Anti-inflammatory and anti-migratory properties of wild-grape-derived ε-viniferin in human endometrial cells: A potential therapy for endometriosis

YINZHI LIN[1,2], YOSHIKAZU NAIKI[3], SHIORI KOJIMA[1,4], MASATOSHI HAKAMATA[1,5], HITOMI FUKATSU[4], YOSHIAKI HASEGAWA[3], AKIHIKO WAKATSUKI[6], NAOKI KOIDE[2] and KAZUO UMEZAWA[1]

[1]Department of Molecular Target Medicine, Aichi Medical University, Nagakute, Aichi 480-1195, Japan; [2]Department of Microbiology and Immunology, Aichi Medical University, Nagakute, Aichi 480-1195, Japan; [3]Department of Microbiology, School of Dentistry, Aichi Gakuin University, Nagoya, Aichi 464-8650, Japan; [4]Fukuyu Medical Institute, Fukuyu Medical Corporation, Nisshin, Aichi 470-0103, Japan; [5]Biotechnology Section, Industrial Research Institute of Shizuoka Prefecture, Numazu, Shizuoka 410-0022, Japan; [6]Department of Obstetrics and Gynecology, Aichi Medical University, Nagakute, Aichi 480-1195, Japan

Received March 14, 2025; Accepted June 27, 2025

DOI: 10.3892/mmr.2025.13648

Abstract. Endometriosis is a chronic gynecological disorder characterized by the ectopic growth and proliferation of endometrial tissue outside the uterine cavity, leading to inflammation. Although low-dose contraceptive pills are widely used for its treatment, these therapies are associated with side effects and are contraindicated in women trying to conceive. ε-viniferin is a resveratrol dimer found in wild grapes (*Ampelopsis brevipedunculata*) with demonstrated antioxidant properties and an anti-migratory effect in lung cancer cells. Because abnormal cell migration is a key process in endometriosis, we hypothesized that ε-viniferin could also exhibit anti-migratory effects in endometriotic cells, which remain to be elucidated. To test this hypothesis, the present study investigated the anti-inflammatory and anti-endometriotic effects of ε-viniferin and wild-grape extract. RAW264.7 and THP-1 were used to evaluate the anti-inflammatory effects of wild-grape extract and ε-viniferin by assessing cytotoxicity; nitric oxide (NO), reactive oxygen species and interleukin-6 (IL-6) production; and NF-κB activity. Additionally, human endometrial stromal cells (HESCs) were used to investigate the anti-endometriotic effects of ε-viniferin, including its impact on cell migration, invasion and gene expression, using PCR array analysis. Both ε-viniferin and wild-grape extract significantly reduced lipopolysaccharide-induced NO and IL-6 production, indicating an anti-inflammatory activity. Inhibition of NF-κB activity at the cellular level further supported these findings. Moreover, both ε-viniferin and wild-grape extract effectively suppressed the migration and invasion of HESCs, indicating their potential to alleviate endometriosis symptoms. These findings suggest that ε-viniferin is a promising therapeutic candidate for endometriosis, exhibiting both anti-inflammatory and anti-migratory effects. Our results present a novel approach for developing effective anti-endometriotic therapies.

Introduction

Endometriosis is a prevalent gynecological disorder associated with female infertility. It affects approximately 10% of women of reproductive age and 25-50% of women experiencing infertility. This disorder is characterized by the growth of endometrial tissue outside the uterine cavity, leading to symptoms such as inflammation, dysmenorrhea, infertility, and dyspareunia. These symptoms significantly impair the quality of life for women and are often accompanied by psychological burdens (1,2).

However, effective long-term management of endometriosis remains challenging despite the availability of treatment options that include both surgical and non-surgical approaches. Surgical approaches for endometriosis include minimally invasive techniques such as laparoscopy, laser ablation, and electrocoagulation for early-stage cases, whereas advanced surgical procedures such as laparotomy, oophorectomy, and colorectal surgery are required for deep infiltrating endometriosis. However, these interventions carry risks, including recurrence due to incomplete excision, ovarian dysfunction, voiding impairment, and tissue damage (3-5). Several non-surgical treatment options are currently available for managing endometriosis, with the most common approaches being hormone therapy and pain management. Low-dose oral contraceptives, which contain estrogen and

Correspondence to: Dr Yoshikazu Naiki, Department of Microbiology, School of Dentistry, Aichi Gakuin University, 1-100 Kusumoto-cho, Chikusa, Nagoya, Aichi 464-8650, Japan
E-mail: naikiy@dpc.agu.ac.jp

Abbreviations: THP-1, human monocytic leukemia cell line; HESCs, human endometrial stromal cells

Key words: ε-viniferin, wild-grape exact, endometriosis, inflammation, migration, invasion

progesterone, are widely used and are shown to alleviate menstrual pain, ameliorate infertility, reduce dyspareunia, and shrink endometriotic lesions. However, these treatments are associated with adverse effects, such as nausea, headache, and breakthrough bleeding, and an increased risk of thrombosis with long-term use. Additionally, they are contraindicated in women who wish to conceive (3-6). Other treatment options include aromatase inhibitors and progestin-based medications. Aromatase inhibitors are drugs that inhibit the enzyme aromatase, which converts androgens, the precursors of estrogen, into estrogen, thereby suppressing estrogen production. Although aromatase inhibitors reduce dysmenorrhea and shrink lesions, they are expensive and decrease bone mineral density (7,8). Progestin-based medications, which suppress estrogen activity and inhibit endometrial proliferation, also cause adverse effects such as breakthrough bleeding, and their efficacy is typically lower than that of low-dose oral contraceptives (9). Other treatment options, such as nonsteroidal anti-inflammatory drugs (such as ibuprofen), carry cardiovascular risks with long-term use, whereas neuromodulators and antidepressants (such as gabapentinoids) used for chronic pain management may lead to dependency (10,11). Hence, effective and safe treatment options must be explored for endometriosis.

Recently, considerable progress has been made in understanding the pathophysiology of endometriosis; genetic and immunological factors involved in the ectopic occurrence, proliferation, and invasion of endometrial tissue are gradually being elucidated. For instance, interleukin (IL)-8 and endothelin, secreted by ectopic endometrial tissue, promote inflammatory responses mediated by immune cells and contribute to symptoms such as pain and complications such as adhesions (12-14). These advances in understanding have spurred efforts to develop alternative treatment methods. Subsequently, natural compounds are being researched as effective therapeutic options with minimal side effects for endometriosis, and the potential of resveratrol, an anti-inflammatory and antioxidant compound, for the treatment of endometriosis has been demonstrated (15-17). However, the therapeutic utility of resveratrol is limited by its low absorption rates. In contrast, ε-viniferin, a dimer of resveratrol, exhibits increased lipophilicity, which enhances membrane permeability and absorption in the gastrointestinal tract and liver (Fig. 1). Pharmacokinetic studies have also demonstrated high bioaccumulation of ε-viniferin in white adipose tissue, suggesting that these tissues may serve as a reservoir for its native form, enabling slow release and prolonged systemic presence (18-20).

ε-viniferin is the main functional compound in wild grapes (*Ampelopsis brevipedunculata*), with antioxidant, anti-inflammatory, and anti-tumor effects (21-24). It also has neuroprotective effects, which may be beneficial in the treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases (25), and preventive effects against obesity-related morbidities, such as type 2 diabetes, dyslipidemia, hypertension, and fatty liver (26). ε-viniferin inhibits TGF-β1-induced epithelial-mesenchymal transition, migration, and invasion in lung cancer cells by downregulating vimentin (27). Elevated levels of TGF-β1 have also been implicated in the development of endometriosis by promoting cell migration and invasiveness (28). Given these shared mechanistic pathways, we hypothesized that ε-viniferin exerts beneficial effects in endometriosis by inhibiting key processes involved in the disease. However, its potential effects on endometrial cells remain unclear.

In this study, we aimed to investigate the anti-inflammatory effects of ε-viniferin and wild-grape extract, along with their inhibitory effects on the migration and invasion of human endometrial stromal cells (HESCs). We believe that our findings would contribute to the development of ε-viniferin as a potential therapeutic option for the effective management of endometriosis.

Materials and methods

Materials. Wild-grape extract (cat. no. 30811033) and ε-viniferin (cat. no. NS3013) were purchased from Maruzen Pharmaceuticals Co., Ltd. and Nagara Science Co., Ltd., respectively.

Cell culture. Mouse macrophage-like cell line RAW264.7 (RRID:CVCL_0493, cat. no. TIB-71; American Type Culture Collection) and human monocytic leukemia cell line THP-1 (RRID:CVCL_0006, cat. no. RCB3686; Riken Bioresource Center) were cultured in RPMI1640 medium (cat. no. 189-02025; FUJIFILM Wako Pure Chemical Corporation) supplemented with 10% inactivated fetal bovine serum (cat. no. F7524, non-USA origin; Sigma-Aldrich; Merck KGaA) and 1% (v/v) penicillin-streptomycin (cat. no. 15140122; Gibco; Thermo Fisher Scientific, Inc.) at 37˚C in a humidified incubator with 5% CO_2. THP-1 cells ($1.5x10^4$ cells/well) were seeded into 96-well plates and differentiated into macrophage-like cells using 0.1 μg/ml phorbol 12-myristate 13-acetate (PMA; cat. no. P1585; Sigma-Aldrich; Merck KGaA) for 72 h.

To investigate the potential application of ε-viniferin for its anti-endometriotic effects, we used HESCs, which are derived from ectopic endometrial tissues associated with endometriosis and drive the abnormal migration and invasion of endometrial tissue.

Immortalized HESCs (cat. no. T0533; Applied Biological Materials Inc., Richmond, BC, Canada, https://www.abmgood.com/immortalized-human-endometrial-stromal-cells-hesc.html) were cultured in Prigrow IV medium (cat. no. TM004; Applied Biological Materials, Inc.) supplemented with 2 mM L-Glutamine (cat. no. G275; Applied Biological Materials, Inc.), 10% charcoal-stripped fetal bovine serum (cat. no. 12676-029; Gibco), and 1% (v/v) penicillin-streptomycin at 37˚C in a humidified incubator containing 5% CO_2.

Cell viability. RAW264.7 cells ($3x10^4$ cells/well) were seeded into 96-well plates and incubated for 1 h. Differentiated THP-1 cells were rinsed with phosphate-buffered saline (PBS) and fresh medium before overnight incubation. HESCs ($1.5x10^4$ cells/well) were seeded into 96-well plates and incubated overnight. The test chemicals, namely, wild-grape extract at 0.03, 0.1, and 0.3 mg/ml and ε-viniferin at 3, 10, and 30 μg/ml, were added, and the cells were incubated for an additional 24 h at 37˚C. Dimethyl sulfoxide (DMSO) was used as the control. MTT reagent (working concentration: 0.5 mg/ml; cat. no. 10009591; Cayman Chemical) was added to each well and incubated for 2 h at 37˚C. The supernatant was replaced with 100 μl of DMSO to dissolve the formazan

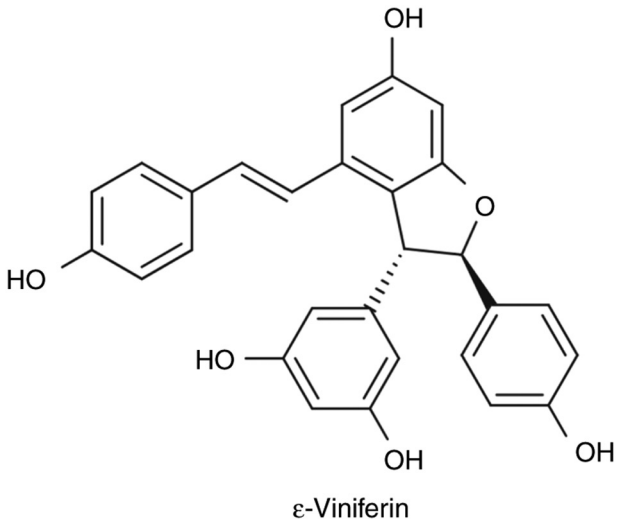

Figure 1. Chemical structure of ε-viniferin.

crystals. Absorbance was measured at 570 nm using a microplate reader (Bio-Rad Laboratories).

Nitric oxide (NO) production. RAW264.7 cells ($3x10^4$ cells/well) were seeded into 96-well plates and incubated for 1 h. Test chemicals were added to each well and incubated for an additional hour, followed by exposure to lipopolysaccharide (LPS, 100 ng/ml; cat. no. L5293; Sigma-Aldrich; Merck KGaA) for 24 h. The supernatant was collected, and 50 μl aliquots were mixed with an equal volume of Griess reagent in a 96-well plate. Absorbance was measured at 570 nm using a microplate reader (Bio-Rad Laboratories).

Measurement of IL-6 production RAW264.7 cells ($3x10^4$ cells/well) were seeded into 96-well plates and incubated for 1 h. Differentiated THP-1 cells were washed with PBS and fresh medium before overnight incubation. The test chemicals, namely, wild-grape extract at 0.1, 0.2, and 0.3 mg/ml and ε-viniferin at 10, 20, and 30 μg/ml, were added to each well and incubated for 1 h, followed by exposure to LPS (100 ng/ml) for 24 h. IL-6 concentration in the supernatants were measured using Mouse IL-6 ELISA Kit (cat. no. M6000B; R&D Systems) for RAW264.7 cells and Human IL-6 ELISA Kit (cat. no. D6050; R&D Systems) for THP-1 cells, following the manufacturer's protocol. Plates were washed four times, and horseradish peroxidase-conjugated IL-6 was added, followed by a 2 h incubation at 23-25˚C. Substrate solutions of tetramethylbenzidine (TMB) and hydrogen peroxide were added to each well after washing four times, and plates were incubated for 20 min at room temperature in the dark. The reaction was quenched using diluted hydrochloric acid. Absorbance was measured at 450 nm using a microplate reader (Bio-Rad Laboratories).

Measurement of reactive oxygen species (ROS) production. RAW264.7 cells ($3x10^4$ cells/well) were seeded into 96-well plates and incubated overnight. Test chemicals at various concentrations were added, and the cells were incubated for 1 h, followed by exposure to LPS (100 ng/ml) for 24 h at 37˚C. ROS production was determined using DCFH-DA (20 μM; cat. no. 35845; Sigma-Aldrich; Merck KGaA), an oxidant-sensitive fluorescent probe. The medium was removed from each well, and the cells were washed twice with Ca^{2+}, Mg^{2+}-free PBS (PBS-) and incubated with DCFH-DA for 30 min. After removing the supernatant and washing twice with PBS-, 200 μl PBS- was added to each well. Fluorescence was measured with excitation and emission wavelengths of 485 nm and 535 nm, respectively, using a fluorescence plate reader (SpectraMax M5; Molecular Devices).

Measurement of DPPH radical scavenging ratio. The scavenging effect was assessed using the DPPH Antioxidant Assay Kit (cat. no. D678; Dojindo). Briefly, 20 μl of the sample solution was added to each well of a 96-well microplate, followed by 80 μl of assay buffer and 100 μl of 2,2-diphenyl-1-picrylhydrazyl (DPPH) working solution. Wild-grape extract solutions (at 0.1, 0.3, and 1 mg/ml) and ε-viniferin (at 3, 10, and 30 μg/ml) were used as samples. Trolox at 80 μg/ml was used as a positive control. The plate was incubated for 30 min at room temperature in the dark. Absorbance was measured at 517 nm using a microplate reader (SpectraMax M5).

RNA isolation, reverse transcription-quantitative polymerase chain reaction (RT-qPCR), and reverse transcription-PCR (RT-PCR)

RAW264.7 cells ($1x10^6$ cells/well) were seeded into 60 mm dishes and incubated for 24 h. Test chemicals were added to each dish and incubated for 1 h, followed by LPS treatment (100 ng/ml) for 4 h at 37˚C. RNA was extracted using TRIzol reagent (1 ml; cat. no. 15596018; Invitrogen; Thermo Fisher Scientific, Inc.) and reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (cat. no. 4368814; Applied Biosystems; Thermo Fisher Scientific, Inc., Vilnius, Lithuania) at 25˚C for 10 min, 37˚C for 120 min, and 85˚C for 5 min. For RT-qPCR, cDNA was amplified in triplicate using KOD FX Neo PCR Buffer (14 μl) and dNTPs (cat. no. KFX-201; Toyobo Co., Ltd.). Target DNA sequences for each primer pair were amplified in triplicate under the following conditions: initial denaturation at 94˚C for 10 sec, followed by 40 cycles of 94˚C for 10 sec, 60˚C for 10 sec, and 70˚C for 20 sec, using the QuantStudio 3 system (Applied Biosystems, Singapore). *mGapdh* and *ACTB* were used as internal controls. Relative mRNA expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method (29).

For RT-PCR, PCR products were separated on a 2% agarose gel, and the band intensities were analyzed using ImageJ software (ImageJ 1.53e with Java 1.8.0_172; National Institutes of Health). The following primer pairs were used: mouse *iNOS*, 5'- GTCTTGCAAGCTGATGGTCA -3' (forward) and 5'- ACCACTCGTACTTGGGATGC -3' (reverse); mouse *Il1β*, 5'-CGTGGACCTTCCAGGATGAG-3' (forward) and 5'-GGAGCCTGTAGTGCAGTTGTC-3' (reverse); mouse *Il6*, 5'-ACCACGGCCTTCCCTACTTC-3' (forward) and 5'-CACAACTCTTTTCTCATTTCCACG-3' (reverse); human *IL6*, 5'-AGACAGCCACTCACCTCTTCAG-3' (forward) and 5'- TTCTGCCAGTGCCTCTTTGCTG-3' (reverse); *mouse Gapdh*, 5'-TGCACCACCAACTGCTTAG-3' (forward) and 5'-GATGCAGGGATGATGTTC-3' (reverse); and human *ACTB*, 5'-CTTCTACAATGAGCTGCGTG-3' (forward) and 5'-TCATGAGGTAGTCAGTCAGG-3' (reverse).

Wound healing assay. HESCs ($1x10^5$ cells/well) were seeded into 24-well plates and cultured overnight until confluence. A uniform scratch was created across the center of the well using a 200 μl pipette tip. Floating cells and the growth medium were removed, and serum-free medium containing the test chemicals was added to each well. Cells were incubated for an additional 8 h, and their movement into the scratched area was recorded every 2 h using a phase-contrast microscope (Nikon Eclipse TS100).

Matrigel invasion chamber assay. HESCs ($5x10^4$ cells/well) suspended in 500 μl serum-free Prigrow IV medium containing test chemicals were seeded into commercial Matrigel-coated chambers (BD Matrigel Basement Membrane Matrix; cat. no. 354480; Corning). The lower chambers were filled with 750 μl of Prigrow IV medium with 10% FBS and were incubated for 16 h at 37˚C. Thereafter, non-invading cells were removed by wiping the upper surface of the membrane with a cotton swab, and invading cells on the lower surface of the membrane were stained with Diff-Quick (cat. no. 16920; Sysmex) according to the manufacturer's instruction and counted under a phase-contrast microscope (Nikon Eclipse TS100).

PCR array. Total RNA was extracted from HESCs using the RNeasy Mini Kit (cat. no. 74106; Qiagen, Hilden, North Rhine-Westphalia, Germany), treated with ε-viniferin for 8 h, and reverse transcribed using the RT^2 First Strand Kit (cat. no. 330401; Qiagen, Germantown, MD, USA). The cDNA was applied to the Human Tumor Metastasis PCR Array (cat. no. 330231; Qiagen, Germantown, MD, USA) using RT^2 SYBR-Green ROX qPCR Mastermix (cat. no. 330520; Qiagen, Germantown, MD, USA). Data were analyzed using the $2^{-\Delta\Delta Cq}$ method (29).

Measurement of NF-κB activity. RAW264.7 cells ($3x10^6$ cells/well) were seeded into 6-well plates and incubated for 1 h. Test chemicals at various concentrations were added to each well and incubated for 1 h, followed by LPS treatment (100 ng/ml) for 2 h at 37˚C. Nuclear extracts were prepared using the Nuclear Extract Kit (cat. no. 40010; Active Motif) according to the manufacturer's instructions. NF-κB binding activity was measured using nuclear extract (5 μg) with the TransAM NF-κB p65 Transcription Factor Assay Kit (cat. no. 40096; Active Motif).

Statistical analysis. Results are presented as mean±standard deviation (SD). Statistical analysis was performed using GraphPad Prism version 10.0 (Dotmatics). Differences between the two groups were analyzed using the Student's t-test. One-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test was used for comparisons among more than two groups. Statistical significance was set at $P<0.05$.

Results

Wild-grape extract suppresses inflammatory regulators and ROS in RAW264.7 cells. The anti-inflammatory effects of wild-grape extract were evaluated in RAW264.7 cells. Wild-grape extract was not cytotoxic to RAW264.7 cells after 24 h of treatment at concentrations below 0.3 mg/ml (Fig. 2A). The extract suppressed LPS-induced NO and ROS production in RAW264.7 cells (Fig. 2B and C) and exhibited significant dose-dependent antioxidant activity in the DPPH assay (Fig. 2D). As inducible nitric oxide synthase (iNOS) is responsible for NO production, we explored the effect of wild-grape extract on LPS-induced iNOS and the expression of downstream inflammatory factors, IL-6 and IL-1β. Although wild-grape extract did not inhibit LPS-induced iNOS or IL-1β expression, it significantly inhibited LPS-induced IL-6 expression (Fig. 2E). We further validated this inhibition using RT-qPCR and ELISA in RAW264.7 cells (Fig. 2F and G). These findings indicate that wild-grape extract suppresses ROS-activated IL-6 inflammatory reactions in RAW264.7 cells.

ε-viniferin reduces inflammation through ROS suppression and NF-κB inhibition in RAW264.7 cells. We investigated the anti-inflammatory action of ε-viniferin in RAW264.7 cells. ε-viniferin was not cytotoxic to RAW264.7 cells after 24 h of treatment at concentrations below 10 μg/ml (Fig. 3A). ε-viniferin, similar to wild-grape extracts, showed significant dose-dependent antioxidant activity in the DPPH assay (Fig. 3B). Although ε-viniferin did not suppress LPS-induced NO production (Fig. 3C), it suppressed LPS-induced ROS production at non-toxic concentrations (Fig. 3D). These results suggest that the anti-inflammatory effects of ε-viniferin partially stem from the suppression of ROS production.

We then explored the effects of ε-viniferin on IL-6 production in RAW264.7 cells because wild-grape extract inhibited LPS-induced IL-6 production and IL-6 production is an early response in LPS-induced inflammatory reaction. Within 4 h of exposure, ε-viniferin was not cytotoxic to RAW264.7 cells at a concentration of 30 μg/ml (Fig. 3E). However, it significantly inhibited LPS-induced IL-6 production both at gene and protein levels (Fig 3F and G).

We also tested the effect of ε-viniferin on NF-κB activity, as this transcription factor is present upstream of IL-6, and NF-κB activation was significantly suppressed by ε-viniferin in a concentration-dependent manner (Fig. 3H). These results suggest that the NF-κB-mediated IL-6 signaling pathway is central to the anti-inflammatory effects of ε-viniferin in RAW264.7 cells.

ε-viniferin exhibits anti-inflammatory effects in human macrophage-like THP-1 cells. To further validate the anti-inflammatory effects of ε-viniferin, we used THP-1 cells, which differentiate into macrophage-like cells upon stimulation with phorbol esters such as phorbol-12-myristate-13-acetate (PMA). ε-viniferin (30 μg/ml) was not cytotoxic to THP-1 cells after 4 h of treatment (Fig. 4A). ε-viniferin significantly suppressed LPS-induced *IL6* mRNA expression at 20 μg/ml and its protein production at 10 μg/ml (Fig. 4B and C). These results confirm that ε-viniferin exhibits anti-inflammatory effects in both mouse- and human-derived macrophage-like cells.

ε-viniferin inhibits migration and invasion of HESCs and modulates inflammatory signaling pathways. ε-viniferin was not cytotoxic to HESCs at concentrations below 10 μg/ml (Fig. 5A). It suppressed HESC migration at a concentration of 1 μg/ml and invasion at concentrations of 3 μg/ml and

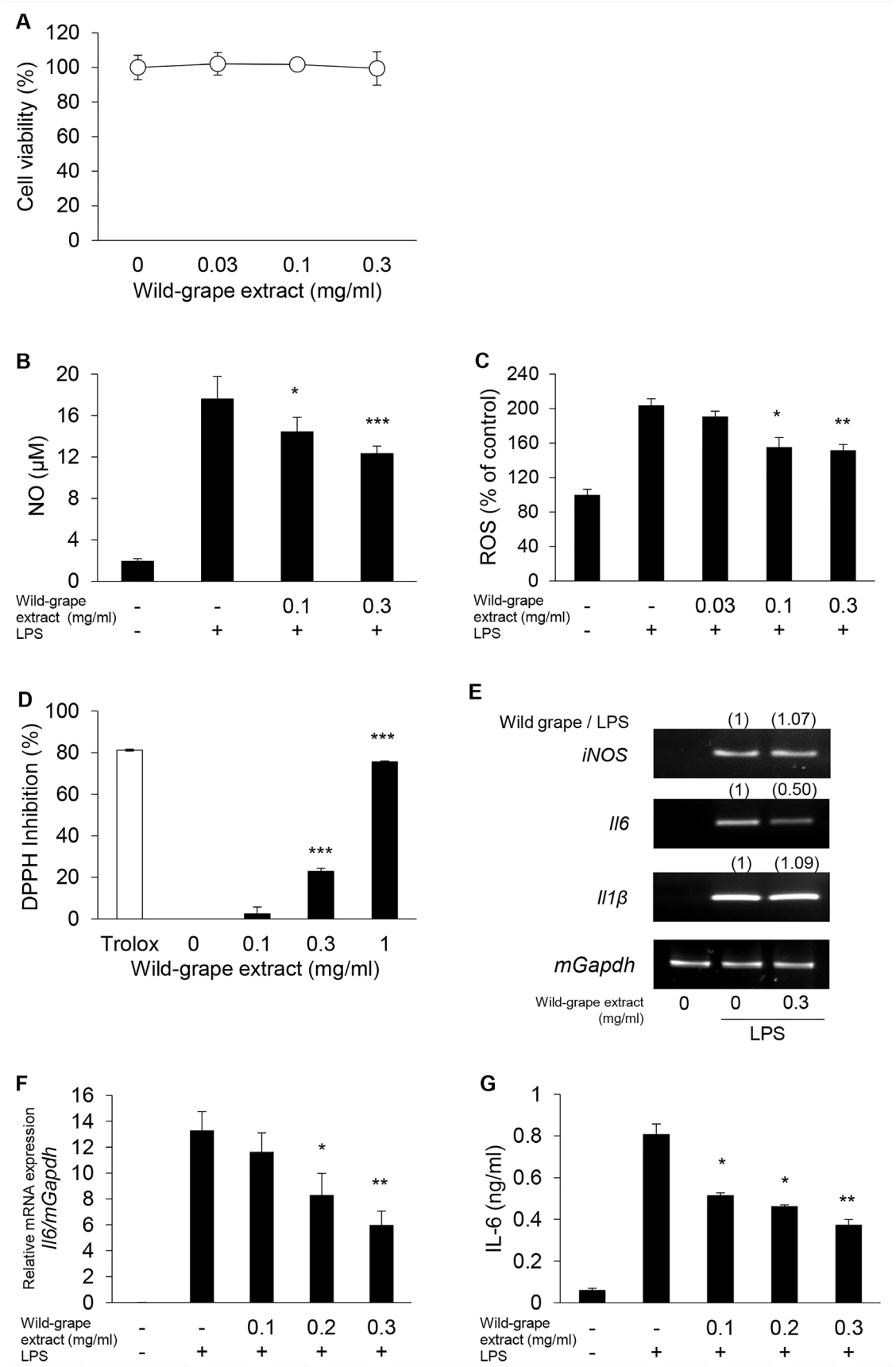

Figure 2. Inhibition of inflammatory regulators and ROS production by wild-grape extract in RAW264.7 cells. (A) Effect of wild-grape extract on cell viability after 24 h of exposure, assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) assay. (B) Inhibition of LPS-induced NO production. (C) Reduction of LPS-induced ROS production, measured based on fluorescent intensity. (D) *In vitro* antioxidant activity determined using the DPPH radical-scavenging assay. (E) Effect on LPS-induced expression of iNOS, IL-6 and IL-1β. (F) Inhibition of LPS-induced IL-6 expression. (G) Inhibition of LPS-induced IL-6 secretion. RAW264.7 cells were stimulated with 100 ng/ml LPS in the presence or absence of wild-grape extract. n=3. *P<0.05, **P<0.01, ***P<0.001 vs. control without the extract. LPS, lipopolysaccharide; NO, nitric oxide; ROS, reactive oxygen species; DPPH, 2,2-diphenyl-1-picrylhydrazyl; iNOS, inducible NOS; IL, interleukin; mGapdh, mouse glyceraldehyde-3-phosphate dehydrogenase.

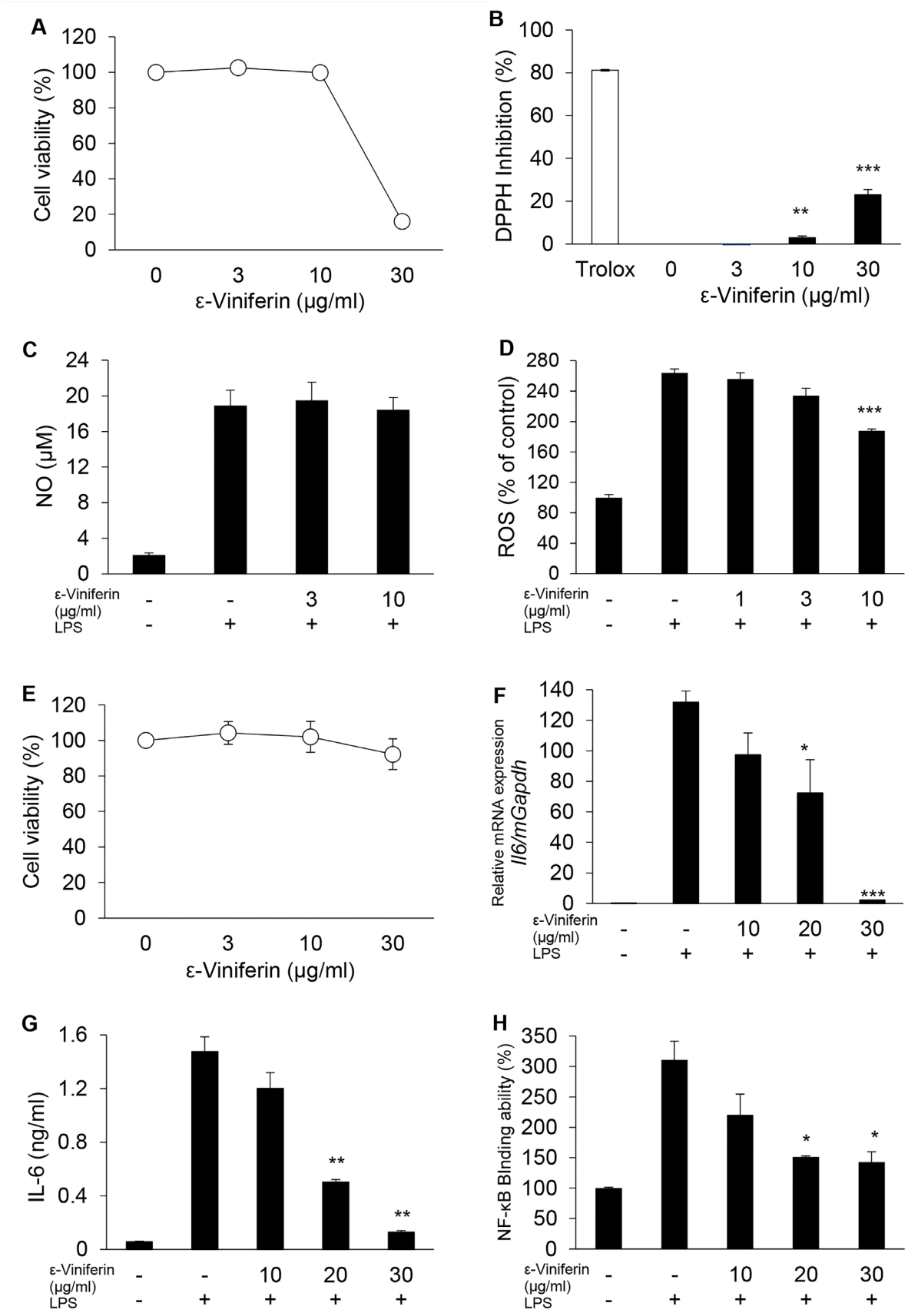

Figure 3. Inhibition of inflammatory regulator production and NF-κB activity by ε-viniferin in RAW264.7 cells. (A) Effect of ε-viniferin on cell viability after 24 h of exposure. (B) *In vitro* antioxidant activity determined using the DPPH radical-scavenging assay. (C) Inhibition of LPS-induced NO production. (D) Inhibition of LPS-induced ROS production, measured based on fluorescent intensity. (E) Effect of ε-viniferin on cell viability after 4 h of exposure. (F) Inhibition of LPS-induced IL-6 expression. (G) Inhibition of LPS-induced IL-6 secretion. (H) Inhibition of LPS-induced NF-κB activity. RAW264.7 cells were stimulated with 100 ng/ml LPS in the presence or absence of ε-viniferin. N=3. *P<0.05, **P<0.01, ***P<0.001 vs. control without ε-viniferin and LPS. LPS, lipopolysaccharide; NO, nitric oxide; ROS, reactive oxygen species; IL, interleukin; DPPH, 2,2-diphenyl-1-picrylhydrazyl; mGapdh, mouse glyceraldehyde-3-phosphate dehydrogenase.

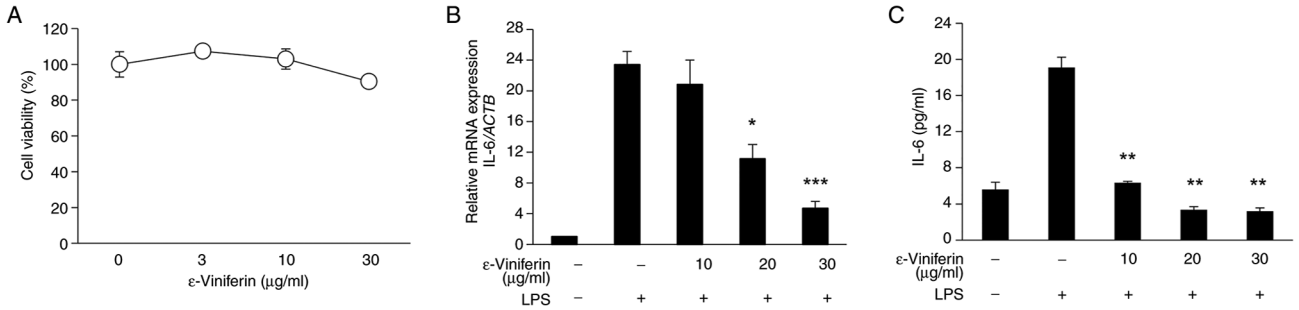

Figure 4. Inhibition of IL-6 expression and secretion by ε-viniferin in THP-1 cells. (A) Effect of ε-viniferin on cell viability after 4 h of exposure. (B) Inhibition of LPS-induced IL-6 expression. (C) Inhibition of LPS-induced IL-6 secretion. THP-1 cells were stimulated with 100 ng/ml LPS in the presence or absence of ε-viniferin. N=3. *P<0.05, **P<0.01, ***P<0.001 vs. control without ε-viniferin and LPS. IL, interleukin; LPS, lipopolysaccharide. ACTB, β-actin.

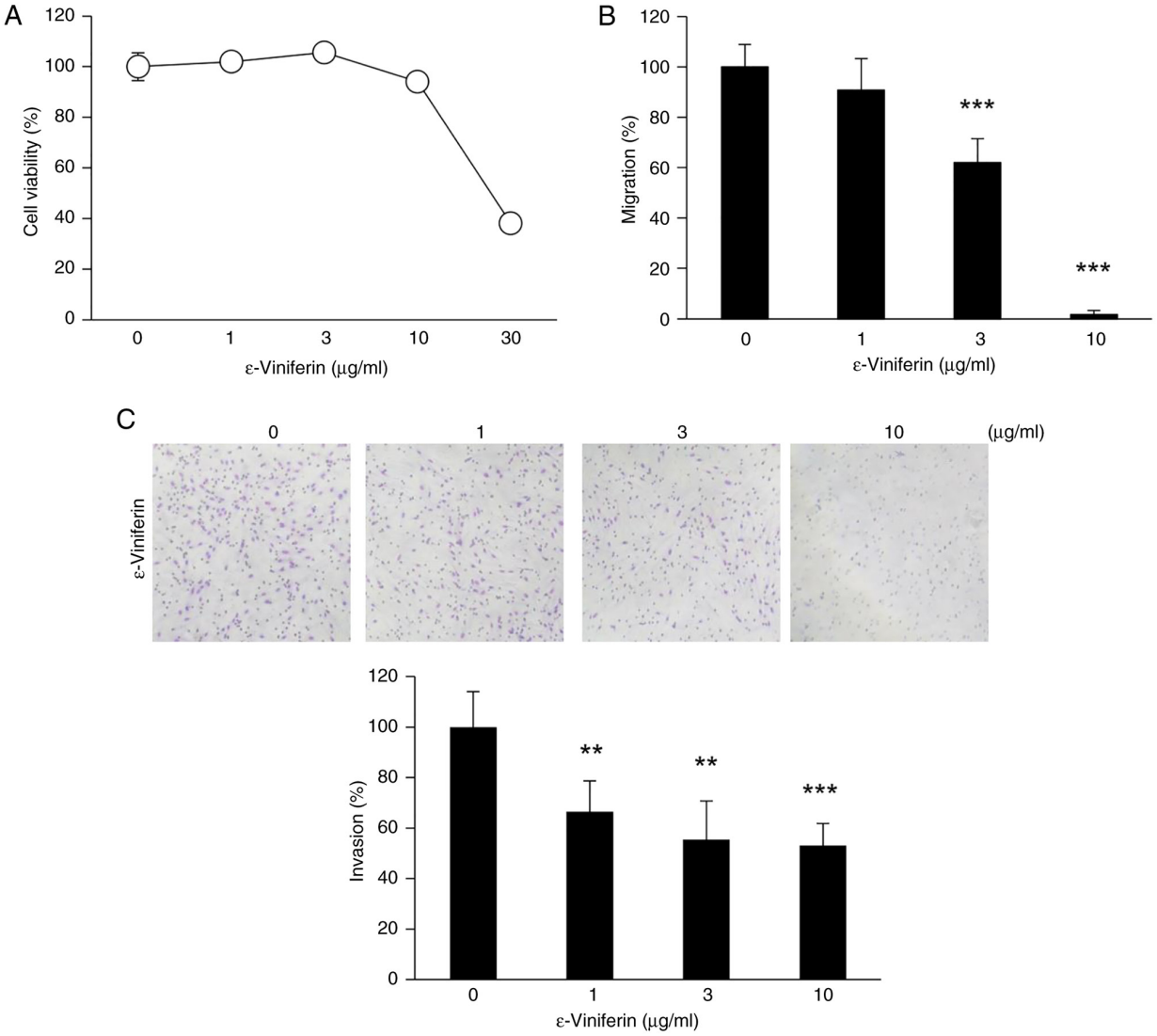

Figure 5. Inhibition of migration and invasion of human endometrial stromal cells by ε-viniferin. (A) Effect of ε-viniferin on cell viability after 24 h of exposure. (B) Inhibition of cell migration assessed using the wound healing assay after 8 h of treatment. (C) Suppression of cell invasion measured using the Matrigel invasion chamber assay after 16 h of treatment. Magnification, x100. N=3. **P<0.01, ***P<0.001 vs. control without ε-viniferin.

above (Fig. 5B and C). Similar inhibitory effects on migration and invasion were observed with wild-grape extract (Fig. S1A-C).

To further investigate the inflammatory signaling pathways potentially involved in the anti-endometriotic effects of ε-viniferin, a PCR array was employed (Fig. S2). Treatment of HESCs with ε-viniferin upregulated *CDKN2A*, a gene that inhibits abnormal cell growth and proliferation, and downregulated *TNFSF10*, a regulator of inflammation. Additionally, ε-viniferin suppressed the

expression of metastasis-promoting genes such as *HPSE* and *FGFR4*.

Discussion

In this study, we investigated the anti-inflammatory and anti-endometriotic effects of wild-grape extract and purified ε-viniferin and demonstrated that both compounds effectively suppressed LPS-induced inflammatory mediators, including NO, ROS, and IL-6, in mouse and human macrophage cell lines. They also inhibited the activation of NF-κB, a transcription factor that regulates the expression of these mediators. These findings suggest that wild-grape extract and purified ε-viniferin exhibit anti-inflammatory activity by inhibiting NF-κB mediated inflammatory pathways. Furthermore, we validated the previously demonstrated radical scavenging activity of ε-viniferin *in vitro*. ε-viniferin consists of two stilbenol units linked together, and its radical-scavenging activity is attributed to the functional groups such as phenolic hydroxyl groups and double bonds within this structure (30,31).

Previously, we reported the potential of NF-κB inhibitor dehydroxymethylepoxyquinimicin (DHMEQ) in the treatment of endometriosis (32). Based on these findings and the observed NF-κB inhibition by ε-viniferin, we further explored the potential application of ε-viniferin for its anti-endometriotic effects. We demonstrated that ε-viniferin effectively inhibited the migration and invasion of HESCs, a key cell type implicated in endometriosis. Thus, ε-viniferin exhibited potential for the treatment of endometriosis. Additionally, PCR array analysis revealed that ε-viniferin suppressed the expression of mobility-related genes, such as *HPSE* and *FGFR4*, and the inflammation-promoting mediator *TNFSF10*. The observed gene expression patterns revealed that ε-viniferin could inhibit the progression of endometriosis by modulating inflammatory regulators and metastasis-related pathways. To gain deeper mechanistic insights into ε-viniferin's molecular targets, future transcriptomic and/or proteomic analyses of HPSE and FGFR4 functions are warranted. In the present study, we focused on HESCs to assess the direct anti-migratory and anti-inflammatory effects of ε-viniferin. To further evaluate its therapeutic relevance in the endometriotic microenvironment, future studies will examine ε-viniferin's effects on other cellular components of endometriotic lesions, including epithelial cells, immune cells, and vascular endothelial cells.

While our current study focused on the NF-κB pathway, it is important to explore broader signaling networks to fully understand the therapeutic effects of ε-viniferin. Previous studies (22,27) reported that ε-viniferin suppresses inflammatory cytokines such as TNF-α and TGF-β, which are associated with the JAK/STAT and TGF-β pathways. These findings suggest that ε-viniferin may have broader anti-inflammatory effects beyond NF-κB inhibition. In addition, our PCR array data showed that ε-viniferin downregulated TNFSF10 (Fig. S2), a gene regulated by TRAIL and involved in immune and apoptotic pathways. These results support the potential of wider regulatory effects. Future studies will investigate the JAK/STAT and TGF-β pathways to further clarify ε-viniferin's therapeutic mechanisms.

Overall, our results suggest that ε-viniferin is a promising anti-endometriosis agent with potent anti-inflammatory effects, which are crucial for managing disease progression. ε-viniferin-based therapy may be a safer, non-hormonal alternative to conventional hormonal therapies, which often cause side effects such as bone loss and menstrual irregularities. While these findings strongly support the therapeutic potential of ε-viniferin, further *in vivo* and clinical studies are necessary to confirm its efficacy and long-term safety. Moreover, previous pharmacokinetic studies have shown that ε-viniferin accumulates in white adipose tissue and remains in the body for extended periods (18-20). However, critical pharmacokinetic parameters-such as absorption rate, metabolism, and plasma half-life-remain uncharacterized in the context of endometriosis. Pharmacokinetic modeling and *in vivo* biodistribution studies are therefore planned to optimize its therapeutic use. As a non-hormonal agent, ε-viniferin may also be considered for combination therapy. Although its interaction with standard hormonal treatments remains unexplored, previous studies have shown that resveratrol, a structurally related polyphenol, enhances the efficacy of hormonal therapies and improves the management of endometriosis-related pain by reducing inflammation (33). These findings raise the possibility that ε-viniferin may offer similar combinatorial benefits. To enhance clinical applicability, we also plan to explore combination therapies using ε-viniferin alongside standard hormonal treatments. Together, our findings present a novel strategy for developing anti-endometriosis therapies by targeting both inflammation and cell migration, paving the way for future translational research.

Acknowledgements

The authors would like to thank Mr. Kazuyuki Ino (General Affairs Department, Fukuyu Hospital, Fukuyu Medical Corporation, Fukuyu, Japan) for providing support in writing and editing the manuscript.

Funding

This work was supported by the Japan Society for the Promotion of Science Kakenhi (grant no. 22H03062) and the Japan Agency for Medical Research and Development (grant no. JP18fk0310118JSPS). Additional financial support was provided by Fukuyu Medical Corporation.

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

YL, KU, SK and YN contributed to the experimental design and manuscript preparation. YL, SK, MH and HF conducted the experiments. YH, AW and NK contributed to the experimental design. YL, YN and SK confirmed the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The Department of Molecular Target Medicine, to which KU belongs, is a funding-supported laboratory financially supported by Fukuyu Medical Corporation (Nisshin, Japan); Brunaise Co., Ltd. (Nagoya, Japan); Shenzhen Wanhe Pharmaceutical Company (Shenzhen, China); and Meiji Seika Pharma (Tokyo, Japan). This study is partly supported by Fukuyu Medical Corporation.

References

1. Zondervan KT, Becker CM and Missmer SA: Endometriosis. N Engl J Med 382: 1244-1256, 2020.
2. Pessoa de Farias Rodrigues M, Lima Vilarino F, de Souza Barbeiro Munhoz A, da Silva Paiva L, de Alcantara Sousa LV, Zaia V and Parente Barbosa C: Clinical aspects and the quality of life among women with endometriosis and infertility: A cross-sectional study. BMC Womens Health 20: 124, 2020.
3. Johnson NP, Hummelshoj L and World Endometriosis Society Montpellier Consortium: Consensus on current management of endometriosis. Hum Reprod 28: 1552-1568, 2013.
4. Taylor HS, Kotlyar AM and Flores VA: Endometriosis is a chronic systemic disease: Clinical challenges and novel innovations. Lancet 397: 839-852, 2021.
5. Hee L, Kettner LO and Vejtorp M: Continuous use of oral contraceptives: An overview of effects and side-effects. Acta Obstet Gynecol Scand 92: 125-136, 2013.
6. De Corte P, Milhoranca I, Mechsner S, Oberg AS, Kurth T and Heinemann K: Unravelling the causal relationship between endometriosis and the risk for developing venous thromboembolism: A pooled analysis. Thromb Haemost 125: 385-394, 2025.
7. Polyzos NP, Fatemi HM, Zavos A, Grimbizis G, Kyrou D, Velasco JG, Devroey P, Tarlatzis B and Papanikolaou EG: Aromatase inhibitors in post-menopausal endometriosis. Reprod Biol Endocrinol 9: 90, 2011.
8. Pavone ME and Bulun SE: Clinical review: The use of aromatase inhibitors for ovulation induction and superovulation. J Clin Endocrinol Metab 98: 1838-1844, 2013.
9. Takagi H, Takakura M and Sasagawa T: Risk factors of heavy uterine bleeding in patients with endometriosis and adenomyosis treated with dienogest. Taiwan J Obstet Gynecol 62: 852-857, 2023.
10. Bally M, Dendukuri N, Rich B, Nadeau L, Helin-Salmivaara A, Garbe E and Brophy JM: Risk of acute myocardial infarction with NSAIDs in real world use: Bayesian meta-analysis of individual patient data. BMJ 357: j1909, 2017.
11. Hägg S, Jönsson AK and Ahlner J: Current evidence on abuse and misuse of gabapentinoids. Drug Saf 43: 1235-1254, 2020.
12. Rahmioglu N, Nyholt DR, Morris AP, Missmer SA, Montgomery GW and Zondervan KT: Genetic variants underlying risk of endometriosis: Insights from meta-analysis of eight genome-wide association and replication datasets. Hum Reprod Update 20: 702-716, 2014.
13. Nishimoto-Kakiuchi A, Sato I, Nakano K, Ohmori H, Kayukawa Y, Tanimura H, Yamamoto S, Sakamoto Y, Nakamura G, Maeda A, *et al*: A long-acting anti-IL-8 antibody improves inflammation and fibrosis in endometriosis. Sci Transl Med 15: eabq5858, 2023.
14. Yoshino O, Yamada-Nomoto K, Kobayashi M, Andoh T, Hongo M, Ono Y, Hasegawa-Idemitsu A, Sakai A, Osuga Y and Saito S: Bradykinin system is involved in endometriosis-related pain through endothelin-1 production. Eur J Pain 22: 501-510, 2018.
15. Bruner-Tran KL, Osteen KG, Taylor HS, Sokalska A, Haines K and Duleba AJ: Resveratrol inhibits development of experimental endometriosis *in vivo* and reduces endometrial stromal cell invasiveness *in vitro*. Biol Reprod 84: 106-112, 2011.
16. Ricci AG, Olivares CN, Bilotas MA, Bastón JI, Singla JJ, Meresman GF and Barañao RI: Natural therapies assessment for the treatment of endometriosis. Hum Reprod 28: 178-188, 2013.
17. Rudzitis-Auth J, Menger MD and Laschke MW: Resveratrol is a potent inhibitor of vascularization and cell proliferation in experimental endometriosis. Hum Reprod 28: 1339-1347, 2013.
18. Zghonda N, Yoshida S, Araki M, Kusunoki M, Mliki A, Ghorbel A and Miyazaki H: Greater effectiveness of ε-viniferin in red wine than its monomer resveratrol for inhibiting vascular smooth muscle cell proliferation and migration. Biosci Biotechnol Biochem 75: 1259-1267, 2011.
19. Courtois A, Atgié C, Marchal A, Hornedo-Ortega R, Lapèze C, Faure C, Richard T and Krisa S: Tissular distribution and metabolism of *trans*-ε-viniferin after intraperitoneal injection in rat. Nutrients 10: 1660, 2018.
20. Kim J, Min JS, Kim D, Zheng YF, Mailar K, Choi WJ, Lee C and Bae SK: A simple and sensitive liquid chromatography-tandem mass spectrometry method for trans-ε-viniferin quantification in mouse plasma and its application to a pharmacokinetic study in mice. J Pharm Biomed Anal 134: 116-121, 2017.
21. Jiménez-Moreno N, Volpe F, Moler JA, Esparza I and Ancín-Azpilicueta C: Impact of extraction conditions on the phenolic composition and antioxidant capacity of grape stem extracts. Antioxidants (Basel) 8: 597, 2019.
22. Sergi D, Gélinas A, Beaulieu J, Renaud J, Tardif-Pellerin E, Guillard J and Martinoli MG: Anti-apoptotic and anti-inflammatory role of trans ε-viniferin in a neuron-glia co-culture cellular model of Parkinson's disease. Foods 10: 586, 2021.
23. Beaumont P, Amintas S, Krisa S, Courtois A, Richard T, Eseberri I and Portillo MP: Glucuronide metabolites of trans-ε-viniferin decrease triglycerides accumulation in an *in vitro* model of hepatic steatosis. J Physiol Biochem 80: 685-696, 2024.
24. Huang C, Lin ZJ, Chen JC, Zheng HJ, Lai YH and Huang HC: α-viniferin-induced apoptosis through downregulation of SIRT1 in non-small cell lung cancer cells. Pharmaceuticals (Basel) 16: 727, 2023.
25. Zhang S, Ma Y and Feng J: Neuroprotective mechanisms of ε-viniferin in a rotenone-induced cell model of Parkinson's disease: Significance of SIRT3-mediated FOXO3 deacetylation. Neural Regen Res 15: 2143-2153, 2020.
26. Gómez-Zorita S, Milton-Laskibar I, Eseberri I, Beaumont P, Courtois A, Krisa S and Portillo MP: Beneficial effects of ε-viniferin on obesity and related health alterations. Nutrients 15: 928, 2023.
27. Chiou WC, Huang C, Lin ZJ, Hong LS, Lai YH, Chen JC and Huang HC: α-viniferin and ε-viniferin inhibited TGF-β1-induced epithelial-mesenchymal transition, migration and invasion in lung cancer cells through downregulation of vimentin expression. Nutrients 14: 2294, 2022.
28. Soni UK, Chadchan SB, Kumar V, Ubba V, Khan MTA, Vinod BSV, Konwar R, Bora HK, Rath SK, Sharma S and Jha RK: A high level of TGF-B1 promotes endometriosis development via cell migration, adhesiveness, colonization, and invasiveness†. Biol Reprod 100: 917-938, 2019.
29. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
30. Szewczuk LM, Lee SH, Blair IA and Penning TM: Viniferin formation by COX-1: Evidence for radical intermediates during co-oxidation of resveratrol. J Nat Prod 68: 36-42, 2005.
31. Privat C, Telo JP, Bernardes-Genisson V, Vieira A, Souchard JP and Nepveu F: Antioxidant properties of trans-ε-viniferin as compared to stilbene derivatives in aqueous and nonaqueous media. J Agric Food Chem 50: 1213-1217, 2002.
32. Lin Y, Kojima S, Ishikawa A, Matsushita H, Takeuchi Y, Mori Y, Ma J, Takeuchi K, Umezawa K and Wakatsuki A: Inhibition of MLCK-mediated migration and invasion in human endometriosis stromal cells by NF-κB inhibitor DHMEQ. Mol Med Rep 28: 141, 2023.
33. Maia H Jr, Haddad C, Pinheiro N and Casoy J: Advantages of the association of resveratrol with oral contraceptives for management of endometriosis-related pain. Int J Womens Health 4: 543-549, 2012.

Copyright © 2025 Lin et al. This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.