4±4.9	143.8±27.5	787.3±124.0	
Substantia nigra				
Control	88.9±10.5	75.1±9.0	388.8±56.4	29.7±3.4
Val	76.6±3.9	87.1±4.2	459.3±52.1	30.7±3.1
Halo−VCM	65.9±2.9	87.5±9.6	400.3±46.5	34.8±9.6
Halo+VCM	66.8±9.3	97.5±9.9	533.0±39.3	24.4±0.6
Val+Halo−VCM	82.2±9.8	89.1±4.2	433.8±59.4	30.5±3.3
Val+Halo+VCM	80.5±10.0	100.6±11.5	481.1±56.3	28.1±3.1

Protein content was measured by method of Lowry et al. (1951) and bovine serum albumin was used as standard.

2.6. Statistical analysis

Data from behavioral parameter were analyzed by one-way or two-way ANOVA. F values are presented in the text only if p value associated with it was <0.05. Prevalence data were analyzed by the Chi-square test. Data from TBARS, ROS quantification, SOD activity, carbonyl content and [^{3}H] dopamine uptake were analyzed by one-way ANOVA, followed by Duncan's Post Hoc tests when appropriate. A possible relationship between oxidative stress parameters, VCM, and [^{3}H] dopamine uptake were also determined using linear regression analysis using SPSS 10.1 for Windows. Significance was considered when $p<0.05$.

3. Results

3.1. Effects of V. officinalis on VCMs induced by long-term treatment with haloperidol

Haloperidol caused a marked increase on VCMs when compared with its vehicle ($F(5,44)=10.41$, $p<0.001$; Fig. 1A). In fact, a significant interaction between haloperidol and time treatment ($F(30,264)=2.27$ and $p<0.001$) was observed in this case. Treatment with haloperidol induced a VCMs prevalence of 40% compared to its vehicle (Chi-square=4.05; $p<0.05$; Fig. 1B), with 4 out of 10 animals actually having VCMs. The treatment with *V. officinalis* was not able to reduce neither the prevalence nor the intensity of VCMs in those rats that developed VCMs. In fact, the co-treatment of haloperidol with *V. officinalis* developed VCMs in 35.7% of the rats.

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

Haloperidol caused a marked and time-dependent decrease on locomotor activity, represented by the number of crossings in the open field test. In fact, a significant interaction between haloperidol x time treatment ($F(3,138)=12.12$ and $p<0.001$) was observed. *V. officinalis* administered alone also caused a significant decrease in locomotor activity only 8 weeks after haloperidol administration ($F(3,46)=15.43$ and $p<0.001$ (Fig. 2). The effect of concomitant treatment with *V. officinalis* and haloperidol was similar to haloperidol treated group.

3.3. Effects of long-term treatment with V. officinalis and haloperidol on plus maze test in rats

There was a significant effect of the time on head dippings ($F(3,138)=5,72$ and $p<0.05$; Fig. 3A). Long-term treatment with haloperidol did not cause any effect on head dippings in rats. Similarly, *V. officinalis* alone or with haloperidol also did not cause any effect on this parameter.

Long-term treatment with haloperidol did not cause any effect neither in the percentage of the time spent on open arm nor in the percentage of entries into the open arm when compared to

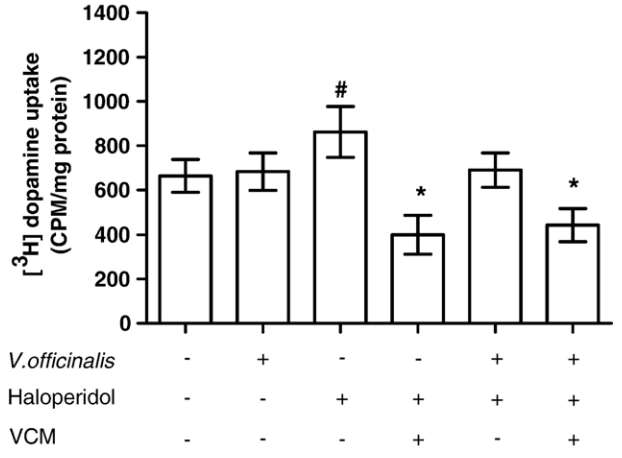

Fig. 4. Effects of long-term treatment with haloperidol and *V. officinalis* on [^{3}H] dopamine uptake (CPM/mg protein) in slices from striatum of rats. Data (mean ± SEM, with control, $n=12$; *V. officinalis*, $n=14$; haloperidol (−VCM), $n=6$; haloperidol (+VCM), $n=4$; haloperidol + *V. officinalis* (−VCM), $n=9$; haloperidol + *V. officinalis* (+VCM), $n=9$) were analyzed by one-way ANOVA followed by Duncan's multiple range tests. * represents significant difference from control group and # represents significant differences from animals treated with haloperidol (+VCMs).

control group (Fig. 3B and C). *V. officinalis* alone caused a significant increase in the percentage of the time spent on open arm 12 weeks after haloperidol administration (Fig. 3B). Furthermore, there was a significant difference of *V. officinalis* from other groups in the percentage of the entries into the open arm 8 and 12 weeks after haloperidol administration (Fig. 3C). Also, a significant effect of the time was observed in the percentage of time spent into the open arm ($F(3,138)=3.99$; $p<0.05$) and in the number of entries into the open arm ($F(3,138)=3.35$; $p<0.05$). The treatment with *V. officinalis* did not cause neither effect in the percentage of the time spent into open (Fig. 3B) nor in the percentage of entries into the open arm in rats treated concomitantly with haloperidol (Fig. 3C).

3.4. Effects of haloperidol and V. officinalis on oxidative stress parameters

There was no significant difference among the groups in DCFH-DA-oxidation levels, TBARS, carbonyl content groups and SOD activity in rats under long-term treatment with haloperidol and *V. officinalis* (Table 1).

3.5. Effects of haloperidol and V. officinalis on [^{3}H] dopamine uptake

Haloperidol treatment in association with VCM development decreased [^{3}H] dopamine uptake in striatal slices when compared to control group ($p<0.05$) (Fig. 4). *V. officinalis* co-treatment did not protect against haloperidol-induced [^{3}H] dopamine uptake reduction in those rats that developed VCM (Fig. 4). In rats co-treated with both drugs that did not develop VCM, the level of [^{3}H] dopamine uptake was similar to vehicle levels (Fig. 4). *V. officinalis* administration alone did not alter [^{3}H] dopamine uptake in rats.

4. Discussion

TD is a serious side effect caused by long-term treatment with neuroleptic drugs. Particularly, it is problematic due to its high prevalence and the lack of effective treatment. Our current study shows that *V. officinalis* was not effective in reducing OD prevalence or intensity in rats under chronic treatment with haloperidol. *V. officinalis* showed a significant effect in to maintain rats on the open arm of the elevated plus maze. The chronic treatment with *V. officinalis* and/or haloperidol did not cause any effect on oxidative stress parameters. Furthermore, the reduction in dopamine uptake in striatum seems to have an important role in the development of OD in rats, an effect not altered by chronic treatment with *V. officinalis*.

It has been demonstrated that long-term treatment with neuroleptic drugs is capable of producing OD in rats and TD in humans. However the mechanisms that can be involved are not clear. In the present study, we found that long-term treatment with haloperidol caused a prevalence of OD in 40% of treated rats. Accordingly, a previous study showed that chronic treatment with haloperidol develops significant OD 45–55% in rats with 6 months of treatment and approximately 65–75% after 12 months of treatment (Kaneda et al., 1992).

We have demonstrated that there was a significant reduction in dopamine uptake in the animals presenting OD in relation to the control group and group that did not develop OD. These results imply that chronic treatment with haloperidol could be causing an overflow of dopamine into the synaptic cleft of extrapyramidal dopaminergic neurons, which may be one of the possible mechanisms of typical neuroleptic-induced TD. Furthermore, *V. officinalis* could not prevent the reduction in dopamine uptake nor OD. In accordance with our findings, recent data from literature have demonstrated that haloperidol can decrease the striatal expression of dopamine transporter in rats (Saldaña et al., 2006). Several factors might explain the reduction of dopamine uptake in the striatum of rats presenting OD, including neurodegeneration of cells that uptake dopamine and alteration in dopamine transport function. Moreover, it has been shown that some neuroleptics, including haloperidol, can directly interact with and inhibit the dopamine transporter in vitro (Lee et al., 1997).

Literature data have shown that oxidative stress can decrease the activity of dopamine transporters (Hashimoto et al., 2004; Huang et al., 2003). Thus, we investigated oxidative stress parameters in this model of OD. A hypothesis has postulated that free radicals could have an important role in the development of TD (Lohr et al., 1990, 2003). In humans, there are some studies showing that patients with TD had an increase in oxidative stress parameters in plasma and cerebral spinal fluid (CSF) (Brown et al., 1998; Lohr et al., 1990; Pall et al., 1987). In rats, acute OD has been related to an increase in oxidative stress parameters (Abílio et al., 2004; Andreassen et al., 2003; Burger et al., 2005a, b; Faria et al., 2005; Naidu et al., 2003) and treatment with antioxidant substances seems to be efficacious to reduce OD (Burger et al., 2003, 2004, 2005a; Naidu et al., 2003; Singh et al., 2003). However, oxidative stress could be important in beginning of the events that culminate in OD. In fact, it was detected increase in OD and oxidative stress in several brain regions one month

after haloperidol treatment in rats (Burger et al., 2005a; Naidu et al., 2003). On the other hand, we have detected increased OD, but not oxidative stress in the same brain regions 7 months after haloperidol treatment (Fachinetto et al., 2005). Here, we did not find any alteration in oxidative stress parameters evaluated after 3 months of neuroleptic treatment, suggesting that oxidative stress seems to be involved in the development of acute OD (Abílio et al., 2004; Andreassen et al., 2003; Burger et al., 2005a,b; Faria et al., 2005; Naidu et al., 2003) but not in the maintenance of chronic OD. Accordingly, Shivakumar and Ravindratnath (1993) have shown that the treatment with haloperidol induced oxidative stress up to 1 month after the administration. However, after this period, authors did not find changes in markers of oxidative stress in mice brain up to 3 months of haloperidol treatment. Furthermore, some studies have demonstrated no correlation between oxidative stress and OD or TD (Boomershine et al., 1999; Sachdev et al., 1999; Tsai et al., 1998).

Although the etiology of TD is unclear, reduction in GABA is thought to be important in this syndrome. In fact, it has been described a decrease in the GAD activity and in the levels of GABA in brain regions of monkeys with dyskinetic symptoms induced by neuroleptics (Gunne et al., 1984). The mechanism of action of *V. officinalis* seems to be related with the potentiation of GABAergic transmission via direct and/or indirect agonist effect (Mennini et al., 1993; Ortiz et al., 1999; Santos et al., 1994). Considering these effects, *V. officinalis* could be efficacious against TD. However, *V. officinalis* treatment was not able to alter the prevalence or the intensity of haloperidol-induced OD, at least in the dose used in this experiment.

V. officinalis is clinically used to relieve anxiety and improve symptoms of insomnia (Della Loggia et al., 1981; Kennedy et al., 2006; McCabe, 2002; Morazzoni and Bombardelli, 1995; Oliva et al., 2004; Sakamoto et al., 1992). Thus, we investigated the effects of *V. officinalis* in the locomotor activity and anxiety-like behavior to evaluate if the treatment was capable of producing pharmacological effects. Supporting the effectiveness of treatment used here, *V. officinalis* was able to produce hypolocomotion and anxiolytic-like effect in the treated rats when assessed in open field and plus maze tests 8 weeks after the beginning of the treatment with haloperidol. It has been reported that anxiolytic effects appears acutely in response to drugs (Carobrez and Bertoglio, 2005). However, Vorbach et al. (1996) reported that approximately 2–4 weeks of therapy with valerian is needed to achieve significant improvements in sleep disturbances. In our study, probably because we used a low and nontoxic dose of *V. officinalis*, the anxiolytic-like effect of this herb appeared only 8 weeks after haloperidol administration. Of particular importance to select the dose used in this study, we have considered the fact that the treatment with a dose of 500 mg/kg of *V. officinalis* during 7 has caused oxidative stress in liver of mice (Al-Majed et al., 2006). Thus, as we used a chronic model, our dose was 200–250 mg/kg to avoid signals of toxicity. In fact, an important finding of our study was that the chronic treatment with *V. officinalis* did not cause any alteration on oxidative stress parameters neither in the CNS nor in liver and kidney (F.A.A Soares, unpublished data). More studies must be carried out to elucidate the toxic potential of *V. officinalis* treatment. However, further studies must be carried out to elucidate the exact mechanisms through haloperidol treatment reduces dopamine uptake.

5. Conclusion

Taken together, our data suggest that the oxidative stress seems not to have an important role in maintenance of OD. Moreover, a mechanism involving the reduction of dopamine transport related with the maintenance of chronic OD in rats can be involved. Therefore, the chronic treatment with *V. officinalis* seems not produce any oxidative damage to CNS. However, *V. officinalis* seems not effective in preventing or treating OD in rats.

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