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Antidepressant-like effect of *Valeriana glechomifolia* Meyer (Valerianaceae) in mice

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ABSTRACT

The antidepressant-like effect of a supercritical CO_2 ($SCCO_2$) *Valeriana glechomifolia* extract enriched in valepotriates was investigated in a mice tail suspension test (TST) and forced swimming test (FST). The $SCCO_2$ extract decreased mice immobility in the FST (0.5–20 mg/kg p.o.) and elicited a biphasic dose–response relationship in the TST (1–20 mg/kg p.o.) with no alterations in locomotor activity and motor coordination (assessed in the open-field and rota-rod tests, respectively). The anti-immobility effect of the $SCCO_2$ extract (5 mg/kg, p.o.) was prevented by mice pre-treatment with yohimbine (1 mg/kg, i.p., an α2 adrenoceptor antagonist), SCH 23390 (15 μg/kg, s.c., D_1 dopamine receptor antagonist) and sulpiride (50 mg/kg, i.p., D_2 dopamine receptor antagonist). However, mice pre-treatments with prazosin (1 mg/kg, i.p., α1 adrenoceptor antagonist) and *p*-chlorophenilalanine methyl ester (4×100 mg/kg/day, i.p., a serotonin synthesis inhibitor) were not able to block the anti-immobility effect of the $SCCO_2$ extract. Administration (p.o.) of the $SCCO_2$ extract (0.25 mg/kg) and imipramine (10 mg/kg), desipramine (5 mg/kg) and bupropion (3 mg/kg) at subeffective doses significantly reduced mice immobility time in the FST. These data provide the first evidence of the antidepressant-like activity of *V. glechomifolia* valepotriates, which is due to an interaction with dopaminergic and noradrenergic neurotransmission.

1. Introduction

Depression is a high prevalence mood disorder (Berton and Nestler, 2006) associated with a heavy social burden (Greenberg et al., 2003). The neurobiology of this disease is still uncompletely understood but the role of monoaminergic system is accepted (Nutt, 2008). In line with this, the antidepressants currently available boost the synaptic action of one or more of the three monoamines (serotonin, noradrenalin and dopamine) by blocking the neuronal reuptake of these neurotransmitters (Lambert et al., 2000; Stahl, 2008). Nevertheless, the pharmacotherapy employed in the treatment of depression produces several adverse side effects (Brunello et al., 2002) and a significant number of individuals do not respond to any antidepressant (Berton and Nestler, 2006; Nestler et al., 2002). Thus, there is a need to search for new compounds and strategies of treatment that could improve conventional therapies. In this context, natural products research has been considered as an option for the development of drugs with innovative mechanisms of action and/or conceivably minimized adverse side effects (Wang et al., 2008).

Among alleged neuroactive natural products, *Valeriana* genus is widely studied, with special focus on anxiolytic properties (Hattesohl et al., 2008; Murphy et al., 2010). Besides being the most popular herbal supplement for the treatment of anxiety and mild sleep disorders, preclinical studies reported the antidepressant-like activity of *V. officinalis* (Hattesohl et al., 2008), *V. wallichii* (Sah et al., 2011; Subhan et al., 2010) and *V. prionophylla* (Holzmann et al., in press). Moreover, the clinical efficacy of these species in improving depression symptoms was also demonstrated (Bhattacharyya et al., 2007).

The molecular mechanisms responsible for these effects have not been completely elucidated, although some studies point to a stimulation of GABA neurotransmission underlying anxiolytic properties (Cavadas et al., 1995; Mennini et al., 1993; Ortiz et al., 2004).

Valeriana contains over 135 chemical constituents (Wang et al., 2010), comprising the volatile oil, flavonoids (Marder et al., 2003) and valepotriates (epoxy iridoid esters) (Backlund and Moritz, 1998). The identity of bioactive substances is somewhat controversial but there is evidence that valepotriates substantially contribute to the pharmacological properties of plants belonging to this genus. Mennini et al. (1993) demonstrated that diidrovaltrate binds to a barbiturate site of a $GABA_A$ receptor and, therefore, contributes to the

Abbreviations: ANOVA, Analysis of variance; FST, Forced swimming test; i.p., Intraperitoneal; OFT, Open field test; pCPA, *p*-chlorophenilalanine methyl ester; p.o., Per os; $SCCO_2$, Supercritical carbon dioxide; SCH23390, R-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride; S.E.M, Standard error of the mean; TST, Tail suspension test.

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pharmacological activity of the *Valeriana* genus. On the other hand, Lacher et al. (2007) showed that isovaltrate is a potent inverse agonist of adenosine A1 receptors, which may counteract the desired sedative effect of the genus. Estrada-Soto et al. (2010) demonstrated that a hydroalcoholic extract from *V. edulis* ssp. *procera* enriched in valepotriates relaxes isolated rat aorta by blocking calcium channels and previous reports have shown that isovaltrate and valtrate cause a suppression of rhythmic contractions in guinea-pig ileum *in vivo* and *in vitro* (Hazelholff et al., 1982). The citotoxic effects of valepotriates *in vitro* were already demonstrated (Bounthanh et al., 1983; Lin et al., 2009). However, *in vivo* experiments have not demonstrated toxicity in rats orally administered with valepotriates (Tufik et al., 1994). In summary, literature data regarding the pharmacological activity of valepotriates are not very extensive and present some dissonant results. Thus, further studies on the central effects of these compounds are needed.

Among the species native to southern Brazil, *V. glechomifolia* is the most studied, and is the only one that accumulates valepotriates in both aerial and subterranean parts (Silva et al., 2002). Although this species is not used in traditional medicine, Maurmann et al. (2009) demonstrated that a valepotriate fraction of *V. glechomifolia* has sedative and anxiolytic effects and induces alterations in mice recognition memory. Recently, Bettero et al. (2011) demonstrated that valtrate, acevaltrate and 1-β-acevaltrate from *V. glechomifolia* show inhibitory activity toward Na^+ K^+ ATPase from rat brain *in vitro*. This could be particularly relevant since this enzyme activity is changed in mood disorders (El-Mallakh and Wyatt, 1995) and the antidepressant imipramine also inhibits its activity in rat cortex *in vitro* (Zanatta et al., 2001).

Considering that the antidepressant-like effect of *V. glechomifolia* has not been explored yet and central effects of valepotriates are not completely ascertained, the aim of this study was to investigate the potential antidepressant effect of a $SCCO_2$ *V. glechomifolia* extract enriched in valepotriates by using tail suspension and forced swimming tests in mice, which are animal models used to screen new antidepressant drugs. The contribution of monoaminergic neurotransmission (serotonergic, noradrenergic and dopaminergic) to the antidepressant-like effect was also investigated.

2. Materials and methods

2.1. Animals

Male CF1 mice (25–30 g) were purchased from Fundação Estadual de Produção e Pesquisa em Saúde, RS (Brazil). The animals were housed in plastic cages (17×28×13 cm), five mice per cage, and kept under a 12 h light/dark cycle (lights on between 7:00 h and 19:00 h) at constant temperature of 23°±1 °C with free access to standard certified rodent diet and tap water. All behavioral experiments were approved by The Animal Care Local Ethical Committee (CEUA-UFRGS; projects approval number 19022) and performed according to the European Communities Council Directive of 24 November 1986 (86/609/EEC). In this study, 570 animals were used. Except for rota-rod experiments, all animals were used only once.

2.2. Plant material

Plants of *V. glechomifolia* Meyer (Valerianaceae) were harvested during its flowering stage, in São José dos Ausentes, Rio Grande do Sul state, Brazil, in 2009, identified by Dr. M. Sobral (Universidade Federal de São João del-Rei, Minas Gerais, Brazil) and a voucher specimen (Sobral, 7733) was deposited in the Herbarium of the Universidade Federal do Rio Grande do Sul (ICN). The plant material was freeze-dried, powdered and kept frozen until analyzed.

2.3. Chemicals and drugs

For the extract characterization, grade HPLC acetonitrile and methanol were purchased from Merck® (Germany). For the behavioral experiments, the following drugs were used: fluoxetine, sulpiride and imipramine hydrochloride from Galena® (Porto Alegre, Brazil), bupropion from Eurofarma® (Brasil) and *p*CPA, SCH23390, prazosin, yohimbine and desipramine which were purchase from Sigma Chemical Co. (St. Louis, MO, USA). All drugs were dissolved in normal saline and the extract was suspended in saline with 1% of polysorbate 80 (vehicle) prior to use. Drug concentrations in saline solution were: imipramine—2 mg/ml and 1 mg/ml; fluoxetine—3 mg/ml and 1.5 mg/ml; desipramine—0.5 mg/ml; bupropion—0.3 mg/ml; SCH23390—0.5 mg/ml; sulpiride—5 mg/ml; prazosin and yohimbine—0.1 mg/ml and *p*CPA—10 mg/ml. The $SCCO_2$ extract was suspended in vehicle at different concentrations depending on the dose required: 2 mg/ml, 1 mg/ml, 0.5 mg/ml, 0.1 mg/ml, 0.05 mg/ml and 0.025 mg/ml.

2.4. Supercritical extraction

2.4.1. Apparatus

The supercritical extraction was performed in a Pilot Equipment (Cassel et al., 2010). The $SCCO_2$ flowed at rate of 6.67×10^{-4} kg s^{-1} through the extraction vessel. The extraction vessel was supplied with a heating jacket and an automated temperature controller, and heating tapes were used throughout the apparatus to maintain constant temperature in the extraction section. To ensure constant and steady solvent delivery the pump head was cooled by a circulating fluid, which passed through a chiller. Flow rates and accumulated gas volumes passing through the apparatus were measured using a flowmeter assay, 1–300 g/min (Thar 06618-2, USA). Ke (USA) micrometering valves (VC1) were used for flow control throughout the apparatus. Heating tapes with automated temperature control were also used around this valve to prevent both freezing of the solvents and solid solute precipitation following depressurization. Pressure in the extractor was monitored with a digital transducer system, Novus 8800021600, acquired from Novus Produtos Eletrônicos (Brazil) with a precision of ±1.0 bar. The temperature controller (TC) was connected to thermocouples (PT-100), with an accuracy of 0.5 K.

2.4.2. Extract preparation

Powdered aerial and underground plant material (120 g dry weight) was used and the extraction was conducted at constant temperature (40 °C) and pressure (90 bar). The extract was collected every 30 min and taken to a circulating air oven (40 °C), until the complete evaporation of the water condensed in the extract due to Joule–Thompson cooling. The $SCCO_2$ extraction yield recovery was 2.72 g%.

2.5. Determination of diene valepotriates content

Diene valepotriate characterization and quantification of the extract were performed by HPLC analysis, using the method previously validated by Silva et al. (2002) using a Shimadzu HPLC system (LC-6AD pump, SPD-20AV UV detector and automatic injector SIL-20 AC). A Waters Nova-Pack C18 column (4 μm, 3.9×150 mm i.d. with Waters Nova-Pack C18 guard column, 60 Å, 3.9×20 mm) was the stationary phase; the mobile phase consisted of acetonitrile and water as isocratic solvent (50:50 v/v); with a flow rate of 1 mL min^{-1} and detection at 254 nm. Valtrate, the valepotriate found in larger quantities in *V. glechomifolia*, was used as external standard. For the calibration curve achievement, valtrate was isolated from the extract through column chromatography, with silica gel stationary phase and mobile phase gradient with *n*-hexane: dichloromethane. The fractions obtained were monitored by thin layer chromatography, with standard valtrate, gathered and taken to dryness in a rotary evaporator. Linearity analysis of the calibration