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## Skeletal muscle relaxant effect of a standardized extract of *Valeriana officinalis* L. after acute administration in mice



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### ABSTRACT

*Valeriana officinalis* L. root extracts are traditionally taken for their sedative and anxiolytic properties and are also used for muscle relaxation. Relaxant effects were clearly observed on smooth muscle whereas data on effects on skeletal muscle are scarce and inconsistent. The aim of this study was to assess whether a standardized extract (SE) of *V. officinalis* had myorelaxant effects by decreasing skeletal muscle strength and/or neuromuscular tone in mice. Mice received an acute dose of *V. officinalis* SE (2 or 5 g/kg per os) or tetrazepam (10 mg/kg ip), a standard myorelaxant drug. Thirty minutes later, the maximal muscle strength was measured using a grip test, while global skeletal muscle function (endurance and neuromuscular tone) was assessed in a wire hanging test. Compared to tetrazepam, both doses of *V. officinalis* SE induced a pronounced decrease in skeletal muscle strength without any significant effects on endurance and neuromuscular tone. This study provides clear evidence that the extract of *V. officinalis* tested has a relaxant effect on skeletal muscle. By decreasing skeletal muscle strength without impacting endurance and neuromuscular tone, *V. officinalis* SE could induce less undesirable side effects than standard myorelaxant agents, and be particularly useful for avoiding falls in the elderly.

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## 1. Introduction

Native from Europe and North Asia, *Valeriana officinalis* L. is a perennial plant with thick roots often used as an herbal supplement for its anxiolytic and sedative properties.<sup>1,9,25</sup> The European Medicine Agency (EMA) deemed that dry ethanol root extracts of *V. officinalis* could be used for the relief of mild nervous tension and sleep disorders on the basis of their ‘well-established use’ (i.e. bibliographic data are available) and that other valerian extracts could be used for the relief of mild symptoms of mental stress and

to aid sleep on the basis of their ‘traditional use’ (>30-year use).<sup>12</sup> Besides these anxiolytic and sedative effects, *V. officinalis* was also shown to have myorelaxant properties. *V. officinalis* is used as a spasmolytic to treat gastrointestinal spasms and menstrual cramps.<sup>18,19</sup> Relaxant effects were observed on smooth muscle such as guinea pig ileum, vascular and bronchial muscle and isolated human uterine muscle.<sup>13,6,10,19</sup> As regards to the skeletal muscular system, *in vivo* studies provided inconsistent results concerning the capability of *V. officinalis* extracts to affect locomotor activity<sup>24,16,15,14</sup> and the only study we found that assessed relaxant properties on the skeletal muscle reported negative results.<sup>14</sup> The objective of this study was to evaluate the relaxant effects of a standardized extract of *V. officinalis* on skeletal muscle after acute administration in mice, in comparison with tetrazepam, a benzodiazepine known to have robust myorelaxant properties.

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### Abbreviations

DMSO	dimethyl sulfoxide
GABA	gamma–amino butyric acid
HRE	hydroethanolic root extract
HPLC	high performance liquid chromatography
HPTLC	high performance thin layer chromatography
UHPLC	ultra-high performance liquid chromatography
SE	standardized extract

## 2. Material and methods

### 2.1. Preparation of the liquid hydroethanolic root extract of *V. officinalis*

Roots of *V. officinalis* were harvested in north-western France in October 2013.

The hydroethanolic root extract (HRE) of *V. officinalis* was obtained according to the patented process WO2001056584A1. *V. officinalis* frozen fresh roots were crushed, then extracted with 20–70% (v/v) ethanolic leaching. The extract was concentrated under reduced pressure to evaporate ethanol. Final concentration is adjusted by adding glycerine to obtain a 0.01–0.02% (w/v) sesquiterpenic acid range. Batch used in this study (batch no. 111) contained glycerine (80%) with a dry drug: dry genuine extract ratio of 4:1. After the addition of glycerine, the HRE is a standardized extract (SE) of *V. officinalis* (EPS *V. officinalis* PiLeJe Laboratoire, France). To ensure a consistent quality and reproducible biological properties of our extracts, at each extraction, sesquiterpenes content is systematically determined using high performance liquid chromatography (HPLC) adapted from the European Pharmacopoeia 8.0 for valerian dry root monograph (07/2010:0453).

### 2.2. Chromatographic analyses of *V. officinalis* HRE for valerenic acid and derivatives

To further characterize the extract in terms of its content in valerenic acid and derivatives, the HRE of *V. officinalis* was analysed by high performance liquid chromatography (HPLC), mass spectrometry and high performance thin layer chromatography (HPTLC).

#### 2.2.1. HPLC and mass spectrometry

The HPLC analyses were performed on an Agilent Technologies 1260 Infinity using a Nucleodur C18 ec column (100Å, 4 × 250 mm; 5 µm). The mobile phase was a mixture of 0.1% (v/v) formic acid in water (phase A) and 0.1% (v/v) formic acid in acetonitrile (phase B). The gradient of phase A was 100% (0–9 min), 100%–90% (10–15 min), 90%–0% (15–45 min) and then was held at 0% for 10 min. The flow rate was 0.8 mL/min, and the injection volume 5 µL.

Mass analyses were performed on a Ultimate 3000 RSLC ultra-high performance liquid chromatography (UHPLC) system (Thermo Fisher Scientific Inc., MA, USA) coupled to a quaternary rapid separation pump (Ultimate autosampler) and a rapid separation diode array detector. Compounds were separated on a Kinetex EVO C18 column (2.1 × 100 mm; 1.7µm; Phenomenex, CA, USA), which was controlled at 30 °C. The mobile phase was a mixture of 0.1% (v/v) formic acid in water (phase A) and 0.1% (v/v) formic acid in acetonitrile (phase B). The gradient of phase A was 95% (0 min), 95%–1% (0–7.5 min), 1% (7.5–8.5 min) 1%–95%

(8.5–9 min) and then was held at 95% for 2 min. The flow rate was 0.45 mL/min, and the injection volume was 5 µL. The UHPLC system was connected to an Orbitrap (Thermo Fisher Scientific Inc., MA, USA) mass spectrometer, operated in negative electrospray ionization mode. Source operating conditions were: 3 kV spray voltage; 320 °C heated capillary temperature; 400 °C auxiliary gas temperature; sheath, sweep and auxiliary gas (nitrogen) flow rate 50, 10 and 2 arbitrary units, respectively; and collision cell voltage between 10 and 50 eV. Full scan data were obtained at a resolution of 70,000 whereas MS<sup>2</sup> data were obtained at a resolution of 17,500. Data were processed using Xcalibur software (Thermo Fisher Scientific Inc., MA, USA).

The quantity of valerenic acid and its derivatives is known to be low in aqueous ethanolic extracts due to slight solubility.<sup>3</sup> For that reason, to identify them, we had to re-extract the HRE (after elimination of the solvent) with an organic solvent (dichloromethane [DCM]) in which the compounds are more soluble.<sup>6</sup> The resulting fraction was analysed by HPLC and mass spectrometry.

Compounds present in the HRE were characterized according to their retention times, mass spectral data and compared with authentic standards.

#### 2.2.2. HPTLC

Analyses were performed using 0.20 mm silicagel 60 F254 (20 × 10 cm) glass HPTLC analytical plates (Merck, Darmstadt, Germany). Material included a Camag HPTLC system (Muttentz, Switzerland) equipped with an automatic TLC sampler (ATS 4), an automatic developing chamber ADC2 with humidity control, a TLC visualizer, WinCATS 1.4.9 software and for derivatization, a Chromatogram Immersion Device III and TLC Plate Heater III.

Test and reference solutions were applied at volumes of 7 µL and as 8 mm bands, 8 mm from the lower edge of the plate. The syringe was rinsed with absolute ethanol between samples. Mobile phase was a mixture of cyclohexane, ethyl acetate and acetic acid (60:38:2 by vol). Plates were developed over a distance of 70 mm from the lower edge using a twin trough glass chamber saturated for 20 min with mobile phase under controlled humidity. After development, plates were dried under a stream of cool air for 10 min in ADC2. Digital images were captured using 366-nm UV light or white light before derivatization.

The plate was first sprayed with HCl reagent (HCl/acetic acid: 8/2 by vol) and heated at 110 °C for 3 min with the TLC Plate Heater. An additional derivatization was required: the plate was immersed into para-anisaldehyde reagent (170 mL iced methanol, 20 mL acetic acid, 10 mL sulfuric acid and 1 mL para-anisaldehyde) using the Chromatogram Immersion Device (immersion time of 1 s and immersion speed of 5 cm/s) and heated at 100 °C for 3 min. Evaluation of the plates after each derivatization was carried out under visible and UV light (366 nm).

### 2.3. Evaluation of myorelaxant effects in mice

#### 2.3.1. Animals

Experiments were performed in 131 male RjORL: Swiss mice (Janvier, Laval, France), aged 6 weeks and weighing 35.8 ± 0.2 g. Mice were housed 6 per cage and maintained on a 12/12 h light/dark schedule in a temperature controlled facility (22 ± 1 °C) with free access to food and water. Animals were kept undisturbed for 7 days before experiments. All procedures were conducted in conformity with European rules for animal experimentation (French Ethical Committee CEEA-PdL-2011-45/CEEA-PdL-01579.01).

#### 2.3.2. Treatment

Animals were divided into 6 groups. Two groups of 24 mice received a single oral dose of the SE of *V. officinalis* at 2 or 5 g/kg.

Two control groups of 18 and 17 mice received glycerine orally, the SE excipient (batch no. 3189711, 3i nature, France) at the corresponding doses of 1.6 or 4 g/kg. A group of 24 mice received tetrazepam (batch no. 0022P8, Sigma-Aldrich, Saint-Quentin Fallavier, France; 1 mL/kg, tetrazepam in 7.5% dimethyl sulfoxide [DMSO] and 3.75% Tween 20 in 0.9% NaCl solution) intraperitoneally at a dose of 10 mg/kg, and a control group of 24 mice was injected with the vehicle solution (both at a volume of 2.5 mL/kg).

### 2.3.3. Behavioural tests

The grip and the wire hanging tests were performed 30 min after treatment for all groups.

The grip test is a non-invasive method designed to evaluate muscle strength *in vivo*, by taking advantage of the mouse's tendency to grasp a grid while suspended by its tail. The grip test is then used to measure the maximal muscle strength of the four limbs. Mice were placed on a 10 × 10 cm grid and were gently pulled horizontally backward until they released their grip.<sup>8</sup> A grip meter (Panlab, BIOSEB, France) attached to a force transducer measured the peak force generated by the four limbs together. Three measures were performed and averaged. The maximal strength developed by these four limbs was expressed in grams (g; absolute strength) and normalized to the body weight (g/g; relative strength).

The wire hanging test is used to assess global skeletal muscle function and endurance over time in mice. This test is also used in pharmacological studies for evaluating the neuromuscular tone, a continuous and passive partial skeletal muscle contraction.<sup>7,23</sup> The wire hanging apparatus is comprised of a stainless steel bar (49 cm length, 1.5 mm diameter), resting on two vertical supports and elevated 45 cm above a flat surface covered with litter in order to cushion falls.<sup>4,23</sup> Mice were hung onto the bar with the two forepaws midway between the supports. The time spent hanging was recorded until they fall, before being manually hung again. For each mouse, a score equalling to 10 was given at the beginning of the test. Each fall decreased this score by one unit. The test ended when the animal had felt 10 times or after 3 min. For each second of the

test duration, a mean score was calculated for all animals from one group, and the mean score obtained at the “100 s” time point was used as an indicator of endurance. Moreover, the impulse parameter, which represents the ability to maintain a sufficient strength to stay on the bar, was calculated (g.s; body weight [g] multiplied by maximal duration between two falls [s]).<sup>5</sup>

### 2.3.4. Statistical analysis

The results are presented as means ± sem. Normal distribution of data was verified with a D'Agostino-Pearson test. When data were not normally distributed, the significance of differences between groups was evaluated using the Kruskal-Wallis ANOVA followed when significant, by a Mann-Whitney *post hoc* test between test groups and their respective controls. When data were normally distributed, the significance of differences between groups was evaluated using a one-way ANOVA followed when significant, by a Student t-test. Level of significance was set at  $p < 0.05$ . Statistical analyses were carried out with GraphPad Prism version 5.0.

## 3. Results

### 3.1. Content in valerenic acid and derivatives

Valerenic acid and its derivative, hydroxyvalerenic acid, were clearly identified by HPLC in comparison to standards. The HPLC analysis of the DCM fraction and standards in the same conditions confirmed the presence of valerenic and hydroxyvalerenic acids in that fraction and so, in the *V. officinalis* HRE (Fig. 1). Under the extraction conditions used to obtain the DCM fraction, acetoxvalerenic acid underwent degradation and was thus not detected.

The mass spectra showed that the major compounds of the DCM fraction were valerenic acid derivatives; the other compounds could not be identified as known compounds in *V. officinalis* extracts. The presence of valerenic and hydroxyvalerenic acids was confirmed by mass spectrometry.

HPTLC analysis confirmed the presence of the two sesquiterpenes, valerenic and hydroxyvalerenic acids and showed the

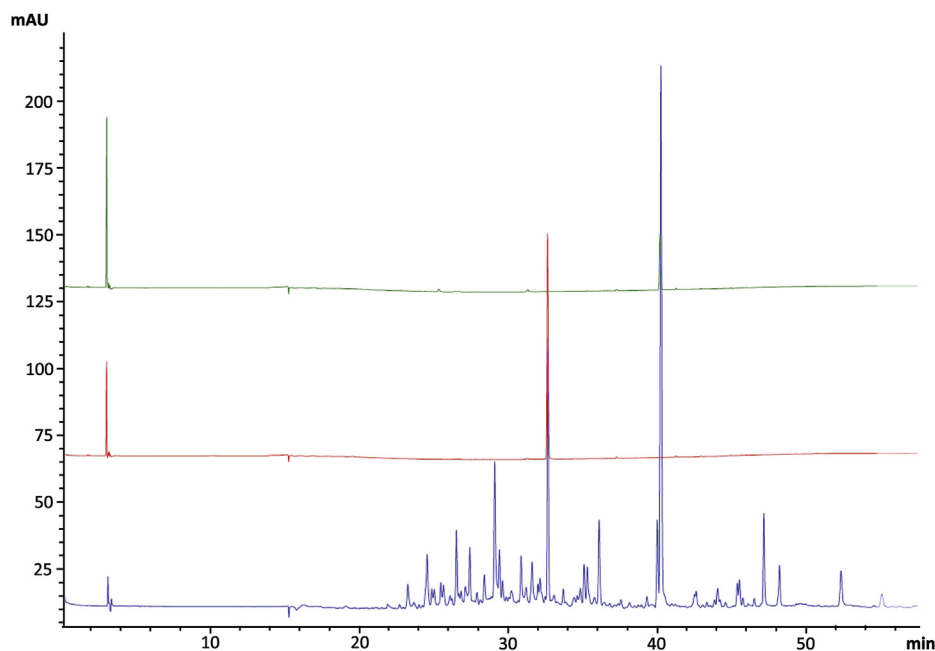
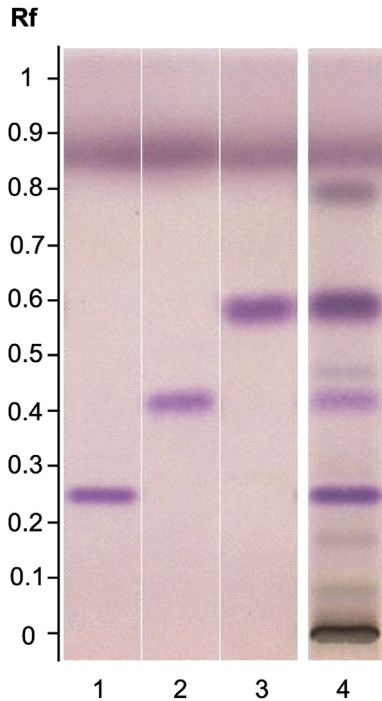


Fig. 1. Superposition of the *V. officinalis* HRE DCM fraction (blue) with hydroxyvalerenic acid standard (red) and valerenic acid standard (green).



**Fig. 2.** HPTLC fingerprint (sesquiterpenes) of *V. officinalis* HRE. Track assignment: 1 hydroxyvaleric acid standard; 2 acetoxyvaleric acid standard; 3 valeric acid standard; 4 *V. officinalis* HRE.

presence of acetoxyvaleric acid. White light observation of the plate after derivatization with para-anisaldehyde allowed the detection of hydroxyvaleric, acetoxyvaleric and valeric acids at retention factor (Rf) 0.25, 0.43 and 0.61, respectively (Fig. 2). These sesquiterpenes were clearly identified in the *V. officinalis* HRE fingerprint in comparison with standards; other weak zones correspond to nonidentified minor substances.

### 3.2. Myorelaxant effect in mice

In the grip test, treatment with the SE of *V. officinalis* at the two doses tested significantly reduced the maximal skeletal muscle strength when compared to the respective control groups (Fig. 3). Specifically, the 2 g/kg dose induced a significant decrease in maximal strength of 17.6% for the absolute ( $p < 0.001$ , Fig. 3A) and 18.2% for the relative strength in comparison with the control groups ( $p < 0.001$ , Fig. 3B). The higher dose of 5 g/kg was also effective: the absolute and relative strengths were significantly reduced by 15.9% ( $p < 0.01$ , Fig. 3A) and 14.5% ( $p < 0.01$ , Fig. 3B), respectively. As usually observed, maximal skeletal muscle strength

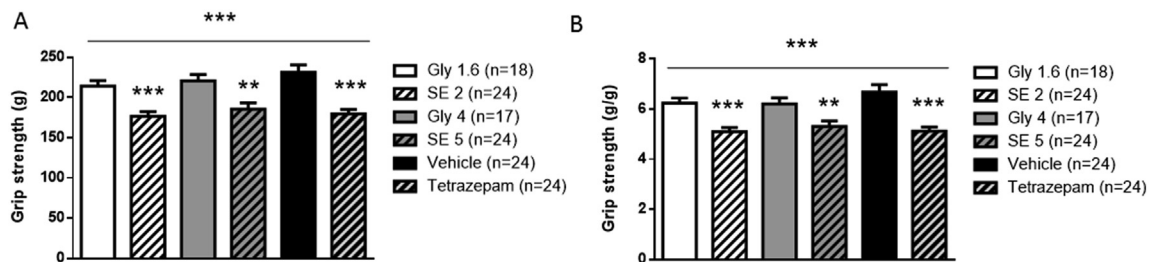
was also significantly decreased with tetrazepam; we measured a decrease of 22.4% for the absolute and 23.3% for the relative strength in comparison with the vehicle ( $p < 0.001$ , Fig. 3). At the two doses tested, the SE of *V. officinalis* induced a significant decrease in the maximal skeletal muscle strength which was similar to that observed with tetrazepam.

In the wire hanging test, mice treated with the SE of *V. officinalis* exhibited a reduced latency to fall (Fig. 4A). However, although the “100 s” scores were decreased by approximately 20% at the two doses, the decrease was not significantly different from those observed in the control groups. In good agreement, the impulse parameter was also decreased by approximately 14% in the two SE groups but the difference with controls was not statistically significant (Fig. 4D). By contrast, the latency to fall was significantly reduced in mice treated with tetrazepam (Fig. 4B). The “100 s” score was strongly and significantly decreased by 83.3% ( $p < 0.001$ , Fig. 4C) and impulse parameter by 67.3% ( $p < 0.001$ , Fig. 4D).

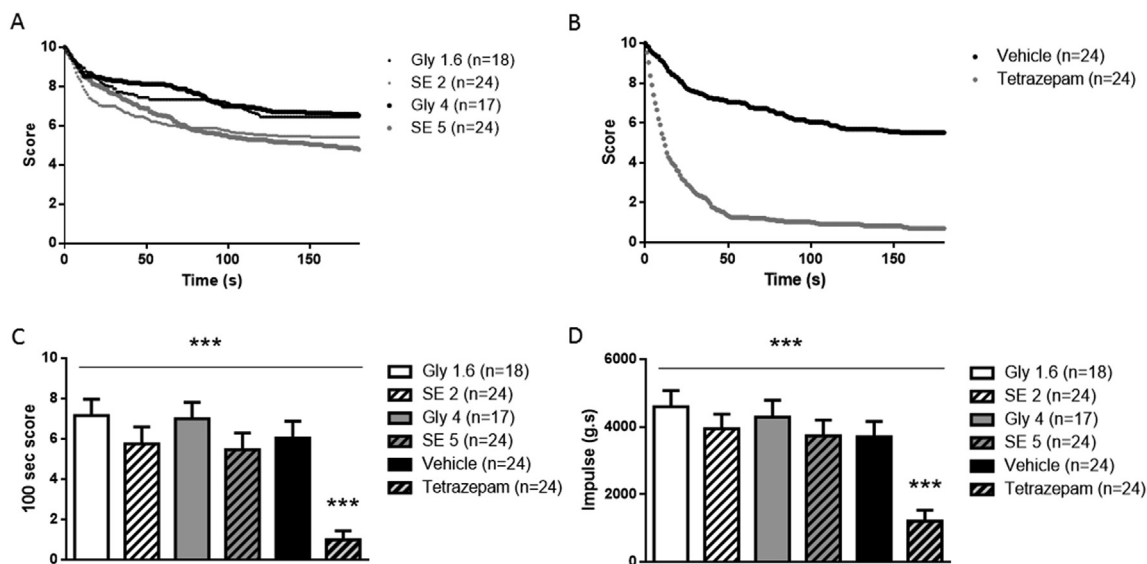
## 4. Discussion

Our results provide clear evidence that the SE of *V. officinalis* tested has a relaxant effect on skeletal muscle. This effect of *V. officinalis* had never been clearly shown previously. These results suggest that *V. officinalis* could not only be used for its anxiolytic and sedative properties but also for its relaxant effects on the skeletal muscle. We observed that the SE of *V. officinalis* reduces skeletal muscle strength: the SE of *V. officinalis* led to a marked impairment of the performance of mice in the grip test at an extent similar to that of tetrazepam. By contrast, the impact of the SE of *V. officinalis* on global skeletal muscle function (endurance and neuromuscular tone) measured in the wire hanging test was much less severe than that induced by tetrazepam. This selective effect of *V. officinalis* SE on the skeletal muscle is interesting because tetrazepam (withdrawn from the market because of an unpredictable risk of serious skin reactions)<sup>11</sup> and in a general way benzodiazepines are commonly used in the elderly to treat sleep and anxiety problems but were reported to increase the risk of falls by approximately 50% in that population.<sup>17,21</sup> By decreasing skeletal muscle strength without impacting endurance and neuromuscular tone, *V. officinalis* SE could induce less undesirable side effects than benzodiazepines and therefore, be a useful alternative myorelaxant, particularly for avoiding falls in the elderly.

In the study by Hattesoehl and colleagues,<sup>14</sup> several *V. officinalis* extracts were tested and reported to have anxiolytic and antidepressant properties but no myorelaxant properties. It should be noted that although the term “strength” was used throughout the article, the design was more for measurement of tone than strength. The effect of *V. officinalis* was indeed evaluated in a test similar to the wire hanging test used in our study: the authors assessed the percentage of mice that had lost the ability to grasp a



**Fig. 3.** Mean ( $\pm$ sem) grip strength developed by the four paws 30 min after acute treatment with *V. officinalis* SE (2 g/kg, SE 2 and 5 g/kg, SE 5), glycerine (1.6 g/kg, Gly 1.6 and 4 g/kg, Gly 4), tetrazepam (10 mg/kg) or vehicle, in grams (g) (A, absolute values) and grams per gram of body weight (g/g) (B, relative values). \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , one-way ANOVA (overall symbol) followed by post-hoc Student t-test (test group versus corresponding control group).



**Fig. 4.** Score evolution on the wire hanging test 30 min after acute treatment with (A) *V. officinalis* SE (2 g/kg; SE 2 and 5 g/kg; SE 5) or glycerine (1.6 g/kg, Gly 1.6 and 4 g/kg, Gly 4) or (B) tetrazepam (10 mg/kg) or vehicle. C: Mean score at 100 s ( $\pm$ sem). D: mean impulse ( $\pm$ sem) in grams second (g.s). \*\*\* $p < 0.001$ , Kruskal-Wallis ANOVA (overall symbol) followed by Mann-Whitney post hoc test (test group versus corresponding control group).

wire with at least one paw, when tested consecutively every 10 min over a period of 1 h. These results are therefore in agreement with ours showing that the SE of *V. officinalis* had no effect on neuromuscular tone. These results are also in good agreement with an *in vivo* study on *V. officinalis* extracts that demonstrated no effect on mice locomotor activity.<sup>15</sup>

The phytochemical analysis of our extract revealed the presence of the main family of compounds known to be present in *V. officinalis*. The three main sesquiterpenes corresponding to the reference substances, hydroxyvaleric acid, acetoxyvaleric acid and valeric acid, were found. The mechanisms involved in the myorelaxant effects of *V. officinalis* SE remain to be elucidated. However, available data suggest that *V. officinalis* SE and notably the sesquiterpenes identified in the extract could be responsible for the effects we observed via an action on the central nervous system and the gamma-aminobutyric acid (GABA) system. An aqueous extract was shown to increase the release and inhibit the recapture of GABA.<sup>3</sup> *V. officinalis* was shown to increase GABA levels in rat brain homogenate and hippocampal neuronal cultures.<sup>2,20</sup> Valeric acid was found to have an inhibitory effect on muscimol-sensitive neurons *in vitro* via GABA<sub>A</sub>-receptors<sup>26</sup>; to be a subunit-specific allosteric modulator of GABA<sub>A</sub> receptors; and extract fractions with high content of valeric acid exhibited strong receptor activation.<sup>22</sup>

We report here clear evidence for the relaxant effect on skeletal muscle of a standardized extract of *V. officinalis*, the effect being mild compared to that of tetrazepam. By decreasing skeletal muscle strength without impacting endurance and tone, the extract could induce less undesirable effects than standard myorelaxants. Current knowledge suggests an implication of valeric acid and the GABA system. However, other compounds and mechanisms could be involved such as effects on blood pressure. Next step would be to study more closely the compounds and mechanisms at the origin of the myorelaxant effect observed.

### Conflict of interest

Isabelle Guinobert is a scientific project manager at PiLeJe. Valérie Bardot, César Cotte, Isabelle Ripoche and Pierre Chalard

were involved in the chromatographic analyses. Dorian Caudal, Aude Lafoux and Corinne Huchet performed the animal experiments for PiLeJe.

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### Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jtcme.2017.06.011>.

### References

- Andreatini R, Sartori VA, Seabra ML, et al. Effect of valepotriates (valerian extract) in generalized anxiety disorder: a randomized placebo-controlled pilot study. *Phytother Res.* 2002;16:650–654.
- Awad R, Levac D, Cybulska P, et al. Effects of traditionally used anxiolytic botanicals on enzymes of the gamma-aminobutyric acid (GABA) system. *Can J Physiol Pharmacol.* 2007;85:933–942.
- Bruneton J. *Pharmacognosie: phytochimie, plantes médicinales*. 5th ed. Paris, France: Lavoisier; 2016:920–928.
- Carre-Pierrat M, Lafoux A, Tanniou G, et al. Pre-clinical study of 21 approved drugs in the mdx mouse. *Neuromuscul Disord.* 2011;21:313–327.
- Carlson CG, Rutter J, Bledsoe C, et al. A simple protocol for assessing inter-trial and inter-examiner reliability for two noninvasive measures of limb muscle strength. *J Neurosci Methods.* 2010;186:226–230.
- Circosta C, De Pasquale R, Samperi S, et al. Biological and analytical characterization of two extracts from *Valeriana officinalis*. *J Ethnopharmacol.* 2007;112:361–367.
- Crestani F, Löw K, Keist R, et al. Molecular targets for the myorelaxant action of diazepam. *Mol Pharmacol.* 2001;59:442–445.
- De Luca A. *Use of Grip Strength Meter to Assess the Limb Strength of Mdx Mice*; 2008. SOP DMD.M.2.2.001. Version 2.0 <http://www.treat-nmd.eu/downloads/file/sops/dmd/MDX/DMD.M.2.2.001.pdf>. Accessed 15 February 2017.
- Dimpfel W, Suter A. Sleep improving effects of a single dose administration of a

- valerian/hops fluid extract – a double blind, randomized, placebo-controlled sleep-EEG study in a parallel design using electrohypnograms. *Eur J Med Res.* 2008;13:200–204.
10. Emami-Abarghouei M, Vafaei A, Akhavan M, et al. The effect of hydro-alcoholic extract of *Valeriana officinalis* on the ileum movement of Guinea pig. *J Gorgan Uni Med Sci.* 2009;11:1–5.
  11. European Medicines Agency (EMA). *Co-ordination Group for Mutual Recognition and Decentralised Procedures – Human (CMDh). Recommendation to Suspend Tetrazepam-containing Medicines Endorsed by CMDh.* EMA/256383/2013 dated; April 29, 2013. [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/Press\\_release/2013/04/WC500142552.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/Press_release/2013/04/WC500142552.pdf). Accessed 15 February 2017.
  12. European Medicines Agency (EMA). *Committee on Herbal Medicinal Products (HMPC). European Union Herbal Monograph on Valeriana Officinalis L., Radix.* EMA/HMPC/150848/2015 dated; February 02, 2016. [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/Herbal\\_-\\_Herbal\\_monograph/2016/04/WC500205376.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/Herbal_-_Herbal_monograph/2016/04/WC500205376.pdf). Accessed 15 February 2017.
  13. Hazelhoff B, Malingré TM, Meijer DK. Antispasmodic effects of valeriana compounds: an in-vivo and in-vitro study on the guinea-pig ileum. *Arch Int Pharmacodyn Ther.* 1982;257:274–287.
  14. Hattesoil M, Feistel B, Sievers H, et al. Extracts of *Valeriana officinalis* L. s.l. show anxiolytic and antidepressant effects but neither sedative nor myorelaxant properties. *Phytomedicine.* 2008;15:2–15.
  15. Hiller KO, Zetler G. Neuropharmacological studies on ethanol extracts of *Valeriana officinalis* L.: behavioural and anticonvulsant properties. *Phytother Res.* 1996;10:145–151.
  16. Leuschner J, Müller J, Rudmann M. Characterisation of the central nervous depressant activity of a commercially available valerian root extract. *Arzneimittelforschung.* 1993;43:638–641.
  17. Maxwell CJ, Neutel CI, Hirdes JP. A prospective study of falls after benzodiazepine use: a comparison of new and repeat use. *Pharmacoepidemiol Drug Saf.* 1997;6:27–35.
  18. Monograph. *Valeriana officinalis.* *Altern Med Rev.* 2004;9:438–441.
  19. Occhiuto F, Pino A, Palumbo DR, et al. Relaxing effects of *Valeriana officinalis* extracts on isolated human non-pregnant uterine muscle. *J Pharm Pharmacol.* 2009;61:251–256.
  20. Ortiz JG, Nieves-Natal J, Chavez P. Effects of *Valeriana officinalis* extracts on [3H] flunitrazepam binding, synaptosomal [3H]GABA uptake, and hippocampal [3H] GABA release. *Neurochem Res.* 1999;24:1373–1378.
  21. Pariente A, Fourrier-Réglat A, Letenneur L. Benzodiazepine use and occurrence of severe falls in community dwelling elderly: data from the PAQUID study. *Pharmacoepidemiol Drug Saf.* 2005;14:S193–S194.
  22. Trauner G, Khom S, Baburin I, et al. Modulation of GABAA receptors by valerian extracts is related to the content of valerianic acid. *Planta Med.* 2008;74(1): 19–24.
  23. van Putten M. *The Use of Hanging Wire Tests to Monitor Muscle Strength and Condition over Time;* 2011. SOP DMD\_M.2.1.004 Version 4.0 [http://www.treat-nmd.eu/downloads/file/sops/dmd/MDX/DMD\\_M.2.1.004.pdf](http://www.treat-nmd.eu/downloads/file/sops/dmd/MDX/DMD_M.2.1.004.pdf). Accessed 15 February 2017.
  24. Wagner H, Jurcic K, Schaette R. Comparative studies on the sedative action of *Valeriana* extracts, valepotriates and their degradation products. *Planta Med.* 1980;39:358–365.
  25. Wheatley D. Medicinal plants for insomnia: a review of their pharmacology, efficacy and tolerability. *J Psychopharmacol.* 2005;19:414–421.
  26. Yuan CS, Mehendale S, Xiao Y, et al. The gamma-aminobutyric acid effects of valerian and valerianic acid on rat brainstem neuronal activity. *Anesth Analg.* 2004;98:353–358.