

Vasorelaxant Effect of the Ethanol Extract from *Valeriana fauriei* Briquet Root and Rhizome on Rat Thoracic Aorta

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Submitted: 06-04-2018

Revised: 08-05-2018

Published: 23-01-2019

ABSTRACT

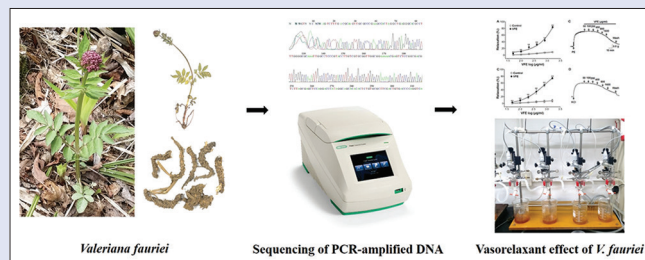
Background: *Valeriana fauriei* has been used to treat anxiety and cardiovascular disease in many countries; however, the mechanisms of the vasorelaxant effect of *V. fauriei* roots and rhizomes have not been elucidated. **Purpose of the Study:** Considering the increased use of traditional medicines for treating hypertension, the aim of this study was to examine the vasorelaxant effect and investigate the mechanisms of *V. fauriei* root and rhizome. **Materials and Methods:** Using an isolated organ-chamber technique, the vasorelaxant effects of 70% ethanol extract of *V. fauriei* root and rhizome (VFE) were tested in rat aortic ring segments. **Results:** VFE concentration dependently relaxed both endothelium-intact and endothelium-denuded aortic rings that were precontracted by phenylephrine (PE) or potassium chloride (KCl). VFE relaxed the aortic rings precontracted by PE (1 μ M) irrespective of K⁺ channel blocker pretreatment such as tetraethylammonium, glibenclamide, or 4-aminopyridine. VFE inhibited vasoconstriction caused by extracellular Ca²⁺ influx through receptor-operative Ca²⁺ channels (ROCCs) or voltage-dependent Ca²⁺ channels (VDCCs) activated by PE or KCl, respectively, in Ca²⁺-free Krebs–Henseleit buffer. **Conclusion:** These results suggested that the vasorelaxant effects of VFE were endothelium independent and not related to K⁺ channels, such as Ca²⁺-activated K⁺ channels, ATP-sensitive K⁺ channels, and Voltage-gated K⁺ channels. In addition, VFE relaxed the aortic rings by blocking the influx of extracellular Ca²⁺ through ROCCs and VDCCs.

Key words: Calcium channels, hypertension, receptor-operative Ca²⁺ channels, *Valeriana fauriei*, vasorelaxation, voltage-dependent Ca²⁺ channels

SUMMARY

- The vasorelaxant effects of the 70% EtOH extract of *Valeriana fauriei* root and rhizome (VFE) were endothelium independent
- VFE-induced vasorelaxation was not related to K⁺ channels, such as Ca²⁺-activated K⁺ channels, ATP-sensitive K⁺ channels, and voltage-gated K⁺ channels

- VFE relaxed the aortic rings by blocking the entry of extracellular Ca²⁺ through receptor-operative Ca²⁺ channels and voltage-dependent Ca²⁺ channels.



Abbreviations used: ACE: Angiotensin-converting enzyme; VFE: The 70% EtOH extract of *Valeriana fauriei* root and rhizome; KH: Krebs–Henseleit; PE: Phenylephrine; KCl: Potassium chloride; TEA: Tetraethylammonium; 4-AP: 4-Aminopyridine; CaCl₂: Calcium chloride; EGTA: Ethylene glycol-bis (2-aminoethylether)-*N, N, N, N*-tetraacetic acid; DMSO: Dimethyl sulfoxide; ROCCs: Receptor-operative Ca²⁺ channels; VDCCs: Voltage-dependent Ca²⁺ channels; K_{IR}: Inward-rectifier K⁺ channels; K_{ATP}: ATP-sensitive K⁺ channels; K_V: Voltage-gated K⁺ channels; K_{Ca}: Ca²⁺-activated K⁺ channels.

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DOI: 10.4103/pm.pm_152_18

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INTRODUCTION

Hypertension, or high blood pressure, is a major risk factor for the global burden of disease. Blood pressure is related to the amount of blood in the heart and the resistance of the arteries. More blood in the heart and narrower arteries cause higher blood pressure. Even if hypertension progresses without any other symptoms for years, damage to the blood vessels and heart increases over time and serious cardiovascular disorders, such as heart attacks (coronary heart disease) and strokes (cerebrovascular disease), can occur.

Hypertension is defined as a systolic blood pressure (SBP) higher than 140 or diastolic blood pressure (DBP) higher than 90 mmHg, whereas prehypertension is defined as an untreated SBP of 120–139 mmHg or untreated DBP of 80–89 mmHg.^[1] The one-third of adults in the US (approximately 75 million) affected by hypertension, 11.5 million are

not aware of their hypertension, and 18 million are not being treated.^[2] Drugs such as angiotensin (AT)-converting enzyme inhibitors, AT-1 receptor antagonists, beta-blockers, calcium antagonists, and diuretics are used as monotherapy for the treatment of hypertension. In addition,

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Cite this article as: Kim B, Ma SS, Jo C, Lee S, Choi H, Lee K, *et al.* Vasorelaxant effect of the ethanol extract from *Valeriana fauriei* briquet root and rhizome on rat thoracic aorta. Phcog Mag 2019;15:59-65.

combination treatment with these drugs can significantly improve the overall response.^[3] However, a recent clinical study indicated that 19.01% of hypertensive patients receiving treatment at a hypertension clinic experienced side effects, including dizziness (56.8%), cough (44.6%), edema (28.4%), and rash/itch (17.7%).^[4]

Although synthetic drugs are available to treat hypertension, these drugs cause a variety of side effects. Currently, the use of herbal medicine is increasing because of its safety, efficacy, lower incidence of side effects, and increased cultural acceptability.^[5] Herbs have long been an integral part of human society. Herbal medicines, including reserpine from *Rauwolfia serpentina*, salicin from *Salix alba*, ephedrine from *Ephedra sinica*, and digitoxin from *Digitalis purpurea*, have made great contributions to the pharmaceutical industry.^[6]

In the Korean Herbal Pharmacopoeia, the plant origin of *Valeriana Radix et Rhizoma* (Korean name, Gil-Cho-Gun) is known as *Valeriana fauriei* Briquet. *V. fauriei* is a perennial plant belonging to the family *Valerianaceae*. *V. fauriei* is an important traditional medicine used as a mild sedative and tranquilizer in various cultures.^[7,8] A few studies regarding *V. fauriei* reported that the plant had antidepressant activity,^[9,10] an anxiolytic effect,^[11] a relaxation effect in prenatal stress-related psychiatric disorders,^[12] and nerve growth factor-potentiating activity.^[13,14] More than 150 compounds from the genus *Valeriana* were isolated, including alkaloids, iridoids, and terpenes. Valerianic acid derivatives, valerenic acid derivatives, and kessane compounds are the main compounds found in *Valeriana* plants that have exhibited an antidepressant effect.^[15,16] Moreover, the root and rhizome of *V. fauriei* contain compounds such as kessane, kessanyl acetate, cyclohexyl acetate, maaliol, kanokonyl acetate, patrinoside, kanokoside, caryophyllene oxide, valerianin E, and monovalerianester A.^[17-19] Among these compounds, kessglycol diacetate, kessglycol, and 8-monoacetate are responsible for the sedative effect.^[20]

V. fauriei has been commonly used to treat anxiety and cardiovascular disease in many countries.^[16] Although the vasodilatory effect of 70% methanol (MeOH) extract of *V. fauriei* root was previously reported,^[21] additional or follow-up studies on various mechanisms of the vasorelaxant effect of *V. fauriei* root and rhizome have not been reported yet. Moreover, due to the high toxicity of MeOH relative to ethanol (EtOH) as the extraction solvent of natural products or herbal medicines, we tested *V. fauriei* extracts with EtOH instead of MeOH. Therefore, the aim of this study was to examine the vasorelaxant effect and investigate the various mechanisms of 70% EtOH extract of *V. fauriei* root and rhizome.

MATERIALS AND METHODS

Materials and equipment

NucleoSpin Plant II kit (MACHERRY-NAGEL, GmbH and Co. KG, Germany), Blend Taq (pfu) (Toyobo, Japan), Midori green direct (Nippon genetics, Japan), NucleoSpin Gel and PCR Clean-up (MACHERRY-NAGEL, GmbH and Co. KG, Germany) were used for DNA extraction. For the *trnH-psbA* spacer region a PCR was performed using primers *trnHf* (5'-CGCGCATGGTGGATTCCAC AATCC-3') and *psbA3f* (5'-GTTATGCATGAACGT AATGCTC-3'). For the *rpoC1* spacer region a PCR was performed using primers *rpoC1_2f* (5'-GGCAAAGAGGGAAGATTTTCG-3') and *rpoC1_4r* (5'-CCATAAGCATATCTTGAGTTGG-3'). Krebs-Henseleit (KH) buffer powder, phenylephrine (PE), potassium chloride (KCl), tetraethylammonium (TEA), glibenclamide, 4-aminopyridine (4-AP), calcium chloride (CaCl₂), ethylene glycol-bis (2-aminoethylether)-N, N, N', N'-tetraacetic acid, and dimethyl sulfoxide were purchased from

Sigma-Aldrich (St. Louis, USA). All other reagents were of analytical purity. Rotary vacuum evaporator (NN series, Eyela Co., Japan) and Freeze-dryer (Operon™, Seoul, Korea) were used for the extraction of plant samples. TissueLyser II (Qiagen GmbH, Germany), T100 Thermal cycler (Bio-Rad, USA), and Gel Doc EZ imager (Bio-Rad, USA) were used for DNA sequencing. Isometric force transducer (Grass instrument Co., Rhode Island, USA) and PowerLab data acquisition system (ADI Instrument Co., New South Wales, Australia) were used to measure the tension developed by the contracting/relaxing vessel.

Plant material and extract preparation

V. fauriei, lying 37°14'N latitudes and 128°90'E longitudes, was collected in Manhangjae, Jeongseon County, Gangwon Province, Republic of Korea, in May 2016. Professor Kyungjin Lee of Kyung Hee University identified the plant. The whole-plant voucher specimen (VS16061618) was deposited in the herbarium of the College of Korean Medicine, Kyung Hee University, Seoul, Republic of Korea [Figure 1]. Dried root (100.0 g) of *V. fauriei* was extracted once with 1 L of 70% EtOH for 3 h in a reflux apparatus at 70°C ± 5°C. After filtration, the filtrate was evaporated to dryness using a rotary vacuum evaporator (NN series, Eyela, Japan) at 60°C and lyophilized in a freeze-dryer (Operon™, Seoul, Korea) to obtain a dark brown powder (19.2 g) of crude extract. The 70% EtOH extract of *V. fauriei* (VFE) powder (0.1 g) was accurately weighed into 1 mL KH buffer and placed in an ultrasonic bath for 10 min. The powder was completely dissolved, and the color was dark brown.

Reverse transcription polymerase chain reaction

The dried leaf tissues of *V. fauriei* were ground into fine powder through the Qiagen TissueLyser II. DNA extraction was constructed using the NucleoSpin Plant II kit. The primers, *trnH-psbA* and *rpoC1*, were used for getting sequence of *V. fauriei*. The PCR reaction mixture contained 20 ng/μL of DNA template and 10 pmole/μL of primer, 5 μL 2 mM-dNTP, 5 μL × 10 buffer, and 0.5 μL of Blend Taq (pfu). For reactions, PCR conditions were as follows: predenaturation at 94°C for 3 min, 94°C 30 s; 34 cycles of denaturation, annealing, and extension were repeated at 94°C for 30 s, at 55°C for 30 s, and at 72°C for 1 min and final extension at 72°C for 5 min per 1 kb. 1 μL of PCR products, 3 μL of 100 bp ladder, and 0.5 μL of Midori Green Direct were loaded onto 1.5% agarose gel for electrophoresis (135V, 25 min). DNA band of PCR



Figure 1: The morphology of *Valeriana fauriei* Briquet

products was detected under UV light. The amplified DNA band was purified with the PCR Clean-up kit and DNA sequence was analyzed at Macrogen Inc., Korea.

Animals and preparation of rat aortic rings

Male Sprague-Dawley rats, 8 weeks of age, weighing 240–290 g were provided by Raon Bio, Gyeonggi Province, Korea. All animal procedures were conducted according to the approval from KHUASP(SE)-16-129 by the Kyung Hee University Institutional Animal Care and Use Committee. The animals were housed under standard laboratory conditions (22°C ± 2°C; lighting, 07:00–19:00) with free access to food and water. Rats were anesthetized by ethyl ether; the thoracic aorta was removed and placed in KH buffer (composition [mM]: NaCl, 118.0; KCl, 4.7; MgSO₄, 1.2; KH₂PO₄, 1.2; CaCl₂, 2.5; NaHCO₃, 25.0; and glucose, 11.1; pH 7.4), and continuously aerated with a gas mixture of 95% O₂ and 5% CO₂ at 37°C. The protocol used in this study has been previously described.^[22]

The equation for percentage changes of vasorelaxation after treatment with extracts on aortic rings were precontracted by PE or KCl as follows:

$$\text{Percentage of vasorelaxation} = \frac{[(A - C) - (B - C)]}{(A - C)} \times 100$$

Where A is the maximal contraction of aortic rings after precontraction by PE or KCl; B is the contraction of aortic rings with drug treatment; C is the contraction of aortic rings before precontraction by PE or KCl.

Experimental protocols

Effect of the 70% EtOH extract of *Valeriana fauriei* root and rhizome on phenylephrine- or potassium chloride-induced contraction

We tested the concentration-dependent vasorelaxant effects of VFE (50–1600 µg/mL) on endothelium-intact aortic rings precontracted with PE (1 µM) or KCl (60 mM) in standard KH buffer. Compared to the control group (without VFE), the vasorelaxant effect of VFE was calculated as a percentage of the relaxation in response to PE or KCl.

Effect of the 70% EtOH extract of *Valeriana fauriei* root and rhizome on endothelium-intact or endothelium-denuded aortic rings

We tested the concentration-dependent vasorelaxant effect of VFE (50–1600 µg/mL) on endothelium-intact and endothelium-denuded aortic rings precontracted with PE (1 µM) or KCl (60 mM) in standard KH buffer. The vasorelaxant effect of VFE was calculated as a percentage of the relaxation in response to PE or KCl.

Effect of the 70% EtOH extract of *Valeriana fauriei* root and rhizome on endothelium-intact aortic rings preincubated with various K⁺ channel blockers

We investigated the vasorelaxant effect of VFE (50–1600 µg/mL) in endothelium-intact aortic rings, that were preincubated with various K⁺ channel blocker, TEA (5 mM), glibenclamide (10 µM), or 4-AP (1 mM) for 20 min before the addition of PE (1 µM). The vasorelaxant effect of VFE was calculated as a percentage of the relaxation in response to K⁺ channel blockers pretreatment on the aortic rings.

Effect of the 70% EtOH extract of *Valeriana fauriei* root and rhizome on contraction evoked by extracellular Ca²⁺ influx

We investigated the vasorelaxant effect of VFE (200–800 µg/mL) on extracellular Ca²⁺-induced contractions through receptor-operative Ca²⁺ channels (ROCCs) or voltage-dependent Ca²⁺ channels (VDCCs)

by PE or KCl pretreatment, respectively. We tested the contraction response induced by CaCl₂ (0.3–10 mM) in the endothelium-denuded aortic rings precontracted by PE (1 µM) or KCl (60 mM) in Ca²⁺-free KH solution after preincubation for 10 min with and without (control) VFE. When compared to the control group, the contraction responses induced by CaCl₂ were calculated in the presence and absence (control) of VFE.

Statistical analysis

All results were expressed as mean ± standard error of mean. Statistical comparisons were made using Student's *t*-test. All statistical analyzes were performed using IBM SPSS version 23.0 (SPSS Inc., Chicago, IL, USA). *P* < 0.05 was considered statistically significant.

RESULTS

Sequencing of polymerase chain reaction-amplified DNA from *Valeriana fauriei*

The dried leaf of *V. fauriei* was amplified by PCR reaction and subjected to DNA sequencing. NCBI nucleotide blast search showed 98% (*trnH-psbA*) and 99% (*rpoC1*) sequence identity of *V. fauriei* (GenBank ID: KJ025054.1) and *V. fauriei* (GenBank ID: JF269866.1), respectively.

The DNA sequence of *V. fauriei* was as follow:

>161111-057_M18_A-1_psbA.ab1 296

CCCTATCTGCTCTTGAGCTCCATCTACAATGGATAAGACCTGTT
CTTAGT

GTATATGAGTTTTTGAACAAAGAAAAAAAAAAGGAAAA
AAAAT TTCCCTT

GAGTCTAGGGGGGCTTTATTTCTCCTTTTTTTTCTTTTATTATA
ATGAAA

TAATGAAAATGCATAGTATTTAACTGACATATGTTCTTG
AAAAA ACAGTC

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GCCAAGTGGCTCAAGGCAGTGGATTGTGAATCCACCATGCG
CG AAA

>161111-057_A20_A-3_rpoC1.ab1 536

TAGGCAACGAGTCGATTATTCAGGGCGTTCTGTTATT GTCGTG
GGTCCTT

CACTCTCATTACATCGATGTGGCTTGCCTCGCGAAATA
GCAATA GAGCTT

TTCCAGCCATTTCTAATTCGCGGTCTAATTAGAAAAGC ATCTTG
CTTTGAA

CCTAGGAGTTGCCAAGACTCAAATTCGGGAACGGGA AAAAGA
ACCAATTC

TATGGCAAATACTTCAGGAAGTTATGCAGGGGCATC CTGTATT
GCTGAAT

AGAGCGCCCACTCTGCATAGATTAGGCATACAAGCATT TCAGC
CCGTTTT

AGTGAAGGGCGCGCTATTTGTTTACATCCGTTAGTT TGTA
GGATTCA

ATGCAGACTTTGACGGGGATCAAATGGCTGTTTCATGT GCCTTT
ATCTTTG

GAGGCTCAAGCAGAGGCACGTTTACTTATGTTTTTCTC ATATGA
ATCTTTT

GTCTCCAGCTATTGGGAATCCCATTTCGGTACCAACT CAAGAT
TGGCCTT
ATTGGAAAAAATAGAAACTATGAGAAAAACTATTG

Vasorelaxant effect of the 70% EtOH extract of *Valeriana fauriei* root and rhizome on phenylephrine (or potassium chloride)-induced contraction

VFE caused vasorelaxation on endothelium-intact aortic rings precontracted by PE (1 μM) or KCl (60 mM) treatment. The maximal relaxant effect was 83.9% ± 3.1% and 74.4% ± 2.5% at the concentration of 1600 μg/mL [Figure 2].

Vasorelaxant effect of the 70% EtOH extract of *Valeriana fauriei* root and rhizome on endothelium-intact or endothelium-denuded aortic rings precontracted with phenylephrine or potassium chloride

VFE caused vasorelaxation on the endothelium-intact or endothelium-denuded aortic rings precontracted by PE (1 μM) or KCl (60 mM). The maximal relaxant effect on PE-induced contraction was 83.9% ± 3.1% and 83.9% ± 2.6% for endothelium-intact and endothelium-denuded aortic rings at the concentration of 1600 μg/mL, respectively [Figure 3]. The maximal relaxant effect on KCl-induced contraction was 74.4% ± 2.5% and

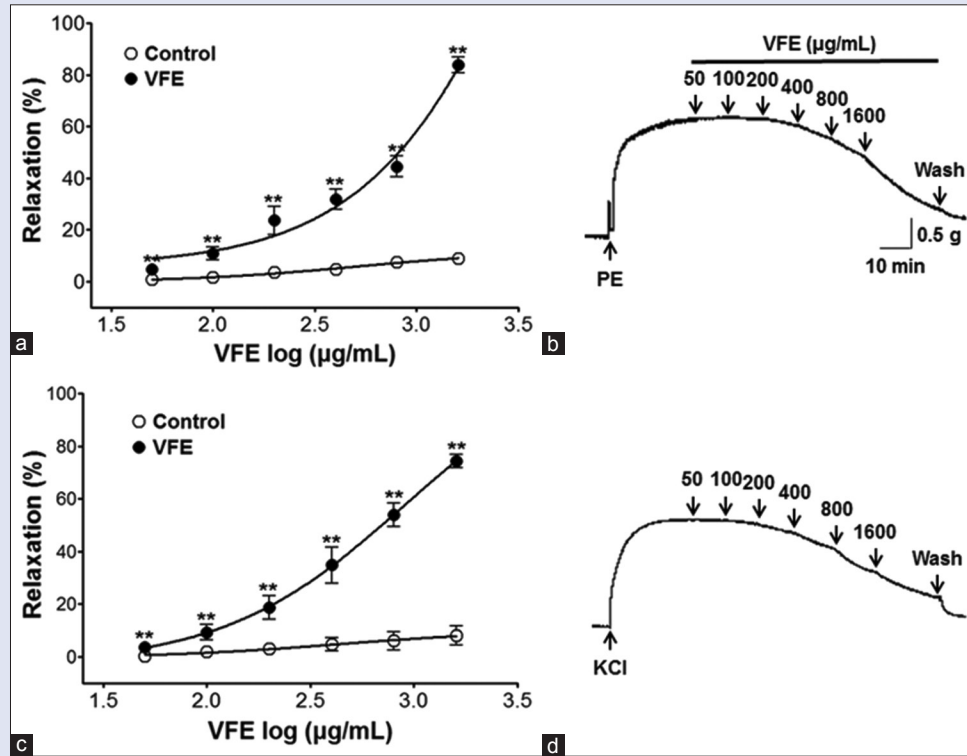


Figure 2: The concentration-dependent relaxation effect and representative traces of the 70% EtOH extract of *Valeriana fauriei* root and rhizome (50–1600 μg/mL) with or without (control) the 70% EtOH extract of *Valeriana fauriei* root and rhizome on phenylephrine (1 μM) (a and b) or potassium chloride (60 mM) (c and d) – precontracted aortic rings. Values are expressed as mean ± standard error of mean (n = 5–9). **P < 0.01 versus control

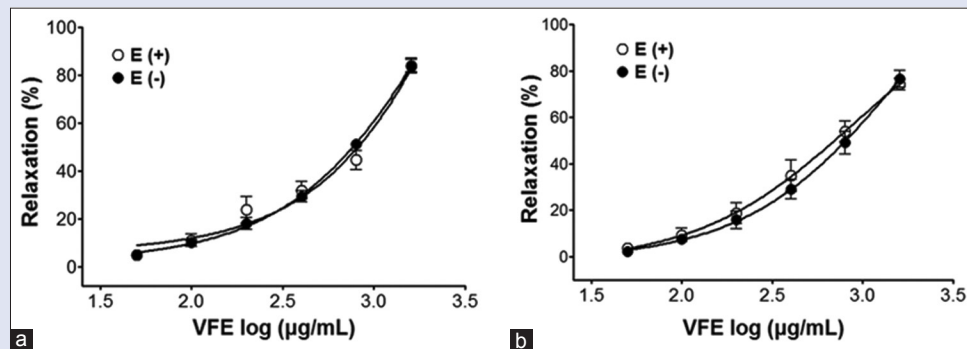


Figure 3: Vasorelaxant effect of the 70% EtOH extract of *Valeriana fauriei* root and rhizome on phenylephrine (1 μM) (a) or potassium chloride (60 mM) (b) – precontracted aortic rings with (E+) or without (E-) endothelium. Values are expressed as mean ± standard error of mean (n = 7–10)

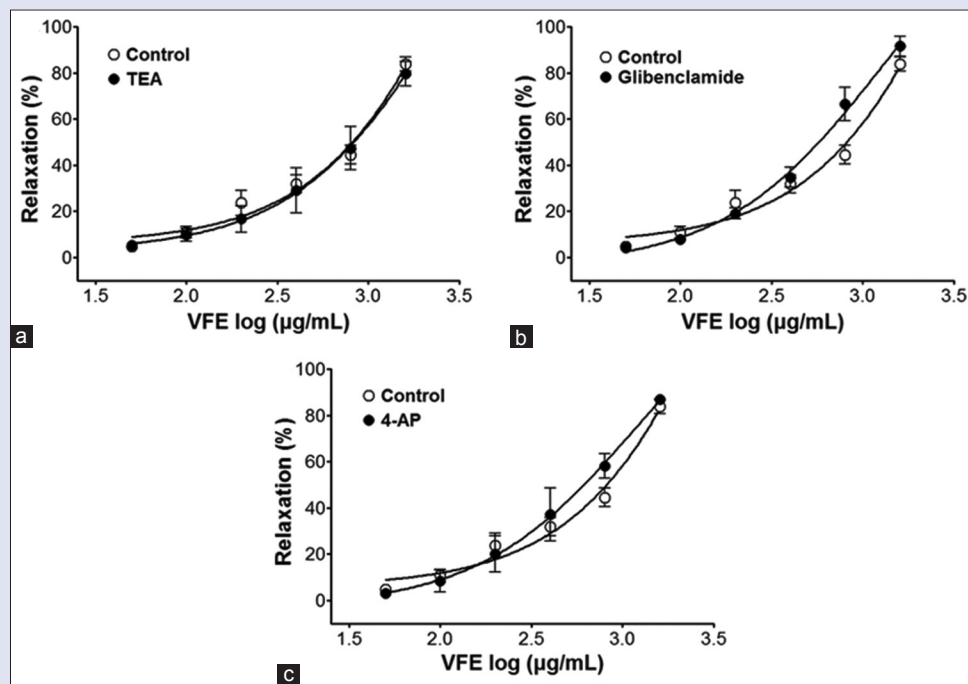


Figure 4: Cumulative concentration-response curves to the 70% EtOH extract of *Valeriana fauriei* root and rhizome on endothelium-intact aortic rings precontracted with phenylephrine (1 μ M) in the presence or absence (control) tetraethylammonium (5 mM) (a), glibenclamide (10 μ M) (b), or 4-aminopyridine (1 mM) (c). Values are expressed as mean \pm standard error of mean ($n = 5-7$)

76.6% \pm 3.7% for endothelium-intact and endothelium-denuded aortic rings at the concentration of 1600 μ g/mL, respectively [Figure 3].

Vasorelaxant effect of the 70% EtOH extract of *Valeriana fauriei* root and rhizome on endothelium-intact aortic rings preincubated with various K^+ channel blockers (tetraethylammonium, glibenclamide, or 4-aminopyridine)

Incubation with K^+ channel blockers such as TEA (Ca^{2+} -activated K^+ channel [K_{Ca}] blocker, 5 mM), glibenclamide (ATP-sensitive K^+ channel [K_{ATP}] blocker, 10 μ M), or 4-AP (voltage-gated K^+ channel [K_v] blocker, 1 mM) did not affect VFE-induced relaxation on endothelium-intact aortic rings pre-contracted by PE (1 μ M) treatment. In the presence of TEA, glibenclamide, or 4-AP, the VFE-induced relaxant effect was of 79.7% \pm 5.3%, 91.5% \pm 4.3%, 86.8% \pm 2.2% versus the control group 83.9% \pm 3.1% at the concentration of 1600 μ g/mL, respectively [Figure 4].

Effect of the 70% EtOH extract of *Valeriana fauriei* root and rhizome on extracellular Ca^{2+} -induced contraction (via receptor-operative Ca^{2+} channels or voltage-dependent Ca^{2+} channels)

In Ca^{2+} -free KH solution, the cumulative addition of $CaCl_2$ (0.3–10 mM) to the organ baths induced gradually increased tension on the rat thoracic aorta by PE (1 μ M) or KCl (60 mM) treatment. Preincubation with VFE (200–800 μ g/mL) significantly inhibited the contractions induced by $CaCl_2$ (10 mM). The contraction at VFE (800 μ g/mL) treatment was decreased to 0.19 \pm 0.17 g and -0.15 \pm 0.04 g versus the control group 1.74 \pm 0.08 g and 1.27 \pm 0.07 g, on aortic rings precontracted by PE and KCl, respectively [Figure 5].

DISCUSSION

Previous studies indicated that the root and rhizome of *V. fauriei* mainly contain iridoid glycosides and kessane sesquiterpenoids and the herb has been used to treat anxiety and convulsions.^[14] Previous studies have shown that the maximal vasorelaxant effects of the chloroform ($CHCl_3$) and water layers of the 70% MeOH extract of *V. fauriei* root were 98.1 \pm 0.7 (1 mg/mL) and 96.9 \pm 2.6 (10 mg/mL), respectively. The relaxant effects of the $CHCl_3$ layers were more effective than those of the water layers on rat thoracic aortas contracted by 5-HT (100 μ M).^[21] In the present study, we used 70% EtOH as the extraction solvent instead of MeOH because MeOH can cause metabolic acidosis, neurologic sequela, and even death. VFE concentration dependently caused gradual relaxation of endothelium-intact aortic rings contracted by PE (1 μ M) and KCl (60 mM). The maximal relaxant effects were 83.9% \pm 3.1% and 74.4 \pm 2.5% at concentration of 1600 μ g/mL. *V. fauriei* may be a good natural medicine candidate for the treatment of hypertension.

PE is an $\alpha 1$ -adrenergic agonist that contracts vascular smooth muscle cells (VSMCs) by extracellular Ca^{2+} entry through ROCCs and by intracellular Ca^{2+} release from the sarcoplasmic reticulum through IP3R pathway,^[23] whereas high concentration KCl contracts VSMCs by extracellular Ca^{2+} influx through VDCCs after depolarization of the cell membrane.^[24] Therefore, PE and KCl were used as vasoconstrictors to investigate the vasorelaxation of VFE.

Normal endothelial cells, which exist in the vascular walls of the smooth muscle cells, are important regulators of vascular homeostasis. Moreover, they are related to changes in blood composition and blood flow. Endothelial dysfunction causes several pathological conditions, including atherosclerosis, owing to coagulation, inflammation, and vasoconstriction.^[25] It is well known that vascular endothelial cell plays an important role in regulating the function of cardiovascular system by secretion of vasodilator such as endothelium-derived relaxing factor (NO), prostacyclin, and endothelium-derived hyperpolarizing

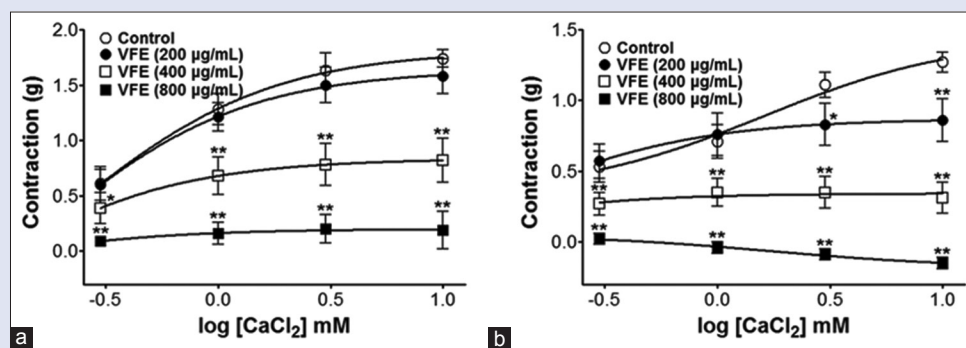


Figure 5: Inhibitory effect of the 70% EtOH extract of *Valeriana fauriei* root and rhizome (200–800 µg/mL) on the contraction induced by Ca^{2+} influx in endothelium-denuded rat thoracic aorta rings that were precontracted with phenylephrine (1 µM) (a) or potassium chloride (60 mM) (b) in the presence or absence (control) of the 70% EtOH extract of *Valeriana fauriei* root and rhizome. Values are expressed as mean \pm standard error of mean ($n = 5-6$). * $P < 0.05$, ** $P < 0.01$ versus control

factor (EDHF) and vasoconstrictors including endothelin.^[26] In this study, VFE concentration dependently relaxed both endothelium-intact and endothelium-denuded aortic rings that were precontracted by PE or KCl. This result indicated that the activity of VFE was not related to endothelial function.

K^+ channels are the main regulators of vascular tone and diameter and activation of K^+ channels can lower blood pressure through vasodilation. Conversely, deactivation of K^+ channels can raise blood pressure through vasoconstriction of VSMCs. Four types of K^+ channels have been identified, including nonselective K_{Ca} , K_{ATP} , K_{V} , and inward rectifier K^+ channels.^[24] Along with argument that the efflux of K^+ from the potassium channels in the endothelium cell is partly related to a part of EDHF causing vasorelaxation,^[27] large conductance K_{Ca} channels, K_{ATP} channels, and K_{V} channels are especially abundant in VSMCs.^[28] In this study, endothelium-intact aortic rings without unintended wounds by removing the endothelium on the VSMCs were used to evaluate the involvement of various potassium channels and VFE-induced vasorelaxation effects. We tested various K^+ channel blockers, including TEA (K_{Ca} channel blocker), glibenclamide (K_{ATP} channel blocker), and 4-AP (K_{V} channel blocker). VFE relaxed the aortic rings that were precontracted by PE (1 µM), irrespective of K^+ channel blockers pretreatment. Therefore, VFE-induced relaxation was not related to K^+ channels such as K_{Ca} channels, K_{ATP} channels, and K_{V} channels.

Ca^{2+} is a major factor involved excitation-contraction coupling that initiates contraction of smooth muscle. Considering the influx of extracellular calcium ion into the cytosol, there are typically three types of calcium channels such as ROCCs, L-type VDCCs, and store-operated calcium channels (SOCCs).^[29] The mechanism of vascular smooth muscle contraction and relaxation involves various signal transduction pathways, which are mainly regulated by the influx of extracellular Ca^{2+} through ROCCs or VDCCs.^[30] In the present study, we denuded the endothelium to achieve more precise VFE-caused blocking results at the entry of extracellular Ca^{2+} through ROCCs or VDCCs in VSMCs, even though VFE-induced vasorelaxation effects were endothelium independent. VFE concentration dependently inhibited vasoconstriction caused by extracellular Ca^{2+} influx through ROCCs or VDCCs activated by PE or KCl, respectively, in Ca^{2+} -free KH buffer.

More than 150 compounds from the genus *Valeriana* were isolated. *V. fauriei* contains a variety of compounds, including kessane, kessanyl acetate, patrinolide, kanokoside, caryophyllene oxide, and valerianin E. However, more pharmacological studies are needed regarding the vasorelaxation mechanism of these compounds. Therefore, further

research should be carried out to determine the mechanism of action of the diverse compounds from this plant on rat aortic rings.

CONCLUSION

We concluded that the vasorelaxant effects of VFE were endothelium independent and (2) not related to K^+ channels, such as K_{Ca} , K_{ATP} and K_{V} channels, and (3) VFE relaxed the aortic rings by blocking the entry of extracellular Ca^{2+} through ROCCs and VDCCs.

Acknowledgements

This research was supported by a grant (15172MFDSD184) from the Ministry of Food and Drug Safety in the study on improvement for quality standards of the official document on herbal medicine (2015–2017).

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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