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Research Paper

Vasorelaxant effect of *Valeriana edulis* ssp. *procera* (Valerianaceae) and its mode of action as calcium channel blocker

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Abstract

Objectives The aim was to evaluate the relaxant effect of extracts from *Valeriana edulis* and determine the possible mechanism of action of the hexanic extract as vasorelaxant agent.
Methods Extracts from rhizomes obtained by maceration (hexanic (HEVe), dichloromethanic (DEVe), methanolic (MEVe) and hydroalcoholic (HAEVe) (3.03–500 μg/ml)) were evaluated on aortic rat rings with and without endothelium.
Key findings Extracts induced a significant concentration-dependent and endothelium-independent relaxation on isolated rat aorta pre-contracted with noradrenaline (0.1 μM). HEVe, the most potent extract (0.15–50 μg/ml), induced relaxation in aortic rings pre-contracted with KCl (80 mM), with an IC50 value of 34.61 ± 1.41 μg/ml and E_{max} value of 85.0 ± 4.38%. Pretreatment with HEVe (30 μg/ml) also inhibited contractile responses to noradrenaline and $CaCl_2$. HEVe (9.98 ± 2.0 μg/ml) reduced noradrenaline-induced transient contraction in Ca^{2+}-free solution, and inhibited contraction induced by KCl (80 mM). In endothelium-denuded rings, the vasorelaxant effect of HEVe was not modified by 1-*H*-[1,2,4]-oxadiazolo-[4,3a]-quinoxalin-1-one (1 μM), tetraethylammonium (5 mM), glibenclamide (10 μM) or 2-aminopyridine (100 μM).
Conclusions Our results suggest that HEVe induces relaxation through an endothelium-independent pathway, involving blockade of Ca^{2+} channels, and this effect could be related to the presence of valepotriates.
Keywords aortic rings; calcium channels; *Valeriana edulis*; vasorelaxant

Introduction

The genus *Valeriana*, with about 200 species, belongs to the family Valerianaceae and has a worldwide distribution. The roots of *Valeriana* species comprise the drug valerian, which has been used as a sedative since it was described by ancient Greeks and Romans.[1] Plants of this genus have sedative, antispasmodic and relaxing properties, which have been ascribed to the presence of iridoid esters (valepotriates), as well as isovalerenic acid and borneol derivatives, which are predominately produced in the roots and rhizomes of the intact plants.[2]

Valeriana edulis ssp. *procera* (Valerianaceae) is native to North America and is found in temperate zone pine and oak forests. The subspecies *V. edulis* ssp. *procera*, commonly named 'valeriana mexicana', is endemic to Mexico and is mainly distributed in the central zone of the country.[2] Phytochemical analysis has shown that this plant contains high concentrations of valepotriates, from which valtrate is perhaps the most important active compound of this group.[2] Previous pharmacological studies have been carried out with *V. edulis*, such as investigation of its anticonvulsant and sedative effects in mice.[3] Also, it caused a significant reduction in sleep latencies and nocturnal time awake, lengthening total sleep time and improving sleep quality.[4] Furthermore, Herrera-Arellano *et al.*, using polysomnographic recordings, demonstrated that a hydroalcoholic extract from *V. edulis* reduced

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the number of waking episodes, increased the sleep efficiency index and reduced morning sleepiness without modifying the anterograde memory in humans.[5] Recently, Ugalde *et al*. showed, using isobolographic analysis, that co-administration of hydroalcoholic extract from *V. edulis* with some CNS depressant drugs, such as haloperidol, diazepam, buspirone, pentobarbital, diphenhydramine and ethanol, produce a sedative interaction in mice.[6] Nevertheless, there is no investigation that offers a scientific basis for the traditional use of *V. edulis* as an antihypertensive agent. There are some reports, however, about the antispasmodic and hypotensive effects of *Valeriana wallichii*, suggesting that these effects are mediated through K^+-ATP channel activation, which justify its use in gastrointestinal and cardiovascular disorders.[7] Finally, the ethanolic extract and lignans isolated from *V. prionophylla* showed a significantly vasorelaxant effect on rat isolated aorta rings pre-contracted with phenylephrine.[1]

Therefore, the aim of this study was to evaluate the vasorelaxant activity of some extracts from *V. edulis* to rationalize some folkloric uses, and also to determine the possible mechanism of action of the hexanic extract as a vasorelaxant agent.

Materials and Methods

Chemicals and drugs

Carbamylcholine HCl (carbachol), noradrenaline HCl (NA), nitrendipine, 1-*H*-[1,2,4]-oxadiazolo-[4,3a]-quinoxalin-1-one (ODQ), glibenclamide, tetraethylammonium (TEA), 2-aminopyridine (2-AP), chlorogenic acid, hesperidin and valerenic acid were purchased from Sigma-Aldrich Co. (St Louis, USA). All other reagents were analytical grade from local sources.

Plant material and extraction

Rhizomes of *V. edulis* were collected on July 2005 from a wild population in the village of San Felipe Neri, Tlalnepantla, Morelos, Mexico (19°05′35.78″ N; 98°56′41.99″ E; 2876 m.s.n.m; 2005 Google Earth). The identification and collection of the plant material was carried out by Dr Patricia Castillo-España.[8] A voucher specimen (No. 22446) was deposited at the HUMO-Herbarium (CEAMISH) of the Morelos State University. Briefly, the dried plant material was pulverized (203 g) and crude extracts (HEVe, DEVe, MEVe) were prepared by successive maceration with hexane, dichloromethane and methanol (three times for 72 h at room temperature). Also, a hydroalcoholic extract, HAEVe (30 : 70), was obtained from 100 g of *V. edulis*. After filtration, extracts were concentrated *in vacuo* at 40°C. Finally, yields of 6.6, 9.4, 8.94 and 8.4 g, respectively, were obtained.

Animals

To determine the vasorelaxant effect and the mode of action of HEVe, male Wistar rats, 250–350 g, were used. They were maintained under standard laboratory conditions with free access to food and water. All animal procedures were conducted in accordance with our Federal Regulations for Animal Experimentation and Care (SAGARPA, NOM-062-ZOO-1999, México) approved by the Institutional Animal Care and Use Committee, and also were consent with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, revised 1996).

Ex-vivo aortic rings test

Rats ($n = 6$) were sacrificed by cervical dislocation and thoracic aortas were removed and immersed in Krebs solution at room temperature. Aortic rings (3–5 mm) were obtained free from connective tissue and fat (in some rings the endothelium was removed), and suspended by platinum hooks under an optimal tension of 3 g in a Krebs solution (composition, mM: NaCl 118; KCl 4.7; $CaCl_2$ 2.5; $MgSO_4$ 1.2; KH_2PO_4 1.2; $NaHCO_3$ 25.0; EDTA 0.026; glucose 11.1, pH 7.4), maintained at 37°C and gassed with a mixture of 95% O_2 and 5% CO_2. Isometric tension was measured and recorded using Grass-FT03 force transducers (Astromed, West Warwick, USA) connected to a MP100 analyser (BIOPAC Instruments, Santa Barbara, USA), as previously described.[9] After equilibration, rings were contracted by noradrenaline (0.1 μM) and washed every 30 min for 2 h. The absence of endothelium was confirmed by the lack of a relaxing response to carbachol (1 μM). After pre-contraction with noradrenaline, the test samples (extracts, vehicle and positive control) were added to the bath in a volume of 100 μl; then cumulative concentration–response curves were obtained for each ring (3.03–500 μg/ml). To avoid fatigue of the arterial preparation, a 60-min recovery period was allowed between curves. The relaxant effect of extracts and positive controls were determined by comparing the muscular tone of the contraction before and after addition of the test materials. Muscular tone was calculated from the tracings, using Acknowledge software (Biopac).

The underlying mode of action of HEVe was determined using functional experiments.[10,11]

Effect of hexanic extract of *Valeriana edulis* on the contraction induced by KCl (80 mM)

The aortic rings without endothelium were pre-contracted with high KCl (80 mM). Once a plateau was attained, concentration–response curves of HEVe-induced relaxation (0.15–50 μg/ml) were obtained by adding cumulative concentrations of the extract to the bath.

Effect of hexanic extract of *Valeriana edulis* on the contraction induced by noradrenaline

Endothelium-denuded aortic rings were incubated with 30 μg/ml of HEVe for 15 min, and then noradrenaline was added at different concentrations (0.1 nM to 60 mM). Finally, the contractile effect induced by noradrenaline was compared in absence (control group) and presence of the extract.

Effect of hexanic extract of *Valeriana edulis* on extracellular Ca^{2+}-induced contraction activated by KCl

To determine whether the inhibition of extracellular Ca^{2+} influx was involved in HEVe-induced relaxation, the

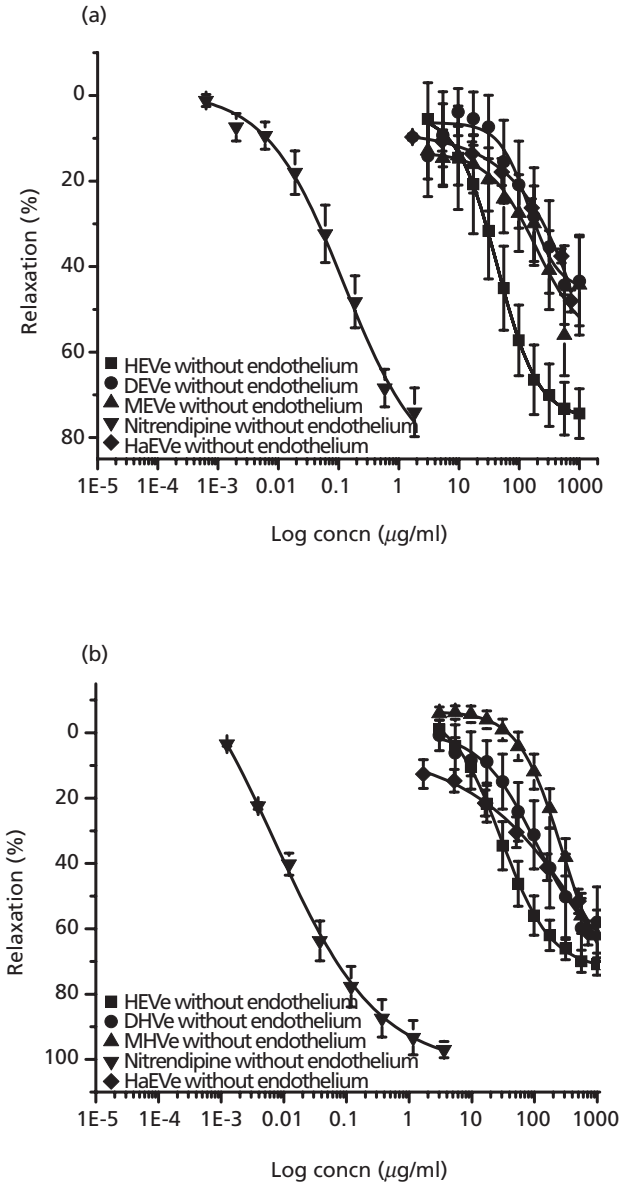

Figure 1 Concentration–response curves of the relaxant effect of hexanic, dichloromethanic, methanolic and hydroalcoholic extracts from *Valeriana edulis* on the contraction induced by noradrenaline (0.1 μM) in rat isolated aortic rings. HEVe, hexanic extract from *V. edulis*; DEVe, dichloromethanic extract; MEVe, methanolic extract; HaEVe, hydroalcoholic extract. (a) Endothelium-intact aortic rings. (b) Endothelium-denuded aortic rings. Results are presented as mean ± SEM, $n = 6$.

experiments were carried out in Ca^{2+}-free Krebs solution. Endothelium-denuded aortic rings were washed with Ca^{2+}-free solution (approximately 20 min) and then rinsed with Ca^{2+}-free solution containing KCl (80 mM). The cumulative concentration–response curves for $CaCl_2$ (1 mM to 0.01 M) were obtained in the absence of HEVe (control group) or after 15 min incubation with the extract (30 μg/ml). Finally, the contractile effect induced by $CaCl_2$ was compared in absence and presence of HEVe.

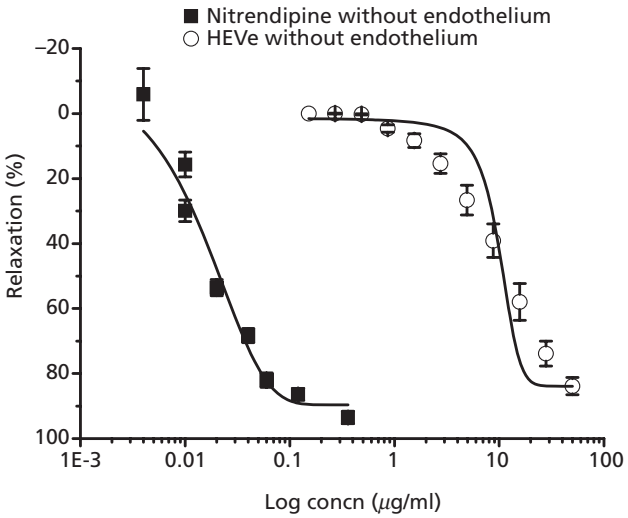

Figure 2 Concentration–response curve of the relaxant effect of hexanic extract of *Valeriana edulis* on the contraction induced by KCl (80 mM) in rat isolated endothelium-denuded aortic rings. HEVe, hexanic extract from *V. edulis*. Results are presented as mean ± SEM, $n = 6$.

Effect of hexanic extract of *Valeriana edulis* on the sarcoplasmic reticulum calcium release induced by noradrenaline

To clarify whether the relaxation induced by the extract was related to inhibition of intracellular Ca^{2+} release, the experiments were carried out in Ca^{2+}-free Krebs solution. Endothelium-denuded rings were washed with Ca^{2+}-free Krebs solution for 10 min, then HEVe (30 μg/ml) was present for 20 min and, finally, noradrenaline (0.1 μM) was added to stimulate the release of intracellular Ca^{2+}. The maximal tension induced by noradrenaline in the control group (without HEVe) was considered as 100%.

Role of K^+ channels in relaxation induced by hexanic extract of *Valeriana edulis*

To investigate the role of K^+ channels on HEVe-induced relaxation, endothelium-denuded arterial rings were pre-incubated with the K^+ channel blockers TEA (5 mM), glibenclamide (10 μM) and 2-AP (100 μM) for 15 min before noradrenaline (10 μM) was added; when a plateau was attained, cumulatively (0.15–50 μg/ml) HEVe was added.

Role of soluble guanylate cyclase (sGC) in relaxation induced by hexanic extract of *Valeriana edulis*

Endothelium-denuded aortic rings were incubated with ODQ, a specific inhibitor of soluble guanylyl cyclase (1 μM) for 15 min. After that, aortic rings were pre-contracted with noradrenaline (10 μM) and once a plateau was attained, concentration–response curves of HEVe-induced relaxation (0.15–50 μg/ml) were obtained by adding cumulative concentrations of the extract to the bath. Finally, the relaxant effect induced by HEVe was compared in absence (control group) and presence of ODQ.

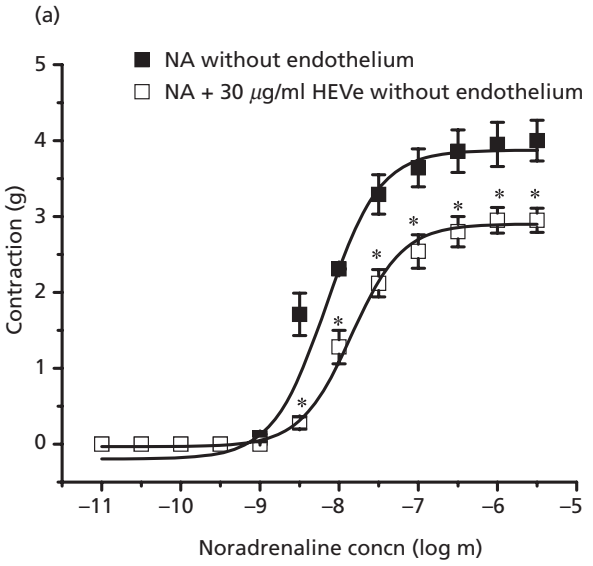

Figure 3 Inhibitory effect of hexanic extract of *Valeriana edulis* (30 μg/ml) in rat isolated endothelium-denuded aortic rings on: (a) the contraction induced by noradrenaline (0.1 nM to 60 mM) and (b) the cumulative contraction curve dependent on extracellular Ca^{2+} influx ($CaCl_2$) induced by 80 mM KCl in Ca^{2+}-free solution. NA, noradrenaline; HEVe, hexanic extract from *V. edulis*. Results are presented as mean ± SEM, $n = 6$. *$P < 0.05$ compared with control.

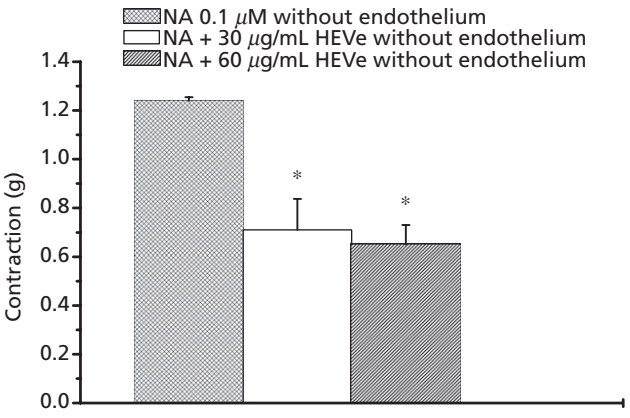

Figure 4 Inhibitory effect of hexanic extract of *Valeriana edulis* (30 and 60 μg/ml) on the noradrenaline (0.1 mM)-induced contraction in rat isolated endothelium-denuded aortic rings in Ca^{2+}-free solution. NA, noradrenaline; HEVe, hexanic extract from *V. edulis*. Results are presented as mean ± SEM, $n = 6$. *$P < 0.05$ compared with control.

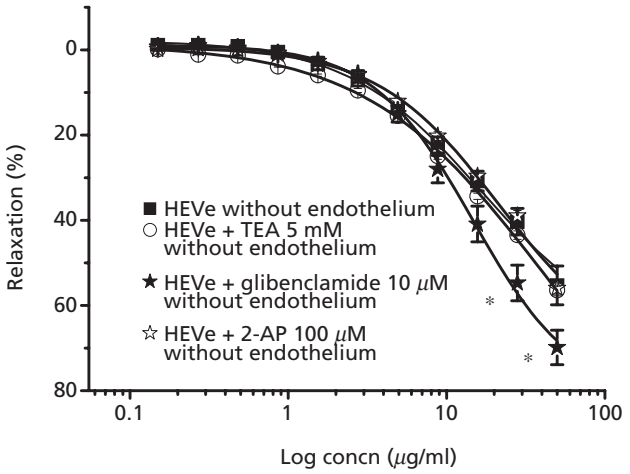

Figure 5 Effect of glibenclamide (10 μM), tetraethylammonium (5 mM) and 2-aminopyridine (100 μM) on the relaxation induced by the hexanic extract of *Valeriana edulis* in rat endothelium-denuded aortic rings pre-contracted by noradrenaline (0.1 μM). TEA, tetraethylammonium; 2-AP, 2-aminopyridine; HEVe, hexanic extract of *Valeriana edulis*. Results are presented as mean ± SEM, $n = 6$. *p < 0.05 compared with control.

Instrumentation and chromatographic conditions

The HPLC system consisted of a Perkin-Elmer Series 200 with a quaternary pump, UV–Vis detector and Rheodyne injector with a loop of 20 μl. Separation was achieved with a MICROSORB MV C18, 4.5 × 150 mm and 5 μm pore, operated at 36°C. The mobile phase consisted of water (A) and acetonitrile–methanol (1 : 1) (B), both containing 0.05% phosphoric acid, which were applied in the gradient elution sequences. Each run was followed by a 5-min wash with 100% of acetonitrile and an equilibration period of 15 min. The flow rate was adjusted to 0.8 ml/min, detected at 225 nm and the injection volume was 20 μl.[12] Chlorogenic acid, hesperidin and valerenic acid were detected at t_R of 11.9, 20.8 and 33.2 min, respectively. All standards were dissolved and mixed up in methanol (HPLC grade) at final concentration of 50 μg/ml.

Data analysis

Results are expressed as the mean of six experiments ± SEM. Concentration–response curves (CRC) were plotted and the experimental data from the CRC were adjusted by the nonlinear, curve fitting program (ORIGIN 8.0). The statistical

significance ($P < 0.05$) of differences between means was assessed by analysis of variance followed by Tukey's test.

Results

Effect of *V. edulis* extracts on the contraction induced by noradrenaline

HEVe, DEVe, MEVe and HAEVe relaxed noradrenaline-precontracted (0.1 μM) aortic rings with (E+) and without (E-) endothelium, in a concentration-dependent and endothelium-independent manner. HEVe was the most potent (IC50 = 61.48 ± 2.86 and 40.41 ± 6.24 μg/ml, with and without endothelium, respectively) and efficient (Emax = 75.80 ± 0.28 and 66.66 ± 1.52%, with and without endothelium, respectively) relaxant agent. All extracts were less potent and efficient than carbachol and nitrendipine (IC_{50} = 0.01 ± 0.0025 μg/ml and Emax = 69.17 ± 1.02% with endothelium and IC_{50} = 0.005 ± 0.001 µg/ml and Emax = 86.42 ± 4.08% without endothelium respectively), a calcium channel blocker agent (Figure 1a and 1b; $P < 0.05$).

Effect of hexanic extract of *Valeriana edulis* on the contraction induced by KCl

HEVe induced a significant relaxant effect on KCl-precontracted (80 mM) aortic rings without endothelium in a concentration-dependent fashion with an IC50 value of 9.98 ± 2.0 μg/ml and Emax value of 83.87 ± 3.15% (Figure 2). HEVe was less active than nitrendipine (IC50 = 0.023 ± 0.015 µg/ml and Emax = 89.63 ± 3.58%) ($P < 0.05$).

Effect of hexanic extract of *Valeriana edulis* on the cumulative contraction induced by noradrenaline

HEVe (30 μg/ml) inhibited the concentration–response contraction of noradrenaline in a non-parallel manner (EC50 = 85 ± 4.1 nM for control vs 78 ± 2.1 nM for HEVe) and depressed the maximum effect (Emax = 4 ± 0.6 g for control vs 2.95 ± 0.7 g for HEVe) ($P < 0.05$; Figure 3a).

Effect of hexanic extract of *Valeriana edulis* on extracellular Ca^{2+}-induced contraction activated by KCl

In the Ca^{2+}-free solution plus 80 mM of KCl, cumulative addition of $CaCl_2$ (0.3–200 mM) induced a stepwise tension increase in aortic rings. Pre-treatment with HEVe (30 μg/ml) attenuated $CaCl_2$-induced contraction of the control group in a non-parallel manner (EC50 = 3.2 ± 0.4 mM for control vs 4 ± 0.5 mM for HEVe) and depressed its maximal response (Emax = 3.56 ± 0.02 g for control vs Emax = 2.39 ± 0.03 g for HEVe), suggesting that Ca^{2+} influx was reduced by the extract (Figure 3b).

Effect of hexanic extract of *Valeriana edulis* on the sarcoplasmic reticulum calcium release induced by noradrenaline

In the Ca^{2+}-free solution, noradrenaline (0.1 μM) induced a brief contraction due to the release of intracellular Ca^{2+}. Pre-incubation with HEVe (30 and 60 μg/ml) for 15 min significantly attenuated noradrenaline-induced contraction (~ 50%) compared with control (1.24 g for control and 0.71 and 0.65 g, for 30 and 60 μg/ml of HEVe, respectively) ($P < 0.05$). However, the contraction inhibition produced by 30 and 60 μg/ml of HEVe did not show differ significantly (Figure 4), suggesting that the extract, at these concentrations, provoked a reduction on the sarcoplasmic reticulum calcium release that might not be concentration dependent.

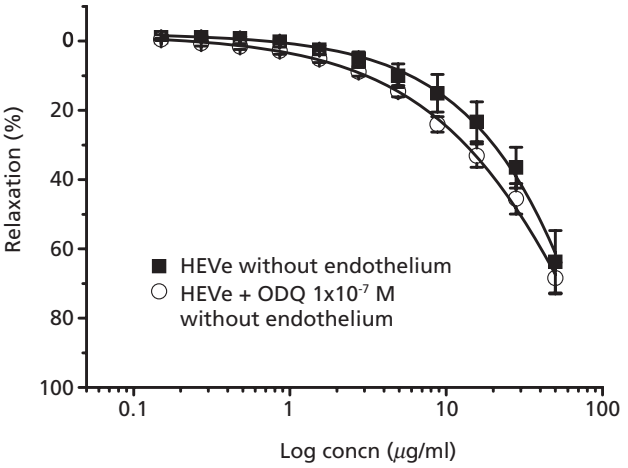

Figure 6 Effect of 1-*H*-[1,2,4]-oxadiazolo-[4,3a]-quinoxalin-1-one (1 μM) on relaxation induced by hexanic extract of *Valeriana edulis* in rat endothelium-denuded aortic rings pre-contracted by noradrenaline (10 μM). ODQ, 1-*H*-[1,2,4]-oxadiazolo-[4,3a]-quinoxalin-1-one; HEVe, hexanic extract of *Valeriana edulis*. Results are presented as mean ± SEM, $n = 6$. *$P < 0.05$ compared with control.

Role of K^+ channels in relaxation induced by hexanic extract of *Valeriana edulis*

All three K^+-channel blockers, TEA (5 mM), glibenclamide (10 μM) and 2-AP (100 μM), did not inhibit significantly HEVe-induced relaxation in endothelium-denuded rings pre-contracted by noradrenaline (0.1 μM) (Figure 5).

Role of soluble guanylate cyclase in relaxation induced by hexanic extract of *Valeriana edulis*

Noradrenaline (1 μM) did not significantly reduce HEVe-induced relaxation in endothelium-denuded rings pre-contracted by noradrenaline (0.1 μM) (Figure 6).

Chemical fingerprints of different extracts from *V. edulis*

Based on a previous report by Navarrete *et al*. we reproduced HPLC conditions to identify the presence of chlorogenic acid, hesperidin and valerenic acid in extracts of *V. edulis*.[12] Chromatograms obtained at 225 nm for HEVe, DEVe, MEVe and HAEVe showed that the hexanic extract contain the highest amounts of valerenic acid and related valepotriates (t_R = 33.2 min) followed by the dichloromethanic extract. Moreover, traces of valerenic acid were found in MEVe and HAEVe. Finally, hesperidin and chlorogenic acid were only found in MEVe and HAEVe (Figure 7).

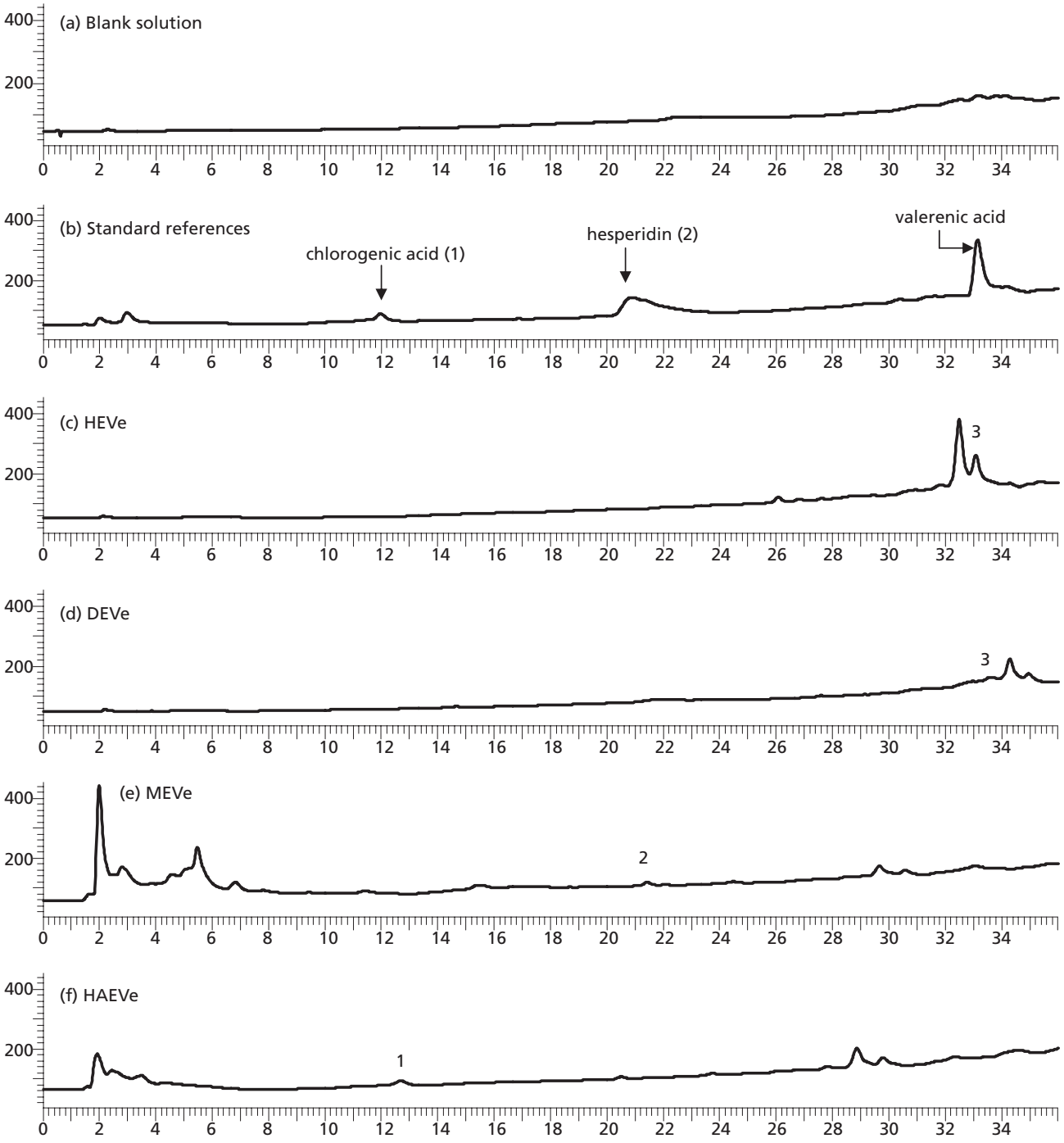

Figure 7 Comparison of HPLC chromatograms at 225 nm of *V. edulis* extracts to detect chlorogenic acid, hesperidin and valerenic acid (3). Chromatograms are as follows: (a) blank solution, (b) standard references, (c) hexanic, (d) dichloromethanic, (e) methanolic and (f) hydroalcoholic (3 : 7) extracts.

Discussion

The rhizome of *Valeriana edulis* ssp. *procera* is commonly known as 'valeriana mexicana' and 'raiz de gato' and is used as infusion for the treatment of insomnia, headache, hypertension and related ailments. Once prepared, a cup of this beverage is taken after each meal to control hypertension.[13,14] Because of its folkloric reputation as antihypertensive agent, organic and hydroalcoholic extracts from the rhizome were tested for their possible vasorelaxant effect in rat isolated aortic preparations.

All extracts induced a vasorelaxant action in a concentration-dependent and endothelium-independent manner on aortic rat rings pre-contracted with noradrenaline. These results indicated that the effect of all extracts is not associated with the synthesis of vasodilator factors produced by the endot-

helium,[9] and also suggested that the extracts induced their effect against contraction through mechanisms contained in vascular smooth muscle cells.[10] The hexanic extract, HEVe, was the most active extract, although it was less efficient and potent than nitrendipine (a calcium-channel blocker). Thus, we decided to determine the mode of action of HEVe as a vasorelaxant agent.

HEVe 30 μg/ml inhibited the concentration–response contraction of noradrenaline and $CaCl_2$ in a non-parallel manner and depressed the maximal responses in rat endothelium-denuded aortic rings, suggesting that vasodilation is produced by interference with a common pathway which several receptor agonists exert such as the augmentation of free cytosolic Ca^{2+} levels.[11,15,16] In this context, it is important to mention that in smooth muscle cells there are two kinds of Ca^{2+} channels: voltage-dependent Ca^{2+} channels (high KCl-induced contraction is due to membrane depolarization, leading to increased Ca^{2+} influx through voltage-dependent channels) and receptor-operated Ca^{2+} channels (contraction induced by noradrenaline in Ca^{2+}-free medium is due to intracellular Ca^{2+} release, through sarcoplasmic reticulum Ca^{2+} channels activated by IP_3).[17,18] Under this condition, HEVe was capable of inhibiting contractility induced by KCl (80 mM) in a concentration-dependent and endothelium-independent manner in rat aortic rings, signifying that HEVe might obstruct both voltage-dependent and receptor-operated Ca^{2+} channels, supporting the idea that HEVe possesses a Ca^{2+} entry blocking activity.[17] Additionally, HEVe (30 and 60 μg/ml) inhibited those contractions triggered by noradrenaline in endothelium-denuded rings in Ca^{2+}-free medium, indicating that it may also inhibit Ca^{2+} mobilization from intracellular stores by a possible IP_3 signalling blockade. On the other hand, the opening of K^+ channels in vascular smooth muscle cells induces hyperpolarization of the membrane, which provides an important mechanism to dilate arteries.[11,17] The relaxant effect of HEVe was not significantly inhibited by the non-specific ATP-sensitive K^+-channel blocker glibenclamide, the Ca^{2+}-activated K^+-channel blocker TEA or the voltage-dependent K^+-channel blocker 2-AP.

To discard or confirm the cGMP participation in the vasorelaxant effect of HEVe, we decided to evaluate its effect in the presence of ODQ (a specific guanylyl cyclase inhibitor). This last experiment was designed because modulation of vascular tone is also mediated by an augmentation of cGMP concentration into the smooth muscle cells.[19] In this context, pretreatment with a soluble guanylyl cyclase inhibitor (ODQ, 1×10^{-6} M) did not inhibit the concentration–response relaxation of HEVe and neither did it depress the maximal response in endothelium-denuded aortic rings, suggesting that the cyclic nucleotide pathway (cGMP) is not involved in extract-induced relaxation in endothelium-denuded aorta rings.

The results obtained are in agreement with recent reports that describe the in-vitro vasorelaxant effect of some *Valeriana* species and isolated compounds on rat thoracic aorta rings. In one report, Gillani *et al*. reported that antispasmodic and hypotensive effects of *V. wallichii* are produced by possibly K^+-ATP channel activation, which justify its use in gastrointestinal and cardiovascular disorders.[7] A second study showed that lignans isolated from *V. prionophylla* and its ethanolic extract (80%) showed a significantly vasorelaxant effect on rat isolated aorta rings pre-contracted with phenylephrine.[1] Although the actual chemical constituents of HEVe were not established in this study, *V. edulis* has been widely reported to contain many chemical compounds, especially valepotriate derivates.[8] In this context, Castillo *et al.* reported a previous HPLC quantification of valtrate, isovaltrate, dihydrovaltrate, dihydroisovaltrate and valerenic acid in *V. edulis*. They found that rhizomes and roots of the regenerated plants produced the highest valepotriate levels, namely 0.19% (dry weight) of valtrates and 0.21% (dry weight) of dihydrovaltrates.[2] This concentration was similar to that found in the rhizomes and roots of wild plants in the reproductive stage (0.29% of dry weight). Also valerenic acid was not detected in any tissue tested from either wild or regenerated plants.

Recently, Navarrete *et al*. reported an efficient HPLC method to establish the chemical fingerprints of different species of *Valeriana*, including *V. edulis*; they used several standards, such as chlorogenic acid, five lignans (massoniresinol-4′-*O*-β-D-glucoside, berchemol-4′-*O*-β-D-glucoside, pinoresinol-4,4′-di-*O*-β-D-glucoside, 8-hydroxypinoresinol-4′-*O*-β-D-glucoside, pinoresinol-4-*O*-β-D-glucoside), two flavonoids (hesperidin and linarin) and three sesquiterpenes (hydroxyvalerenic acid, acetoxyvalerenic acid and valerenic acid), which were previously isolated from *Valeriana* species.[12] In this context, we reproduced the HPLC conditions to determine the presence of chlorogenic acid, hesperidin and valerenic acid in extracts, where HEVe was the extract that contained the highest amounts of valerenic acid and related valepotriate derivatives. So, we suggest that valepotriates, such as valerenic acid, play a crucial role in the vasorelaxant effect shown by the hexanic extract. Further experiments are in progress to isolate the bioactive principles from the hexanic extract of *V. edulis*.

Conclusions

The hexanic extract of *Valeriana edulis* induces relaxation in rat aortic rings through an endothelium-independent pathway, possibly by obstruction of both voltage-dependent and receptor-operated Ca^{2+} channels, supporting the idea that HEVe possesses a extracellular Ca^{2+}-entry blocking activity and inhibits the intracellular Ca^{2+} release from sarcoplasmic reticulum.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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