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Original Research

Efficient Inhibition of Endometriosis Growth by Cepharanthine *In Vitro*, in Patient-Derived Endometrial Organoids, and *In Vivo*

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Abstract

Background: Available treatments for endometriosis remain unsatisfactory; therefore, there is an urgent demand for novel and effective therapeutic strategies. This study focused on the therapeutic effect of cepharanthine, a monomer derived from a Chinese herb, on endometriosis *in vitro*, in patient-derived eutopic endometrial organoids, and *in vivo*. **Methods:** Patient-derived ectopic endometrial stromal cells were isolated from ovarian endometriomas. Organoids were generated from the eutopic endometrium of patients with endometriosis. Ectopic endometrial stromal cells, eutopic endometrial organoids, and immortalized endometrial stromal cells were used to evaluate the effects of cepharanthine on cell viability, growth, and apoptosis. Female BALB/c mice were used to develop a peritoneal endometriosis model. **Results:** Cepharanthine treatment decreased the viability of immortalized endometrial stromal cells, patient-derived endometriotic stromal cells, as well as eutopic endometrial organoids. It induced DNA damage, downregulated cyclin D1, and caused cell-cycle arrest at the G0/G1 phase. It also promoted apoptosis by enhancing cytochrome C release, activating caspase-9 and caspase-3, increasing the expression of proapoptotic factor Bax, and decreasing the expression of antiapoptotic factor B-cell lymphoma 2 (Bcl-2). Intraperitoneal administration of cepharanthine significantly inhibited the growth of murine peritoneal endometriosis model. The treatment significantly downregulated the protein expression of cyclin D1 and DNA repair protein RAD51 (RAD51), and increased phosphorylated histone H2AX (γ-H2AX) expression in endometriosis lesions, indicating that it induced DNA damage and impaired DNA repair. Additionally, Ki-67 expression was significantly decreased, and apoptosis was markedly increased in the lesions. **Conclusions:** Our results indicate that cepharanthin may represent a promising treatment option for endometriosis.

Keywords: apoptosis; cepharanthine; DNA damage; endometriosis; organoids

1. Introduction

Endometriosis is a prevalent chronic gynecological disorder characterized by the presence of endometrial glands and stroma outside the uterine cavity. It mainly affects about 10% of women of reproductive age [1]. The disease is often accompanied by severe pelvic pain, dysmenorrhea, deep dyspareunia, and infertility, and is also a leading cause of miscarriage and implantation failure. It is a chronic condition without a definitive medical or surgical cure. Treatment of endometriosis consists of surgical removal of lesions and hormone treatment (combined oral contraceptives, progestins, or gonadotropin-releasing hormone agonists), often with side effects and variable efficacy. The recurrence rate is up to 50% within 5 years after surgery [2]. Studies have begun to focus on plant-derived agents as natural treatments, aiming for high efficacy, fewer side effects, and preserved fertility [3].

Stephania cepharantha Hayata has been used by Traditional Chinese Medicine practitioners to treat endometriosis-related symptoms for many years. Cepharanthine has been approved for the treatment of leukopenia in Japan and in China [4,5]. It has anti-cancer, anti-inflammation, anti-virus, and other pharmacological activities without major side effects [6,7]. Cepharanthine can inhibit the proliferation of multiple cell types *in vitro*, including peripheral mononuclear cells and T-cells [7]. It can also induce apoptosis of many cancer cells, including ovarian [8] and breast cancer cells [9].

The survival of ectopic endometriotic lesions relies critically on key biological processes, such as proliferation, apoptosis, inflammation, and angiogenesis [10]. Newly developed endometriotic lesions exhibit more cell proliferation than does eutopic endometrium, and endometrial tissue from affected women is more apoptosis-resistant [3]. This imbalance between cell proliferation and cell death promotes pathological tissue overgrowth. In this regard, we hypothesized that cepharanthine, which increases apoptosis in some cancer cells, has therapeutic value in treating endometriosis.

In 2019, Boretto et al. [11] established organoid models for endometrial diseases (endometriosis, hyperplasia, endometrial cancer) that exhibited long-term expandabil-

ity, genomic/transcriptomic stability, replicated disease diversity, and served as promising preclinical models and tools for drug screening/discovery. Other studies have similarly noted that, as an ideal research model, endometrial organoids hold potential for pathophysiological studies, novel therapeutic drug screening, and personalized medicine drug testing [12,13]. Our team has previously reported the successful establishment and culture of endometrial organoids [14]. We tested cepharanthine *in vitro*, in patient-derived endometrial organoids, and *in vivo*.

2. Materials and Methods

2.1 Clinical Sample Collection

The Ethics Committee of West China Second University Hospital of Sichuan University approved the study on March 7, 2018 (ethical approval number: Medical Research 2018 No. 48). Informed consent was obtained from each participant. Samples of ovarian endometrioma and eutopic endometrium were collected from 3 patients of reproductive age who had not received any medication before surgery. Tissue samples were placed in an iced DMEM/F12 culture medium (Gibco, 11330-032, Melbourne, VIC, Australia) containing 1% penicillin-streptomycin (Gibco, 15140-122) and then were immediately transferred to the laboratory on ice.

2.2 Isolation and Identification of Primary Ectopic Endometrial Stromal Cells

This was done as previously described [15]. After rinsing 3 times with iced sterile PBS, the ovarian endometrioma was cut into 1-mm^3 pieces and digested with 0.1% collagenase IV solution (Gibco, Catalog No. 17104-019, Waltham, MA, USA) for 1 h. The mixture was then filtered through a 70 µm filter (Falcon, 352350, Franklin Lakes, NJ, USA) and a 40 µm filter (Falcon, 352340). After centrifugation at 1000 g for 10 min, stromal cells were collected from the bottom of the tube. The cells were resuspended in DMEM/F-12 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 0.1 mg/mL streptomycin, and 0.25 µg/mL amphotericin B (Sigma-Aldrich, Catalog No. A2942, St. Louis, MO, USA), and then incubated in 5% CO_2 at 37 °C overnight. Cells from two passages were used for subsequent experiments. Isolated ectopic endometrial stromal cells were validated for purity via immunofluorescence staining targeting vimentin, a specific marker of stromal cells.

2.3 Primary Cell Isolation and Eutopic Endometrium Organoid Establishment

This was performed as previously described [14,16]. Endometrial samples were placed in cooled normal saline with 1% antibiotic-antimycotic, transported to the laboratory within 1 h, rinsed with 0.01% benzalkonium bromide, then 3 times with phenol red-free DMEM/F12 plus 1% antibiotic-antimycotic. Tissue was transferred to 100-mm dishes with 10 mL digestion solution (cooled PBS with 0.1% collagenase IV), minced into 1-mm^3 fragments, and incubated at 37 °C under 5% CO_2 for 40 min, before neutralizing medium was added. The suspension was filtered through a 70-µm strainer (Falcon, 431751), then a 40-µm strainer (Falcon, 431750). The 40-µm strainer was inverted and rinsed with 6 mL medium to collect epithelial cells. After centrifugation at 1500 rpm for 10 min, the pellet was resuspended in 1 mL advanced DMEM/F12 (Gibco, 12634010, USA), mixed with Matrigel (Corning, 356231, Corning, NY, USA), and kept on ice. Then, 30-µL droplets were seeded into 24-well plates (1/well), incubated at 37 °C for 15 min, and then overlaid with organoid medium. Organoids formed in 4–7 days. They were passaged when >400 µm in diameter; those from one passage were used for experiments.

2.4 Cell Culture and Reagents

The immortalized endometrial stromal cells (iCell Bioscience Inc., iCell-0187a, Shanghai, China) were confirmed by short tandem repeat (STR) validation. All cell lines tested negative for mycoplasma. Cells were cultured in a humidified atmosphere containing 5% CO_2 at 37 °C in DMEM/F12 supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL of penicillin, and 100 µg/mL of streptomycin. Cepharanthine was purchased from APExBIO, N2771, Houston, TX, USA.

2.5 MTT Assay

This was carried out as previously reported [17,18]. Cells were cultured with different concentrations of cepharanthine for 48 h. Cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent (Sigma). Cell survival was calculated by normalizing the absorbance to that of untreated controls.

2.6 Apoptosis Assay and Cell-Cycle Analysis

Cells were treated with 14.16 µM cepharanthine for 48 h. Quantitative evaluation of cellular apoptosis was carried out by flow cytometric analysis using the Annexin V/propidium iodide (PI) staining method. Briefly, cells were digested with 0.25% trypsin-free EDTA, then washed with PBS once, and then incubated with Annexin V-Alexa Fluor 747/PI Apoptosis Detection Kit (Yeasen, 40304-ES20, Shanghai, China). Cells were then resuspended using 100 µL 1× Binding Buffer, and then 5 µL Annexin V plus 10 µL staining solution were added in the dark for 15 min. The ratio of apoptotic cells was determined by flow cytometry (Beckman Coulter, Model CytoFLEX S, Brea, CA, USA), and data were analyzed by software (Version 2.4, Beckman Coulter, Brea, CA, USA). Cell cycle analysis was performed according to a previously published protocol [19,20].

2.7 Colony-Formation Assay

Cells were incubated with 14.16 μM cepharanthine for 48 h. The medium was then changed with fresh DMEM/F12, and the cells were cultured for another 2 weeks. Cells were then fixed in 4% paraformaldehyde (PFA) for 10 min and stained with crystal violet using ImageJ software (Version 1.54f, National Institutes of Health, Bethesda, MD, USA) for 10 min to visualize and quantify the colonies. Colony numbers were determined both manually and using ImageJ in triplicate.

2.8 Western Blot Analysis

The experiment was conducted as described previously [18,21]. Primary antibodies including cyclin D1 (Abcam, ab134175, Cambridge, UK, 1:1000), γH2AX (Abcam, ab26350, 1:1000), caspase-9 (Proteintech, 10380-1-AP, Rosemont, IL, USA, 1:1000), caspase-3 (Proteintech, 66470-2-Ig, 1:1000), B-cell lymphoma 2 (Bcl-2) (Proteintech, 12789-1-AP, 1:1000), Bax (Proteintech, 60267-1-Ig, 1:1000), cytochrome C (Proteintech, 66264-1-IG, 1:1000) and β-actin (Atagenix Laboratory, ATPA00014Rb, Wuhan, Hubei, China, 1:1000) were used. Briefly, 20 μg of whole-cell lysate per sample were loaded onto a 12% polyacrylamide gel for electrophoresis and then transferred to a nitrocellulose membrane. The membrane was blocked with 5% nonfat milk in 1× Tris-buffered saline (pH 7.6) containing 0.05% Tween-20, followed by incubation with primary antibodies. Secondary antibodies were used at 1:5000. Proteins were visualized using the Western Bright enhanced chemiluminescence (ECL) detection kit (Junengbio, K-12043-D10, Guangzhou, Guangdong, China) and imaged with a Bio-Rad ChemiDocXRS+ chemiluminescence system (Bio-Rad, 1708265, Hercules, CA, USA). Each band was obtained after 3 independent repeated experiments. Band densities were quantified via ImageJ software.

2.9 Animal Model

The animal model was established using the method of a previously published study [22]. All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of West China Second University Hospital of Sichuan University (Approval No. 2020028) and performed in compliance with the university's Guide for the Care and Use of Laboratory Animals. Female BALB/c mice (6–8 weeks of age) were housed in a specific pathogen-free (SPF) facility and used to establish a mouse model of peritoneal endometriosis. After adaptive feeding for one week, donor mice were randomly selected and euthanized to collect the uteri, all surgery was performed under anesthesia (pentobarbital sodium, i.p., 50 mg/kg). All uterine horns were identically managed, including a longitudinal split to isolate endometrial tissue and careful dissection into consistent pieces smaller than 1 mm. The fragments were then suspended in sterile PBS. The peritoneal endometriosis model was established by intraperitoneal injection of endometrial tissue pieces. Fragments from one donor mouse were injected into the peritoneal cavity of two recipient mice. Fourteen days after injection, 5 mice were euthanized to check the formation of endometriosis lesions. After hematoxylin and eosin (HE) stain verification of the endometriotic lesions (all of the 5 mice had endometriosis lesions), the remaining 12 mice were randomly divided into two groups. In the literature, the doses of i.p. injections of cepharanthine have varied from 10 mg/kg/day to 20 mg/kg/day [23,24,25]; we chose to use an i.p. injection dose of 10 mg/kg/day, and the control group received i.p. injections of saline. Injections began on the 15th day after the operation and lasted for 4 weeks. The mice were then euthanized, and the endometriotic lesions were collected. Mice were euthanized with 100% CO_2 at a flow rate of 30-70% chamber volume per minute until respiratory arrest. Death was verified by the lack of respiration and reflex response.

2.10 Immunohistochemistry Study

Immunohistochemical staining was performed as previously described [18]. Sections were dewaxed with xylene and dehydrated using a graded ethanol series. The primary antibodies, anti-cyclin D1 (CCND1) (ab134175, Abcam), anti-Ki67 (27309-1-AP, Proteintech), anti-DNA repair protein RAD51 (RAD51) (ab133534, Abcam), and anti-γ-H2AX (ab26350, Abcam) were added to the slides and left overnight at 4 °C. After washing with PBS, the secondary antibody (KIHC-5, Proteintech) was added to the slides. One hour later, the signals were magnified by 3,3′-diaminobenzidine (DAB), and the nuclei were counterstained with haematoxylin. At least fifteen non-overlapping, independent fields of view were randomly selected and analyzed under a microscope.

2.11 TUNEL Assay

Analysis of apoptotic cells within endometriotic lesions was done by using the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay (Promega, Madison, WI, USA), following the manufacturer's protocol. The photographs were captured by Olympus FV-3000 confocal microscopy (Olympus Corporation, Tokyo, Japan) and saved in TIFF format. The number of TUNEL-positive nuclei was counted by ImageJ (1.53 K). Six fields of view were randomly selected from each sample, and each group included six independent replicates.

2.12 Statistical Analysis

Data were presented as means ± standard deviation of at least 3 independent experiments. The two-tailed Student's *t*-test was used to compare two independent groups. Statistical significance was set at $p \leq 0.05$. The statistical analyses were done by using SPSS 20.0 (IBM Corp., 20100901, Armonk, NY, USA) and R 2.10.0 (R Foundation for Statistical Computing, Vienna, Austria).

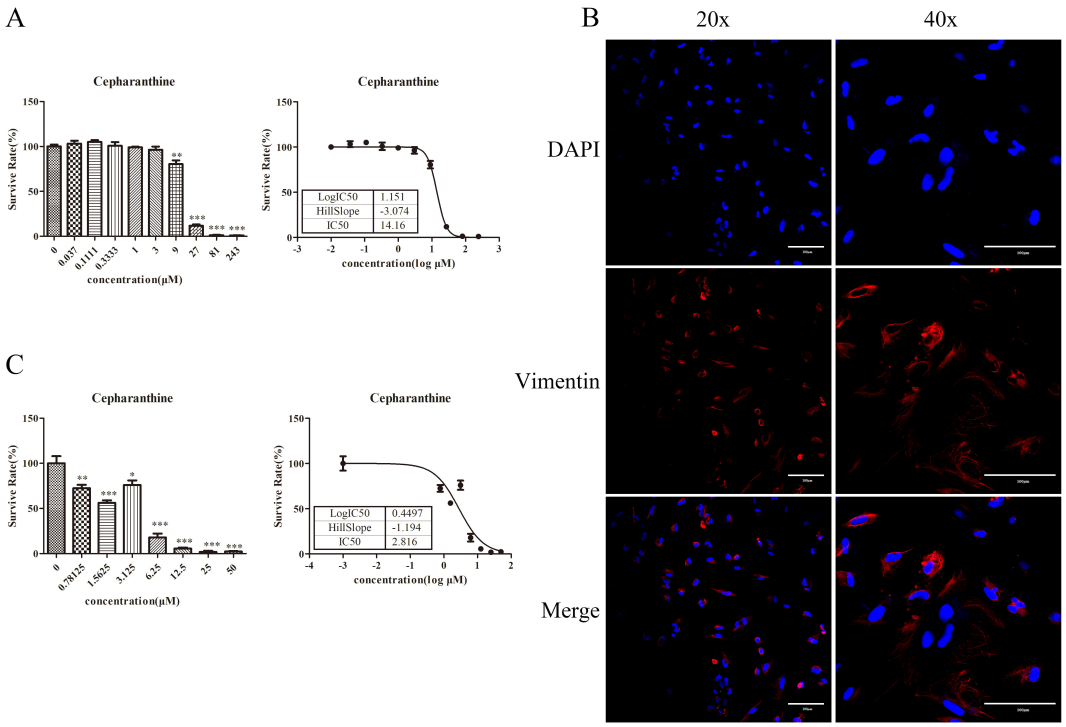

Fig. 1. Cepharanthine decreased the cell viability of immortalized endometrial and patient-derived endometriotic stromal cells. (A) Immortalized endometrial stromal cells were incubated with cepharanthine for 48 h, and cell viability was analyzed by MTT assay. (B) Verification of the patient-derived endometriotic stromal cells. Isolated cells were stained with an antibody to vimentin, a marker of stromal cells, and examined by a laser confocal fluorescence microscope, scale bar = 100 μm. (C) Patient-derived endometriotic stromal cells were incubated with cepharanthine for 48 h, and cell viability was analyzed by MTT assay. Results were mean (SD) (n = 3), *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DAPI, 4′,6-diamidino-2-phenylindole; SD, standard deviation.

3. Results

3.1 Cepharanthine Decreased the Cell Viability of Immortalized Endometrial Stromal Cells and Patient-Derived Ectopic Stromal Cells

We first tested the effect of cepharanthine on an immortalized endometrial stromal cell line. Our results revealed that cepharanthine treatment reduced cell viability in a dose-dependent manner. The half-maximal inhibitory concentration (IC50) was 14.16 μM (Fig. 1A). We then tested the effect of cepharanthine on patient-derived ectopic stromal cells. To verify the primary ectopic stromal cells, we stained the cells with an antibody to vimentin, which is a marker of stromal cells (Fig. 1B). Data showed that cell viability was decreased in a dose-dependent manner by cepharanthine treatment. The IC50 was 2.816 μM (Fig. 1C).

3.2 Cepharanthine Treatment Decreased the Viability of Patient-Derived Eutopic Endometrial Organoids

We previously reported the successful establishment and culture of endometrial organoids [14]. This time, we generated organoids from the eutopic endometrium of patients with endometriosis and investigated the impact of cepharanthine on the viability of these organoids. Fig. 2A shows the growth status of endometrial organoids treated with increasing concentrations of cepharanthine. Cepharanthine treatment resulted in a dose-dependent reduction in the viability of patient-derived eutopic endometrial organoids. The IC50 was 9.058 μM. Fig. 2B shows the morphology of the organoids. Hematoxylin-eosin staining confirmed the presence of well-defined epithelial cells around the lumen in the eutopic endometrial organoid.

3.3 Cepharanthine Inhibited Cell Proliferation by Inducing Apoptosis and Cell Cycle Arrest

The colony-formation assay indicated that the growth ability of the endometrial stromal cells was markedly inhibited by cepharanthine, as indicated by the decreased colony numbers (Fig. 3A). To clarify the association between cepharanthine's antiproliferative activity and apoptosis induction, Annexin V/PI-based flow cytometric analysis was utilized. As shown in Fig. 3B, the number of apoptotic cells was significantly greater in the cepharanthine-treated group than in the control group. Cell-cycle analyses were also conducted, and results showed that cepharanthine-treated cells were blocked at the G0/G1 phase; concomitantly, the percentages of S-phase and G2/M-phase cells were reduced (Fig. 3C).

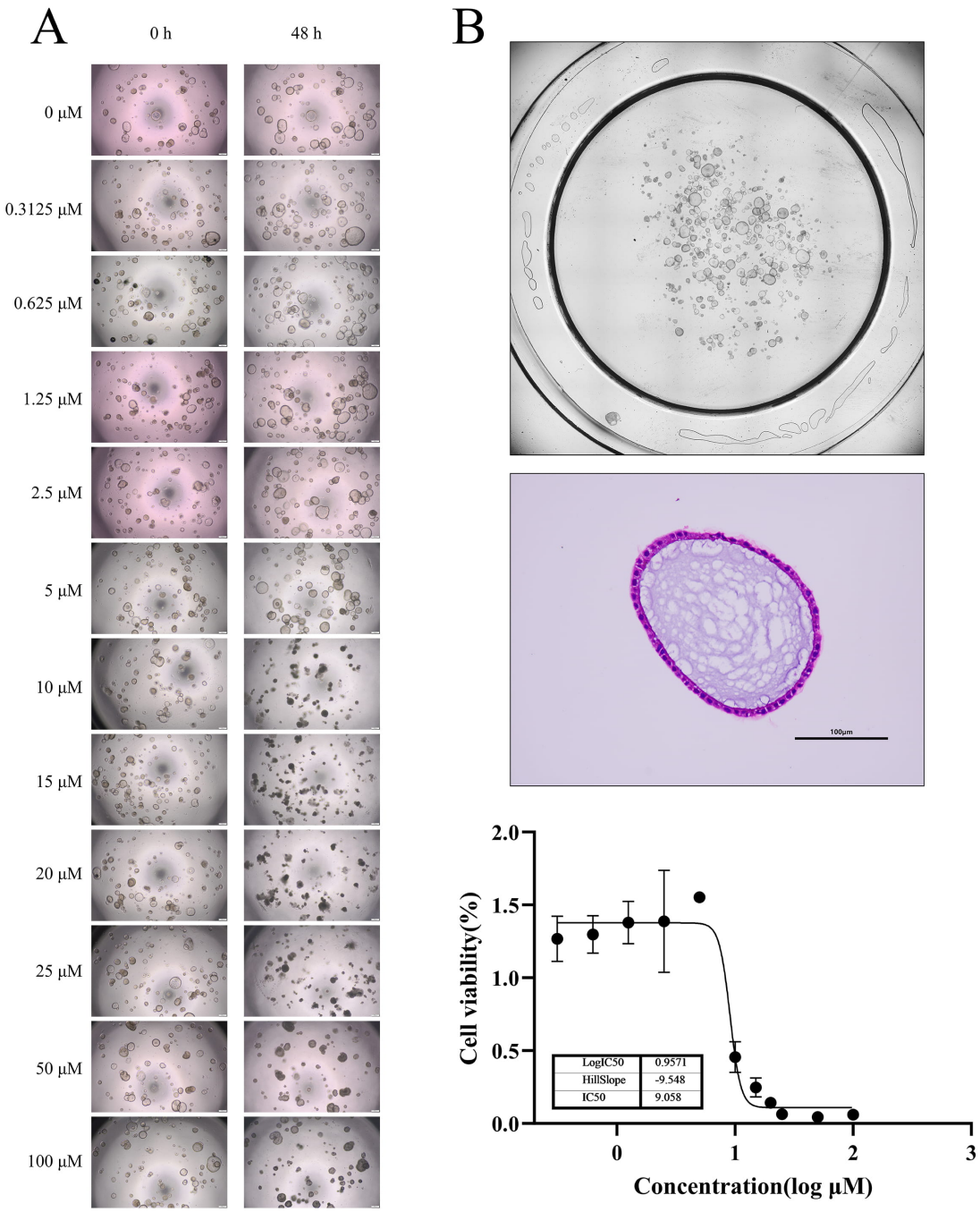

Fig. 2. Cepharanthine decreased the viability of patient-derived eutopic endometrial organoids. (A) Patient-derived eutopic endometrial organoids were incubated with cepharanthine for 48 h, and an MTT assay was subsequently performed. Scale bar = 200 μm. Results were mean (SD) (n = 3). (B) Morphological appearance of endometriosis organoids, scale bar = 100 μm.

3.4 Cepharanthine Blocked Endometriotic Lesion Formation in a Mouse Model of Endometriosis

After HE stain verification of the endometriotic lesions (Fig. 4A), 12 mice were randomized into two groups. The reported doses of intraperitoneal injections of cepharanthine varied from 10 mg/kg/day to 20 mg/kg/day [23,24,25]. The study group chose to use an intraperitoneal injection dose of 10 mg/kg/day, and the control group received an intraperitoneal injection of saline. Compared to the control group (mean lesion weight = 0.445 [0.085] g), treatment with cepharanthine resulted in a statistically significant reduction in lesion weight (mean lesion weight = 0.183 [0.035] g; $p < 0.001$ (Fig. 4B, **Supplementary Fig. 1**). Immunostaining revealed that Ki-67 expression (a proliferation marker) was significantly reduced in the cepharanthine-treated group, $p < 0.01$ (Fig. 4C). The TUNEL assay was used to detect cellular apoptosis within the endometriotic lesion. Fig. 4D shows significantly more

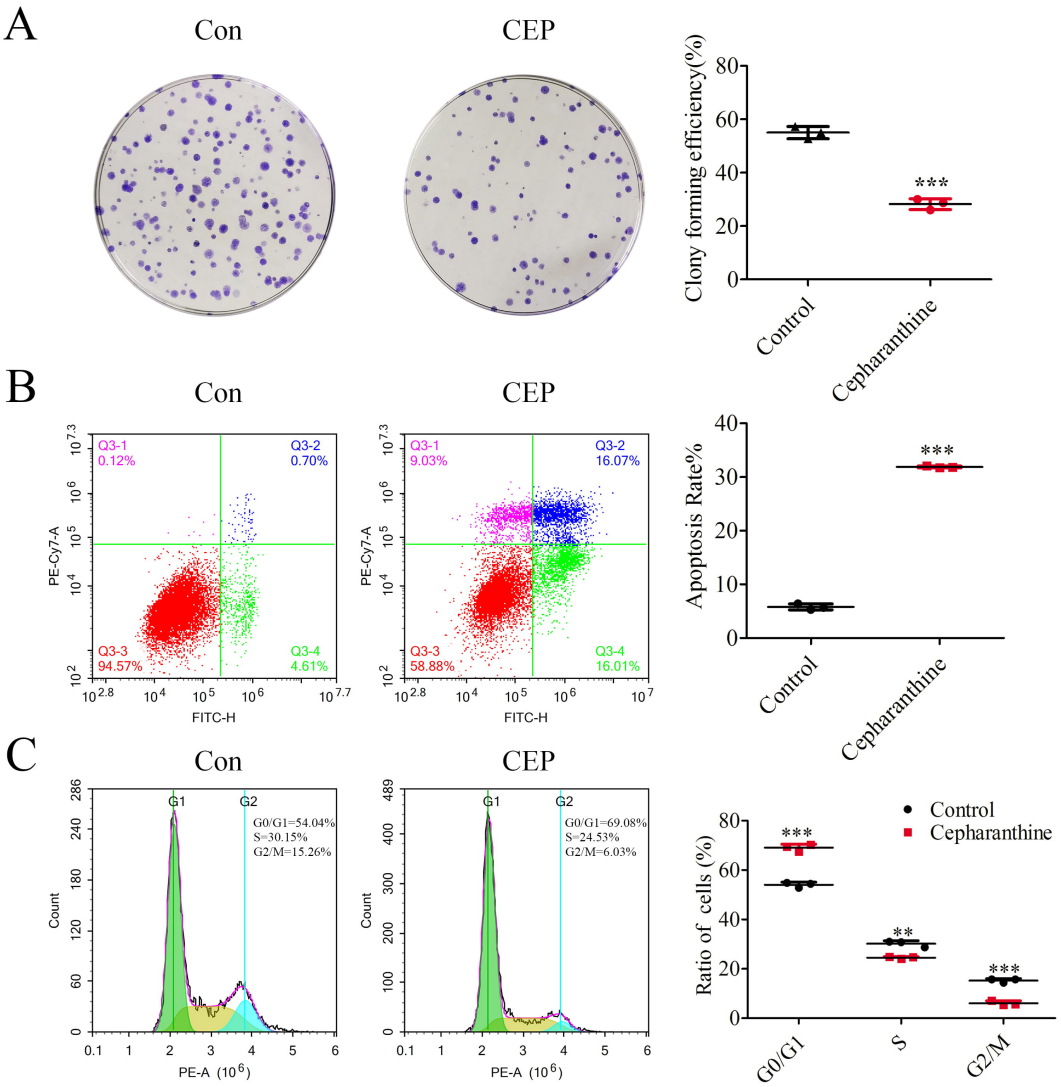

Fig. 3. Treatment with cepharanthine inhibited cell proliferation through the induction of apoptosis and cell-cycle arrest. (A) The colony-formation assay indicated that the growth ability of immortalized endometrial stromal cells was markedly inhibited by cepharanthine, as indicated by the decreased colony numbers. (B) Cells were treated with 14.16 μM cepharanthine for 48 h. Quantitative evaluation of cellular apoptosis was carried out by flow cytometric analysis using the Annexin V/propidium iodide (PI) staining method. (C) Cell cycle analysis was conducted by flow cytometry. Data represented the mean (SD) of 3 independent experiments. Statistical analysis was performed using the Student's *t*-test, *** $p < 0.001$, ** $p < 0.01$.

TUNEL-positive nuclei in the cepharanthine-treated group than in the control group ($p < 0.05$).

3.5 Cepharanthine Caused DNA Damage in Immortalized Endometrial Stromal Cells and Induced Apoptosis Through the Caspase-9/Caspase-3 Pathway

The Western Blot analysis showed that cepharanthine upregulated cytochrome C in the cytoplasm, enhanced the expression of cleaved-caspase-9, cleaved-caspase-3, and Bax, and downregulated the expression of Bcl-2 in endometrial stromal cells. These results suggested that cepharanthine triggered apoptosis via the release of cytochrome C from mitochondria to the cytoplasm and subsequent activation of caspase-9 and caspase-3 [26] (Fig. 5A,B).

Cepharanthine has been reported to induce DNA damage in lung cancer cells [27]. Given that histone H2AX phosphorylation is a well-established marker of DNA double-strand breaks [28], we examined the levels of phosphorylated H2AX (γ-H2AX) in immortalized endometrial stromal cells after cepharanthine treatment. Our results demonstrated that cepharanthine treatment produced significantly more H2AX phosphorylation than in the control group (Fig. 5A,B), indicating its potential to induce DNA damage in immortalized endometrial stromal cells.

Given that cepharanthine treatment induced G0/G1 phase cell-cycle arrest accompanied by decreased proportions of cells in S and G2/M phases, we examined CCND1 expression in the treated cells. The results demonstrated

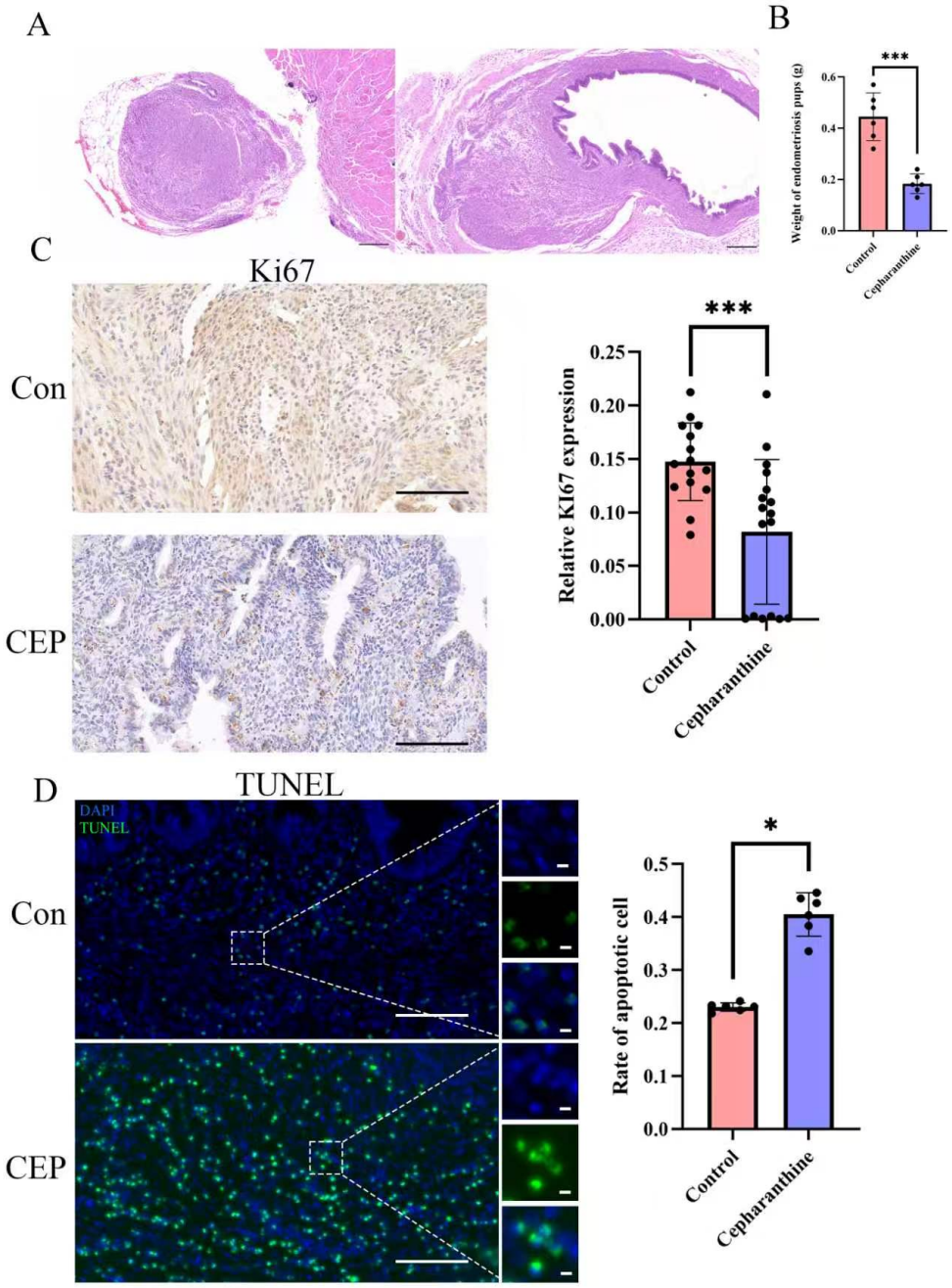

Fig. 4. Cepharanthine decreased endometriotic lesion formation in a mouse model of endometriosis. (A) Hematoxylin and eosin (HE) staining verification of the endometriotic lesions on the 14th day after the peritoneal injection of endometrial fragments. Left image: dense purple tissue mass indicates ectopic endometrial glands and stroma; Right image: irregular serrated glands around the cavity represent an ectopic endometriotic lesion, scale bar = 100 µm. (B) Cepharanthine treatment dramatically decreased the weight of the ectopic lesions. Data represented the mean (SD) of 6 independent experiments. (C) Cepharanthine administration significantly decreased Ki67 expression *in vivo*. The expression level of Ki67 was determined by an immunohistochemistry (IHC) scoring system (0–9 points). The scores were calculated as the chroma of the signal (negative = 0; light yellow = 1; light brown = 2; dark brown = 3) × percentage of positive cells (no signal = 0; weak signal = 0~25%; intermediate signal = 25~50%; strong signal = above 50%). Control: n = 15, Cepharanthine: n = 17, scale bar = 100 µm. (D) Cepharanthine administration significantly increased terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive cells inside the ectopic lesions *in vivo*. Images of TUNEL-positive nuclei were counted by Image J (Version 1.53k, National Institutes of Health, Bethesda, MD, USA), scale bar = 100 µm, n = 6. *** $p < 0.001$; * $p < 0.05$.

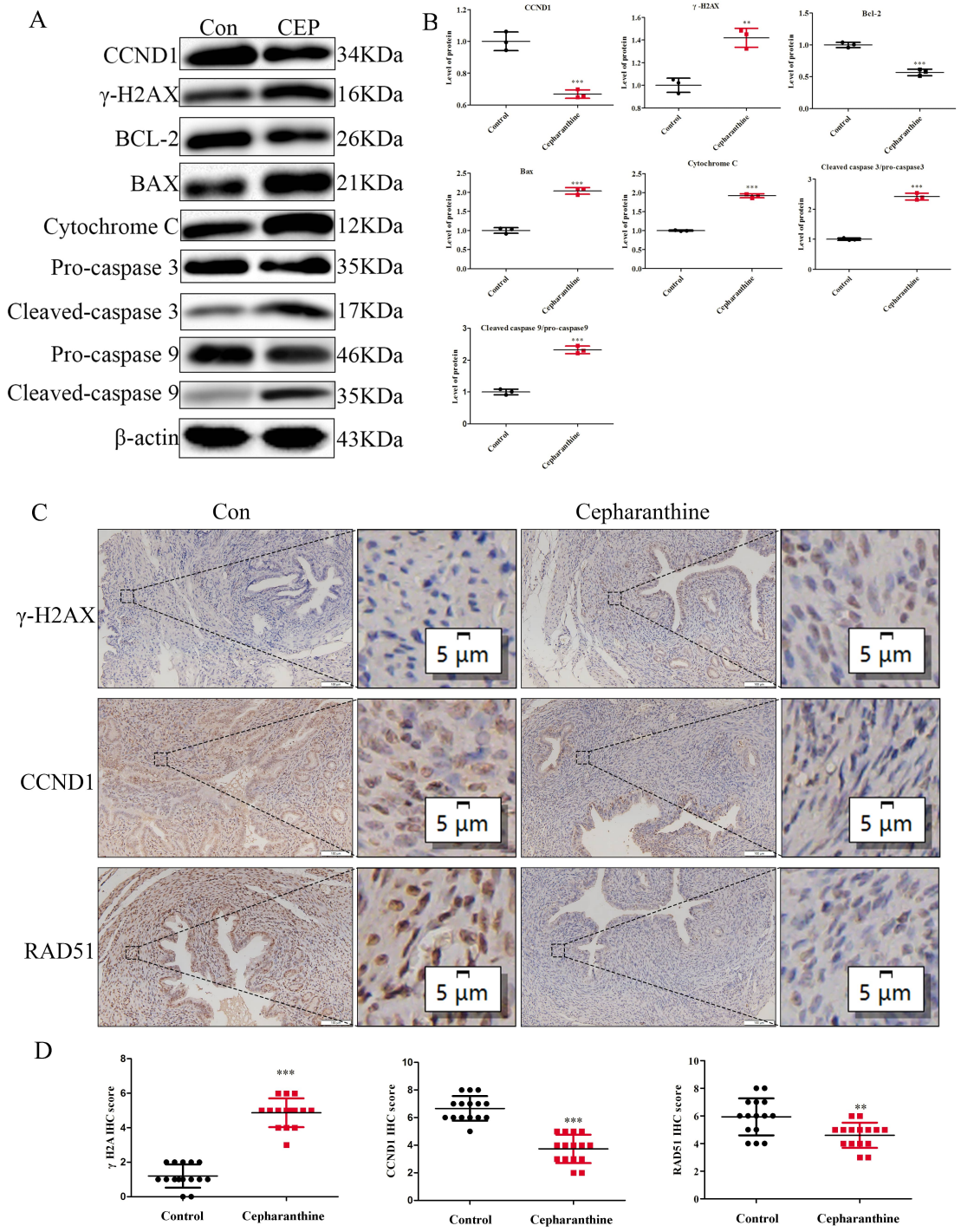

Fig. 5. Cepharanthine inhibited cell proliferation and induced apoptosis through caspase-9/caspase-3 pathway. It also affected homologous recombination-mediated repair in ectopic endometrial cells *in vivo*. (A) Immortalized endometrial stromal cells were treated with cepharanthine or saline. Western Blot analysis was used to determine the levels of proteins in each group. (B) Expression levels of each protein by Western Blot. Data represented the mean (SD) of 3 independent experiments. (C) Representative sections were taken from the cepharanthine-treated endometriosis lesions and the control, scale bar = 5 μm. (D) Expression levels of CCND1, RAD51 and γ-H2AX were quantified using immunohistochemical staining scores (0–9), i.e., scores equaled to the intensity of the signal (0, negative; 1, light yellow; 2, light brown; 3, dark brown) multiplied by the percentage of positive cells (0, no signal, 0%; 1, weak signal, <25%; 2, intermediate signal, 25–50%; and 3, strong signal, >50%). Control: n = 15, Cepharanthine: n = 15. Scale bar = 100 μm. *** $p < 0.001$; ** $p < 0.01$.

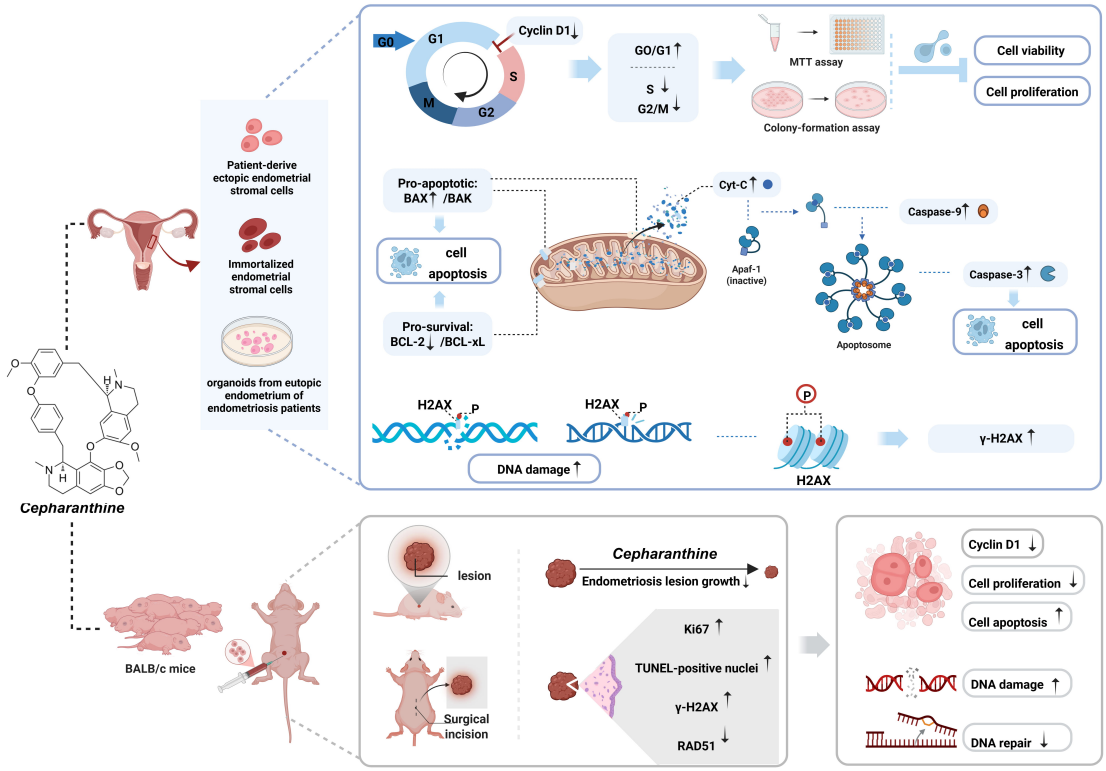

Fig. 6. Diagram of the mechanism by which cepharanthine works against endometriosis. CCND1, cyclin D1; BCL-2, B-cell lymphoma 2; BAX, Bcl-2-associated X protein; BAK, Bcl-2 homologous antagonist/killer; RAD51, DNA repair protein RAD51 homolog 1, ↑, means up-regulated; ↓, means down-regulated.

that cepharanthine significantly downregulated cyclin D1 expression (Fig. 5A,B).

3.6 Cepharanthine Affected Homologous Recombination-Mediated Repair in Ectopic Endometrial Cells

Previous studies have shown that downregulation of cyclin D1 can impair cellular DNA repair [18,21,29]. Since the *in vitro* result showed that cepharanthine treatment could downregulate cyclin D1, we examined cyclin D1 expression in endometriotic lesions from cepharanthine-treated mice. Consistent with the *in vitro* test, the cepharanthine treatment downregulated cyclin D1, concurrently increased the expression of γ-H2AX, and decreased the expression of RAD51 (a protein critical in DNA repair) (Fig. 5C,D). These results were consistent with those of previous studies.

4. Discussion

Endometriosis is a chronic disease that presents with recurring symptoms and is difficult to cure [30]. Available treatments for endometriosis are generally considered to be unsatisfactory [31]. Common medical treatments, such as gonadotropin-releasing hormone agonists, progestins, aromatase inhibitors, and androgens, are associated with various side effects and are not fully effective; the disease frequently returns [32]. Recently, a growing number of studies have identified plant-derived agents as a treatment option for endometriosis to achieve high efficacy and minimize adverse effects [33]. This study focused on the therapeutic effect of cepharanthine, an approved Traditional Chinese Medicine monomer, in treating endometriosis. Cepharanthine was purified by Kondo from *Stephania cepharantha* Hayata in 1934, then tested in humans, based on the traditional use of its original plant [7]. It has been widely used for the past seventy years to treat a variety of acute and chronic diseases, including radiation-induced leukopenia, venomous snakebites, HIV, and the novel coronavirus [4,34], with few known side effects. Studies have already reported various pharmacological effects of cepharanthine, such as antitumor [6,8,9], antimalarial [35], antioxidant [36], anti-allergic [35], and anti-inflammatory [37] effects. However, this drug was not previously tested for endometriosis.

In the present study, cepharanthine inhibited the viability of immortalized endometrial and patient-derived ectopic stromal cells *in vitro*. Recent studies have reported that newly developed endometriosis organoids function as patient-specific avatars by providing expandable biological materials suitable for drug screening [38]. Given that the eutopic endometrium of endometriosis patients exhibits marked changes in proliferation, adhesion, and angiogenesis compared with healthy endometrium [39], we gener-

ated patient-derived eutopic endometrial organoids to test the effects of cepharanthine treatment *in vitro*. Results showed that cepharanthine decreased the viability of endometrial organoids with an IC50 of 9.058 μM. We also established a mouse peritoneal endometriosis model and found that cepharanthine treatment prevented the formation of endometriosis *in vivo*. The formation and survival of endometriotic lesions at ectopic sites have been reported to be mainly dependent on biological processes such as proliferation and apoptosis [40,41,42], and the imbalance between cellular proliferation and cell death contributes to the tumor-like, uncontrolled growth of ectopic tissues. We therefore conducted a series of experiments to illustrate the potential mechanism of cepharanthine against endometriosis. The colony-formation assay indicated that the growth ability of the immortalized endometrial stromal cells was markedly inhibited by cepharanthine treatment. The anti-proliferation and apoptosis-inducing ability of cepharanthine was further confirmed by immunohistochemistry *in vivo*, as indicated by decreased Ki67 expression and increased TUNEL-positive nuclei inside the endometriotic lesions of cepharanthine-treated mice. Cellular apoptosis has been reported to be induced by the downregulation of anti-apoptotic proteins (e.g., Bcl-2) or the upregulation of pro-apoptotic proteins (e.g., Bax) [43]. Our Western Blot results indicated that cepharanthine upregulated cytochrome C in the cytoplasm, enhanced the expression of cleaved-caspase-9, cleaved-caspase-3, and Bax, and downregulated the expression of Bcl-2 in immortalized endometrial stromal cells, consistent with the previous study [44]. Furthermore, cepharanthine caused DNA damage, which was further confirmed by the elevated expression of γ-H2AX (a well-established biomarker for DNA double-strand breaks [45] in cepharanthine-treated immortalized endometrial stromal cells and inside the endometriotic lesions of cepharanthine-treated mice. Previous bioinformatic analysis revealed that the elevated level of cyclin D1 is closely related to the pathogenesis of endometriosis [46]. Studies have also shown that the downregulation of cyclin D1 impairs cellular DNA repair [18,21,29,47]. Our *in vitro* test demonstrated that cepharanthine treatment downregulatedcyclin D1expression, thereby inducing G0/G1 phase cell-cycle arrest. We then checked cyclin D1 expression inside the endometriotic lesions of cepharanthine-treated mice. Consistent with the *in vitro* test, cepharanthine treatment downregulated cyclin D1, concurrently increased the expression of γ-H2AX, and decreased the expression of RAD51 (a protein critical in DNA repair), which was consistent with previous studies [18,21].

Taken together, the mechanisms by which cepharanthine operates against endometriosis involve triggering apoptosis through the caspase-9/caspase-3 pathway, inducing cell cycle arrest, causing cellular DNA damage, and impairing DNA repair by downregulating cyclin D1 (Fig. 6).

However, this study has limitations that must be acknowledged. First, although the mouse model is standard, it does not fully replicate the complex hormonal and immune microenvironment of human endometriosis. Second, our focus was primarily on stromal cells; the effect on epithelial cells within lesions warrants further investigation. Third, the precise upstream signaling responsible for cyclin D1 downregulation by cepharanthine remains to be elucidated. Investigations using more advanced models, such as patient-derived xenografts or humanized systems, would better predict clinical efficacy. Exploring potential synergies between cepharanthine and existing hormonal therapies could reveal combination strategies to prevent recurrence. Moreover, given the known anti-inflammatory properties of cepharanthine [36], its impact on the pelvic inflammatory milieu of endometriosis is a compelling avenue for study.

Limitation

In our research, we verified the cepharanthine therapeutic potential for endometriosis in cell model, animal model and organoid derived from patients, but the clinical trial of this drug monomer on patients has not yet been conducted, and there is a lack of clinical data. The exploration of the pharmacological and mechanistic aspects of drug action will be carried out in future research.

5. Conclusions

Our findings suggested that cepharanthine may serve as a promising cytotoxic agent for the treatment of endometriosis. As cepharanthine tablets are commercially available in Chinese pharmacies, this agent could be rapidly advanced into clinical trials, providing potential therapeutic benefits for patients with endometriosis.

Availability of Data and Materials

All original data generated in this study are available within the manuscript. Further relevant information can be obtained from the corresponding author upon reasonable request.

Author Contributions

WXC researched the literature, conducted the experiments, and drafted the manuscript. HTL conducted literature searches and performed animal experiments. HXY supervised the experiments and conducted part of them. YingX did the surgeries and provided the samples. YangX conceptualized the study and edited the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was carried out in accordance with the guidelines of the Declaration of Helsinki. The use of human resected tissues for this experiment was approved by the Ethics Committee of West China Second University Hospital of Sichuan University on March 7th, 2018 (Ethical Approval No.: Medical Research 2018 No.48). Written informed consent was acquired from each participant before the sample was obtained. All animal procedures were approved by the Institutional Animal Care and Use Committee of West China Second University Hospital of Sichuan University (Approval No.2020028) and conducted in accordance with ethical standards and guidelines. All animal experimental procedures were conducted in accordance with the principles of the 3Rs (Replacement, Reduction, and Refinement).

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Conflicts of Interest

The authors declare no conflicts of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/CEOG48879.

References

[1] Shafrir AL, Farland LV, Shah DK, Harris HR, Kvaskoff M, Zondervan K, et al. Risk for and consequences of endometriosis: A critical epidemiologic review. Best Practice & Research. Clinical Obstetrics & Gynaecology. 2018; 51: 1–15. https://doi.org/10.1016/j.bpobgyn.2018.06.001

[2] Zondervan KT, Becker CM, Missmer SA. Endometriosis. The New England Journal of Medicine. 2020; 382: 1244–1256. https://doi.org/10.1056/NEJMra1810764

[3] Yu Y, Zhu Y, Gao M, Wang Y, Li S, Gou Y. Research progress on natural plant metabolites targeting apoptosis for endometriosis prevention and treatment: a systematic review. Frontiers in Pharmacology. 2025; 16: 1624569. https://doi.org/10.3389/fphar.2025.1624569

[4] Rogosnitzky M, Okediji P, Koman I. Cepharanthine: a review of the antiviral potential of a Japanese-approved alopecia drug in COVID-19. Pharmacological Reports. 2020; 72: 1509–1516. https://doi.org/10.1007/s43440-020-00132-z

[5] Xia B, Zheng L, Li Y, Sun W, Liu Y, Li L, et al. The brief overview, antivirus and anti-SARS-CoV-2 activity, quantitative methods, and pharmacokinetics of cepharanthine: a potential small-molecule drug against COVID-19. Frontiers in Pharmacology. 2023; 14: 1098972. https://doi.org/10.3389/fphar.2023.1098972

[6] Tang ZH, Cao WX, Guo X, Dai XY, Lu JH, Chen X, et al. Identification of a novel autophagic inhibitor cepharanthine to enhance the anti-cancer property of dacomitinib in non-small cell lung cancer. Cancer Letters. 2018; 412: 1–9. https://doi.org/10.1016/j.canlet.2017.10.001

[7] Bailly C. Cepharanthine: An update of its mode of action, pharmacological properties and medical applications. Phytomedicine : International Journal of Phytotherapy and Phytopharmacology. 2019; 62: 152956. https://doi.org/10.1016/j.phymed.2019.152956

[8] Payon V, Kongsaden C, Ketchart W, Mutirangura A, Wonganan P. Mechanism of Cepharanthine Cytotoxicity in Human Ovarian Cancer Cells. Planta Medica. 2019; 85: 41–47. https://doi.org/10.1055/a-0706-7503

[9] Gao S, Li X, Ding X, Qi W, Yang Q. Cepharanthine Induces Autophagy, Apoptosis and Cell Cycle Arrest in Breast Cancer Cells. Cellular Physiology and Biochemistry : International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology. 2017; 41: 1633–1648. https://doi.org/10.1159/000471234

[10] Meresman GF, Götte M, Laschke MW. Plants as source of new therapies for endometriosis: a review of preclinical and clinical studies. Human Reproduction Update. 2021; 27: 367–392. https://doi.org/10.1093/humupd/dmaa039

[11] Boretto M, Maenhoudt N, Luo X, Hennes A, Boeckx B, Bui B, et al. Patient-derived organoids from endometrial disease capture clinical heterogeneity and are amenable to drug screening. Nature Cell Biology. 2019; 21: 1041–1051. https://doi.org/10.1038/s41556-019-0360-z

[12] Gu ZY, Jia SZ, Liu S, Leng JH. Endometrial Organoids: A New Model for the Research of Endometrial-Related Diseases†. Biology of Reproduction. 2020; 103: 918–926. https://doi.org/10.1093/biolre/ioaa124

[13] Garvey M. Endometriosis: Future Biological Perspectives for Diagnosis and Treatment. International Journal of Molecular Sciences. 2024; 25: 12242. https://doi.org/10.3390/ijms252212242

[14] Yang H, Zhang J, Yan F, Chen Y, Wu Y, Luo J, et al. Ciliary IFT-B Transportation Plays an Important Role in Human Endometrial Receptivity Establishment and is Disrupted in Recurrent Implantation Failure Patients. Cell Proliferation. 2025; 58: e13819. https://doi.org/10.1111/cpr.13819

[15] Li Y, Zeng X, Lu D, Yin M, Shan M, Gao Y. Erastin induces ferroptosis via ferroportin-mediated iron accumulation in endometriosis. Human Reproduction (Oxford, England). 2021; 36: 951–964. https://doi.org/10.1093/humrep/deaa363

[16] Turco MY, Gardner L, Hughes J, Cindrova-Davies T, Gomez MJ, Farrell L, et al. Long-term, hormone-responsive organoid cultures of human endometrium in a chemically defined medium. Nature Cell Biology. 2017; 19: 568–577. https://doi.org/10.1038/ncb3516

[17] Zhang W, Zhang F. Effects of quercetin on proliferation, apoptosis, adhesion and migration, and invasion of HeLa cells. European Journal of Gynaecological Oncology. 2009; 30: 60–64.

[18] Zhong Q, Hu Z, Li Q, Yi T, Li J, Yang H. Cyclin D1 silencing impairs DNA double strand break repair, sensitizes BRCA1 wildtype ovarian cancer cells to olaparib. Gynecologic Oncology. 2019; 152: 157–165. https://doi.org/10.1016/j.ygyno.2018.10.027

[19] Zhong Q, Wen YJ, Yang HS, Luo H, Fu AF, Yang F, et al. Efficient inhibition of cisplatin-resistant human ovarian cancer growth and prolonged survival by gene transferred vesicular stomatitis virus matrix protein in nude mice. Annals of Oncology : Official Journal of the European Society for Medical Oncology. 2008; 19: 1584–1591. https://doi.org/10.1093/annonc/mdn167

[20] Zhang XQ, Yang CY, Rao XF, Xiong JP. Plumbagin shows anticancer activity in human breast cancer cells by the upregulation

of p53 and p21 and suppression of G1 cell cycle regulators. European Journal of Gynaecological Oncology. 2016; 37: 30–35.
[21] Zhong Q, Xiong Y, Ling C, Qian Y, Zhao X, Yang H. Enhancing the sensitivity of ovarian cancer cells to olaparib via microRNA-20b-mediated cyclin D1 targeting. Experimental Biology and Medicine (Maywood, N.J.). 2021; 246: 1297–1306. https://doi.org/10.1177/1535370221994077
[22] Yuan M, Li D, An M, Li Q, Zhang L, Wang G. Rediscovering peritoneal macrophages in a murine endometriosis model. Human Reproduction (Oxford, England). 2017; 32: 94–102. https://doi.org/10.1093/humrep/dew274
[23] Jiang X, Huang X, Zheng G, Jia G, Li Z, Ding X, et al. Targeting PI4KA sensitizes refractory leukemia to chemotherapy by modulating the ERK/AMPK/OXPHOS axis. Theranostics. 2022; 12: 6972–6988. https://doi.org/10.7150/thno.76563
[24] Feng F, Pan L, Wu J, Li L, Xu H, Yang L, et al. Cepharanthine inhibits hepatocellular carcinoma cell growth and proliferation by regulating amino acid metabolism and suppresses tumorigenesis *in vivo*. International Journal of Biological Sciences. 2021; 17: 4340–4352. https://doi.org/10.7150/ijbs.64675
[25] Lu YY, Zhu CY, Ding YX, Wang B, Zhao SF, Lv J, et al. Cepharanthine, a regulator of keap1-Nrf2, inhibits gastric cancer growth through oxidative stress and energy metabolism pathway. Cell Death Discovery. 2023; 9: 450. https://doi.org/10.1038/s41420-023-01752-z
[26] Zhang H, Li Y, Huang Q, Ren X, Hu H, Sheng H, et al. MiR-148a promotes apoptosis by targeting Bcl-2 in colorectal cancer. Cell Death and Differentiation. 2011; 18: 1702–1710. https://doi.org/10.1038/cdd.2011.28
[27] Yang ZY, Li LG, Xiong YL, Chen NN, Yu TT, Li HT, et al. Cepharanthine synergizes with photodynamic therapy for boosting ROS-driven DNA damage and suppressing MTH1 as a potential anti-cancer strategy. Photodiagnosis and Photodynamic Therapy. 2024; 45: 103917. https://doi.org/10.1016/j.pdpdt.2023.103917
[28] Kuo LJ, Yang LX. Gamma-H2AX - a novel biomarker for DNA double-strand breaks. In Vivo (Athens, Greece). 2008; 22: 305–309.
[29] Jirawatnotai S, Hu Y, Michowski W, Elias JE, Becks L, Bienvenu F, et al. A function for cyclin D1 in DNA repair uncovered by protein interactome analyses in human cancers. Nature. 2011; 474: 230–234. https://doi.org/10.1038/nature10155
[30] Saunders PTK, Whitaker LHR, Horne AW. Endometriosis: Improvements and challenges in diagnosis and symptom management. Cell Reports. Medicine. 2024; 5: 101596. https://doi.org/10.1016/j.xcrm.2024.101596
[31] Xu H, Becker CM, Lui WT, Chu CY, Davis TN, Kung AL, et al. Green tea epigallocatechin-3-gallate inhibits angiogenesis and suppresses vascular endothelial growth factor C/vascular endothelial growth factor receptor 2 expression and signaling in experimental endometriosis in vivo. Fertility and Sterility. 2011; 96: 1021–1028. https://doi.org/10.1016/j.fertnstert.2011.07.008
[32] Zondervan KT, Becker CM, Koga K, Missmer SA, Taylor RN, Viganò P. Endometriosis. Nature Reviews. Disease Primers. 2018; 4: 9. https://doi.org/10.1038/s41572-018-0008-5
[33] Bina F, Soleymani S, Toliat T, Hajimahmoodi M, Tabarrai M, Abdollahi M, et al. Plant-derived medicines for treatment of endometriosis: A comprehensive review of molecular mechanisms. Pharmacological Research. 2019; 139: 76–90. https://doi.org/10.1016/j.phrs.2018.11.008
[34] Zhang S, Huang W, Ren L, Ju X, Gong M, Rao J, et al. Comparison of viral RNA-host protein interactomes across pathogenic RNA viruses informs rapid antiviral drug discovery for SARS-CoV-2. Cell Research. 2022; 32: 9–23. https://doi.org/10.1038/s41422-021-00581-y
[35] Rogosnitzky M, Danks R. Therapeutic potential of the bisco-claurine alkaloid, cepharanthine, for a range of clinical conditions. Pharmacological Reports : PR. 2011; 63: 337–347. https://doi.org/10.1016/s1734-1140(11)70500-x
[36] Halicka D, Ita M, Tanaka T, Kurose A, Darzynkiewicz Z. Biscoclaurine alkaloid cepharanthine protects DNA in TK6 lymphoblastoid cells from constitutive oxidative damage. Pharmacological Reports : PR. 2008; 60: 93–100.
[37] Huang H, Hu G, Wang C, Xu H, Chen X, Qian A. Cepharanthine, an alkaloid from Stephania cepharantha Hayata, inhibits the inflammatory response in the RAW264.7 cell and mouse models. Inflammation. 2014; 37: 235–246. https://doi.org/10.1007/s10753-013-9734-8
[38] Esfandiari F, Favaedi R, Heidari-Khoei H, Chitsazian F, Yari S, Piryaei A, et al. Insight into epigenetics of human endometriosis organoids: DNA methylation analysis of HOX genes and their cofactors. Fertility and Sterility. 2021; 115: 125–137. https://doi.org/10.1016/j.fertnstert.2020.08.1398
[39] Retis-Resendiz AM, Gómez-Suárez SK, García-Gómez E, Vázquez-Martínez ER. Molecular Basis of Impaired Decidualization in the Eutopic Endometrium of Endometriosis Patients. Cells. 2025; 14: 326. https://doi.org/10.3390/cells14050326
[40] Nasu K, Yuge A, Tsuno A, Nishida M, Narahara H. Involvement of resistance to apoptosis in the pathogenesis of endometriosis. Histology and Histopathology. 2009; 24: 1181–1192. https://doi.org/10.14670/HH-24.1181
[41] Jiang D, Zhang X, Shi J, Tao D, Nie X. Risk factors for ovarian endometrioma recurrence following surgical excision: a systematic review and meta-analysis. Archives of Gynecology and Obstetrics. 2021; 304: 589–598. https://doi.org/10.1007/s00404-021-06129-0
[42] Aznaurova YB, Zhumataev MB, Roberts TK, Aliper AM, Zhavoronkov AA. Molecular aspects of development and regulation of endometriosis. Reproductive Biology and Endocrinology : RB&E. 2014; 12: 50. https://doi.org/10.1186/1477-7827-12-50
[43] Seo JH, Jeong ES, Choi YK. Therapeutic effects of lentivirus-mediated shRNA targeting of cyclin D1 in human gastric cancer. BMC Cancer. 2014; 14: 175. https://doi.org/10.1186/1471-2407-14-175
[44] Seubwai W, Vaeteewoottacharn K, Hiyoshi M, Suzu S, Puapairoj A, Wongkham C, et al. Cepharanthine exerts antitumor activity on cholangiocarcinoma by inhibiting NF-kappaB. Cancer Science. 2010; 101: 1590–1595. https://doi.org/10.1111/j.1349-7006.2010.01572.x
[45] Zhao W, Steinfeld JB, Liang F, Chen X, Maranon DG, Jian Ma C, et al. BRCA1-BARD1 promotes RAD51-mediated homologous DNA pairing. Nature. 2017; 550: 360–365. https://doi.org/10.1038/nature24060
[46] Ping S, Ma C, Liu P, Yang L, Yang X, Wu Q, et al. Molecular mechanisms underlying endometriosis pathogenesis revealed by bioinformatics analysis of microarray data. Archives of Gynecology and Obstetrics. 2016; 293: 797–804. https://doi.org/10.1007/s00404-015-3875-y
[47] Musgrove EA, Caldon CE, Barraclough J, Stone A, Sutherland RL. Cyclin D as a therapeutic target in cancer. Nature Reviews. Cancer. 2011; 11: 558–572. https://doi.org/10.1038/nrc3090