RESEARCH PAPER

Ethacridine induces ROS-mediated apoptosis on ovarian carcinoma cells SKOV3 by suppressing JAK2/STAT3/ERK pathways

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ABSTRACT

Aggressive and recurring gynecological malignancies are associated with a poor prognosis and ineffective treatment. Patients with ovarian cancer (OC) are frequently detected at an advanced stage, when medication resistance, angiogenesis, recurrence, and metastases all have an impact on survival. Ovarian cancer (OC) remains the deadliest gynecologic tumor worldwide. Ethacridine lactate performs a safe and effective method. Ethacridine (ETC) is known for its antiseptic actions. Recently, it has been reported that ETC exerts anticancer effects by inhibiting numerous cancer cells. Thus we explore the anti-tumor efficiency of ETC (2.5-20 µM/ml) on human ovarian cancer (SKOV3) cells and its modulation of janus kinase/signal transducers and activators of transcription/Extracellular signal regulated (JAK/STAT3/ERK) networks. The activity of ETC on SKOV3 cell viability, MMP loss, ROS, apoptotic markers, cytokines, apoptosis, and JAK/STAT3/ERK networks was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test, rhodamine (Rh-123), dichlorodihydrofluorescein diacetate (DCFH-DA), 4',6-diamidino-2-phenylindole (DAPI), propidium iodide (PI), Annexin V–FITC and PI, real time polymerase chain reaction (RT-PCR), western blot, and Flow cytometry analysis. Our results exposed that ETC could reduce SKOV3 cell viability in an amount-reliant way and 10 µM was selected as IC_{50} value for further studies. Our findings established that ETC (10 µM) could inhibit SKOV3 cell viability through enhanced intracellular reactive oxygen species (ROS), mitochondrial membrane potential (MMP) loss, and stimulated apoptosis against control. Likewise, ETC could moderate inflammatory cytokines, generate apoptotic markers, and instigate cell death pathways. Here, we proved that ETC attenuates SKOV3 cell proliferation by ROS-facilitated apoptosis, which suppresses JAK/STAT3/ERK pathways. Hence, ETC may be a promising anticancer agent for OC management.

Keywords: Ethacridine, Ovarian cancer, proliferation, apoptosis, JAK/STAT3/ERK

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INTRODUCTION

Ovarian cancer (OC) is a deadly tumor that occursin the woman's reproductive structure [1] According to the GLOBOCAN reports, OC belongs to the 8thutmost female carcinoma mortality in 2020, accounting for 3.4% of total diagnosed carcinomasworldwide [2]. Accumulating data point out that OC is frequently diagnosed in progressive stages owing to its asymptomatic nature and high rate of metastasis that leads to elevated mortality [3–5]. OC is a cancerous malignancy of the ovary that can grow in the ovary itself or connect with nearby structures, including fallopian tubes and the abdomen lining. It concerning the reproductive system can have a vital influence on a woman's quality of life. Regardless of numerous existing therapeutic methods of surgery and chemotherapy, the 5-year survival fraction of OC is almost 30–40%, due to the lack of substantial signs at former phases and delayed identification [6-9]. To recover the inclusive persistence proportion of patients having OC, contemporary researchers have directed the finding of effective novel remedial approaches including nutraceuticals, which are less harmful [10]. Between these, natural bioactive metabolites have fascinated the consideration of many academics as a result of their comprehensive structural and functional aspects along with less toxicity [11].

Generally, ethacridine (ETC) is known for its antiseptic actions such as antibacterial and antiviral effects [12,13]. ETC (trade name rivanol) is extensively used for second-trimester abortion as a topical antiseptic agent [14]. For the screening for effective medication, ETC has exposed anticancer effects against numerous kinds of malignancies by specially aiming the tumor cells [15]. Previously, it has been investigated that ETC could reduce leukemia cells [15]. Recently, ETC unveiled reduced cell

proliferation together with clonogenicity of thyroid follicular cells and induced apoptosis. Besides, ETC plays a role in thyroid follicular cell discrepancy from human embryonic stem cells [16]. A very recent study has proven that ETC showed anticancer and apoptotic activity against human CRC colon cancer cells. They have established that ETC exhibits antiproliferative, apoptotic, and anti-inflammatory activity by the regulation of JAK2/STAT3/ERK networks in CRC colon cancer cells [17]. Hence, our research is directed to explore the anticancer efficacy of ETC on human SKOV3 cells and evaluate its latent molecular mechanisms.

As typical cell demise, apoptosis occurs in intrinsic and extrinsic pathways, which is observed in numerous pathological conditions. These pathways are regulated by several transduction pathways and coordinated by gene complexes [17]. As a vital procedure, apoptosis is to conserve cellular homeostasis; hence it is a significant target for innovative anti-cancer drug development [18]. During apoptosis, both intra- and extracellularly generate oxidative stress (OS) mostly due to the formation of reactive oxygen species (ROS) [19]. Enhanced ROS production affects the mitochondrial malfunction chiefly superoxide, which stimulates cellular-level oxidative phosphorylation undertaking cell death [20,21]. Additionally, variations in Bcl-2 members originate from the destabilization of the mitochondrial dysfunction, triggering caspase-3 that leads to programmed cell demise [22]. Furthermore, ROS accumulation is an alternative circumstance, which undergoes stress that directs to apoptosis [23]. Several prior pieces of indications also emphasized the vital action of signaling pathways of JAK2/STAT3 in the instigation and diversity of various malignant tumors comprising OC [24,25]. Formerly it has documented those agents that avert JAK/STAT/ERK signaling results in OC cell proliferation reduction and apoptosis [26]. There is no information available on the anticancer efficacy of ETC on human OC cells. Hence forth, we designed to examine the antitumor action of ETC on human OC cells SKOV3 and assess its role in the JAK/STAT/ERK molecular networks.

MATERIALS AND METHODS

1. Chemicals

RPMI-1640 medium, culturing reagents, ETC, bio-chemicals, and antibiotics were attained from Ruicong Ltd (Shanghai, China). For western blot analysis, the antibodies were acquired from Ese-Bio (Shanghai, China).

2. Cell Culture

Human OC cells SKOV3were procured from the National Cell Bank of Iran (NCBI) (Tehran, Iran), and cultivated in RPMI 1640 mediacomprising FBS (10%) and penicillin/streptomycin (1 %) by preservingat 37oCin a CO2 incubator.

3. Cell proliferationassay

SKOV3 cells were sowed in 96-well dishes, at a quantity of 1 × 105 cells/well and conserved for 24h. These SKOV3cells were administered with ETC (2.5 to 20 μM/ml) for one day. According to the previous protocol [27] the MTT test was conducted and calculated the cell viability.

4. Estimation of intracellular ROS level

The formation of intracellular ROS in SKOV3 human OC cells was measured by using the DCFH-DA staining technique [27]. SKOV3 cells were administered with ETC (10 μM) subsequently treated with the DCFH-DA (10 μM) and sustained for 30 mins at 37°C. Measured by using a well plate reader at 485/535 nm wavelength.

5. Assay of mitochondrial membrane potential (*⊿*Ψm)

The *⊿*Ψm was assessed by staining with Rh-123, a well-known cationic lipophilic dye. SKOV3human OC cells were cultivated at 2×105 cells/dish, and added with 10μM/ml of ETC for one day, then administered with 5 μg/ml of Rh-123, subjected to PBS baths, and detected below fluorescencemicroscope. The red/green fluorescence ratio designates the MMP level thus assessing the influence of ETC (10 μM) on mitochondrial dysfunction[27].

6. Apoptosis studies

Apoptotic cell death was inspected by consuming DAPI and PI staining. SKOV3 cells were matured in six-well plates containing ETC (10 μM) at 37oC, and then paraformaldehyde (4%) fixed for half an hour. Cellular morphological modifications during apoptosis by staining with DAPI and PI were perceived using the fluorescence microscope [27].

7. Apoptosis analysis by PI/Annexin V-FITC

1×106 cells per well for a 6-well plate used DMEM cell culture medium, after 18 hours, Replaced medium and treated cells with ETC (10 μM), and incubate for 48 hours, then 500μL of cell suspension with10μL of PI and 5 μL Annexin-V were added. The suspension is incubated for 15 minutes at RT in the dark. Post incubation, the cells were analyzed by flow cytometer.

8. Evaluation of mRNA expression level

Entire RNA was isolated from human SKOV3 cells as per the manufacturer's procedures by using TRIzol ® reagent (Abcam, USA). The isolated RNA undergoes a reverse transcription to from cDNA according to a technique of High-capacity cDNA Reverse Transcription kit (EZ BioResearch, USA). At that point, the Fast Start SYBR Green master mix (EZ BioResearch, USA) was used to examine the cDNA as per the company's techniques. The primers were employed by RT-PCR analysis. Triplet the samples

were run and fold changes expression standardized with β-actin control and estimated by the 2-ΔΔCt method.

9. Western blot analysis

Human OC cells SKOV3 were administered with ETC (10 μM/ml) and cultured for one day. Prepared the cell lysates by the treatment of ice-cold lysis buffer ensuring inhibitor protease and performed analysis of western blot. Total protein was estimated by the procedure of the BCA Test Protein Kit (Pierce Chemical Co, USA). Following, quantified, electrophoretically detached, and moved to a PVDF film. It was blocked at room temperature by using the probe for one hour, treated primary antibodies in 1:1,000 dilutions, and kept at 4oC overnight. Then, HRP-conjugated secondary antibodies were administered. Stained the protein bands and imagined for the recognition of protein. Quantified the protein bands through densitometry with software Image J and homogeneous to β-actin expression.

10. Statistical methods

The analysis of statistics was accomplished by Graphpad prism software version 9.0 and expressed as mean ± SEM. It was executed by an analysis of variance (ANOVA) and subsequently Duncan's test. Student T-test has been conducted and $P<0.05$ was recognized to be significant statistically.

RESULTS

Anti-proliferative efficacy of ETC on SKOV3 cells

The antiproliferative activity of human OC cells SKOV3 was estimated by the MTT experiment with assorted quantities (2.5–20 μM/ml) of ETC. ETC presented a substantial quantity-reliant cell viability reduction. 10 μM ETC was calculated as the IC_{50} in SKOV3 cells. Hence, ETC at a concentration of 10 μM was selected for further study (Fig. 1).

ETC induces the accumulation of intracellular ROS in SKOV3 cells

Fig. 2 depicts that treatment with ETC (10 μM) in SKOV3 cells elevated the fluorescence intensity that exhibited a prominent bright green color in cells versus control cells. The intensity of ROS accumulation in DCFH-DA-branded cells was noticed. The ROS intensity was greatly augmented ($P<0.05$) to 10 μM of ETC in contrast to control.

ETC stimulated mitochondrial damage in SKOV3 cells

Depolarization in the mitochondrial membrane was recognized by the cell's competence to attain Rh-123 dye that emits green fluorescence. The control cells exhibited great strength of Rh-123 in MMP. The Rh-123 fluorescence accretion was reduced in ETC-administered (10 μM) SKOV3 cells (Fig. 3). ETC (10 μM) supplemented SKOV3 cells unveiled considerably great ($P<0.05$) MMP loss caused by deepened depolarization in mitochondria. These results established that ETC-administered SKOV3 cells mitigated the MMP thus enhancing the mitochondrial-facilitated apoptotic cell demise.

ETC stimulated apoptosis on SKOV3 cells exhibited by the staining of DAPI

SKOV3 cells administered with DAPI exposed the existence of characteristic live cells with typical nuclei. Administration of ETC (10 μM) in the SKOV3 cells prompted cell death that intensified the nuclear bodies fragmentation and morphology as related to SKOV3 cells (Fig. 4). These outcomes underscored that ETC-triggered apoptosis was significantly high ($p<0.05$) against control.

ETC-stimulated apoptosis on SKOV3 cells proved by PI

As an effective stain PI was to discriminate the apoptotic and necrotic nucleus differentiation of SKOV3 cells. The stain passes towards the cells when dropping the membrane structures, which is interrelated with the flagging of polarization in the membrane that specifies apoptotic cell disease. The PI uncovered that ETC is prone to apoptotic deeds on SKOV3 (Fig. 5). Supplemented with ETC (10 μM) substantially enhances ($p<0.05$) the apoptotic activity versus control. Hence, ETC-triggered apoptotic cell demise would be one among the deeds of diminishing SKOV3 cell viability.

Flow cytometry identification of apoptotic cells

After 48 h, the early/late apoptotic cell percentages were higher in ETN (10 μM) treated SKOV3 cells compared to the control cells, as shown in Table 1 and Fig. 6. The early/late apoptotic cells increased compared to control cells.

Effect of ETC on apoptotic markers mRNA levels

Apoptotic markers including caspase-3, Bax, Bcl-2, Cyclin-D1, survivin, and C-Myc are documented such as the vital controllers of involuntary cell death (Fig. 7). Human OC cells SKOV3 cells revealed elevated mRNA levels of the Bcl-2, myc, cyclin-D1, and survivin while caspase-3 and Bax were reduced. ETC (10 μM) expressively attenuated ($p<0.05$) the mRNA level of Bcl-2, myc, cyclin-D, and survivin, while Bax and caspase-3 were augmented in contrast to control.

ETC elevates thecaspase-mediated apoptotic pathway

To determine the apoptotic effects of ETC on SKOV3 cells, cells were incubated with ETC (10 μM) and then protein expression of cleaved-caspase-3 and cleaved-PARP were assessed. Western blot analysis data revealed that increased expression of cleaved-caspase-3 and decreased expression levels of cleaved-PARP in ETC (10 μM) treated cells compared to control cells. Administration of ETC (10 μM) substantially suppressed ($p<0.05$) the activities of cleaved-caspase-

3 and cleaved-PARP. These findings emphasized that ETC activated caspase-mediated apoptosis in SKOVO3 cells (Fig. 8).

Anti-inflammatory effect of ETC on SKOV3 cells

Human OC cells SKOV3 cells exhibited high cytokine mRNA levels such as TNF-α, NF-κB, COX-2, and IL-6. Administration of ETC (10 μM) on SKOV3 cells uncovered significantly downregulated (p<0.05) the mRNA level of TNF-α, COX-2, NF-κB, and IL-6. Our findings established the anti-inflammatory action of ETC on human OC cells SKOV3 (Fig. 9).

ETC mitigates the signaling of JAK2/STAT3/ERKpathway

JAK2/STAT3/ERK takes part in carcinoma development, cell linkages, cell propagation, and metastasis. Western blot analysis results exposed that JAK2/STAT3/ERK protein level expressions and phosphorylation were remarkably augmented in SKOV3 cells. Administration of ETC (10 μM) considerably attenuated (p<0.05) JAK2/STAT3/ERK protein networks. These findings indicated that ETC triggered OC cell apoptosis through the suppression of the JAK2/STAT3/ERK activation in the cell signaling pathway (Fig. 10).

DISCUSSION

OC is the foremost cause of gynecological-linked tumor mortalities all over the universe [1,2]. Even with primary responses to chemotherapy, this cancer syndrome steadily degenerates. The survival rates of OC have not altered expressively owing to the complications of early diagnosis [3-5]. To cope with these problems, researchers for finding a suitable remedial approach for OC are enduring. Among them, phytochemicals are more renowned for malignant cure [10,11]. Recently, ETC gained further consideration for its anti-proliferative and apoptotic activity [15,16]. The apoptotic, antiproliferative, and genomic actions of ETC in OC were scrutinized for the first time in the literature. This research work proposes that ETC exerts an anticancer as well as apoptotic effect on OC cells and has the potential for an innovative anti-proliferative agent for epithelial OC treatment. Carrasco-Torres et al., 2017[28] demonstrated the pleiotropic effects of the combination of male anhydride derivatives and quercetin on liver cancer cells and open the possibility of using their effective chemopreventive effects in hepatocellular carcinoma.

Here, the anticancer activity of ETC on human OC cells SKOV3 was explored. ETC exhibited possible growth inhibiting activity against SKOV3 cells as supported by the MTT cell proliferation assay. An earlier study documented that several medications unveil antiproliferative effects through apoptotic cell death [18]. Our outcomes specified that ETC (IC50=10 μM) expressively repressed the growth of SKOV3 cells. These findings are in harmony with other preceding reports, in which ETC has been displayed to subdue the tumor cell proliferation of numerous malignancies [15,16,29]. Administration of ETC on SKOV3 cells also generated some morphological modifications and elevated DNA fragmentations which are typical features of apoptosis presented by DAPI and PI staining. ETC also reduced MMP, which was related to the augmented mRNA level of Bax and the protein expression of Cleaved-caspase-3 and Cleaved-PARP, while diminished Bcl-2, cyclin-D1, myc, and survivin, compared with SKOV3 control. Cyclin-D1 improves cell development by connecting to the cycle-dependent kinases and further elements[30]. The mitochondrial-based apoptotic pathways are regulated by Bcl-2 markers [31]. Bax/Bcl-2 fractions aid in responsible cell existence. Furthermore, upregulation of Bax prompts mitochondrial permeabilization, which results in cytochrome c release into the cytosol, which triggers PARP cleavage and caspase-3 activation. Thus, the activated Caspase-3 leads to DNA damage and apoptosis**.** The cleaved caspase-3 and cleaved-PARP proteins are considered vital cellular markers of apoptosis [31-32]. Survivin and C-myc are significant elements for averting apoptosis. Therefore, survivin, C-Myc, and caspase-3 expression levels are in reversely comparative each other [32]. Administration of ETC also attenuates pro-inflammatory cytokines. Our findings are in line with the previous report on the treatment of ETC on colon cancer cells SW620[29]. As previously, documented medications having apoptosis-inducing properties reveal the possibility of lessening the drug resistance [17,18], and the outcomes of the existing report specified that ETC had apoptosis-stimulating deeds, proposing that ETC may be a vital lead molecule for OC remedy.

Mounting data indicate the action of ROS in the role of signaling pathways, which are essential for cell propagation, diversity, and tumor metastasis [20,21]. Conversely, sustained ROS in high intensity is a sign of the cellular death signal stimulation through triggering death receptors or directly performing on the mitochondria [19,20]. Even though a promising influence of ROS has been detected in the ETC-facilitated apoptotic reaction, by which ETC triggers ROS formation is uncertain. Mitochondria are the main base of ROS, chiefly via electron drip from the respiratory complexes [20]. ROS is a stimulating factor in cell survival, particularly malignancies [20,21]. Prior studies documented that an augmented level of intracellular ROS prompted DNA impairment, which means it moves the clarification and conduction of genetic data [19,21]. Thus, to repress neoplasm, effective agents that prompt intracellular ROS in malignancies are required to be established. Here, we

establish that ETC elevated ROS in intracellular levels of SKOV3 cells. Remarkably, these outcomes intensely revealed that ETC prompted DNA injury in the nucleus. Collective data proposed that ETC could be an ideal candidate that makes ROS levels and DNA impairment in OC. Liu et al., 2023 [33] stated that phytocompound of corilagin on OS cells cytotoxicity, intracellular ROS, MMP, apoptosis, corilagin could suppress OS cell proliferation via enhanced intracellular ROS, and MMP loss, and triggered apoptosis in a dose-dependent has an inferior manner. Corilagin alleviates U2OS and MG-63 cell proliferation by the ROS-mediated apoptosis.

Numerous scientific investigations emphasized the crucial deed of JAK2/STAT3 genetic networks in the progression and disparity of innumerable types of neoplasms comprising OC. The aforementioned experiments also indicated that JAK2/STAT3 is alleviated by tumor healing to restrain OC cell development [24,25]. ERK has a vital action in cell disparity, cell enlargement, and apoptosis [26]. Based on earlier evidence, the findings of the existing works also verified that the antiproliferative impact of ETC on OC cells is caused by the repression of JAK2/STAT3 signaling by attenuating pSTAT3, STAT3, pJAK2, and JAK2 gene levels. Likewise, ETC demonstrated prominent results by constraining JAK2, STAT3, and ERK in an earlier colon cancer study [28]. Taken together, ETC exhibited anti-proliferative and triggered ROS-mediated mitochondrial apoptosis through the suppression of JAK2/STAT3/ERK in SKVO3 human OC. Fig.11. Antonyuk et al., 2021[34] reported the kinetics of a release of ETC in serum blood of rats (free and in the chitosan-ethacridine adduct) differs significantly. It was established that in the case of intraperitoneal administration to rats of ETC lactate, its amount in the blood after 30 min is maximal (≈80% of the injected), remains approximately at this level for 3 hr, and then gradually decreases, and 20 hr after the input is only 1.25% of the input dose. In the case of intraperitoneal administration of the chitosan-ETC adduct, the amount of ETC slowly increases to 8 hours after administration and amount to maximum of ≈16% of the amount of ETC administered, and then slowly decreases, but 72 hours after injection was still recorded at 3% quantity from the entered dose.

CONCLUSION

In conclusion, our results demonstrated the antitumor activity of ETC by inhibiting cell proliferation, inducing ROS formation, loss of MMP, prevention of cell viability, DNA impairment, showed early and late apoptotic cells increased in apoptotic analysis by flow cytometry and stimulating caspase-mediatedapoptosis on human OC cells SKOV3. Together, this report ascertained that ECT acts as a protective candidate by repressing numerous apoptotic markers, cytokines, and cell signaling molecules through the inhibition of JAK2/STAT3/ERK networks in SKVO3 cells, which recommended that ETC might be animpendingbeneficial agent for OC.

Conflict of Interest

There is no conflict of interest.

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Table 1. Early/late apoptotic effects of ETN (IC_{50}: 10 μM) on SKOV3 cells for 48 h. Q1: Necrosis, Q2: late apoptosis, Q3: viability, Q4: early apoptosis.

Groups	Q1	Q2	Q3	Q4
Control (%)	3.78	1.45	94.21	0.56
ETN (%)	0.46	23.42	44.17	67.0

List of figures

Figure 1. ETC inhibits SKOV3 cell proliferation. Human OC cells SKOV3 were administered with diverse amounts (2.5-20μM) of ETC for one day. Cell cytotoxicity was determined by the MTT test. Data were displayed as mean ± SD for triplicate trials and the statistical significance is measured as *p<0.05 against control

Figure 2. ETC augments the accumulation of ROS in SKOV3 cells. Scale bar = 50 μm. Human OC cells SKOV3 were administered with ETC (Control and 10 μM) for one day. The identification of the accumulation of DCFH-DA fluorescence was observed below an inverted fluorescence microscope. Data were displayed as mean ± SD for triplicate trials and the statistical significance is measured as *p<0.05 against control.

Figure 3. ETC enhances the loss of MMP on SKOV3 cells. Scale bar = 50 μm. Human OC cells SKOV3 were administered with ETC (Control and 10 μM) for 24h. An apoptotic action of ETC has been studied by the MMP loss through fluorescent microscopy employing a blue filter (485-530 nm). Data were displayed as mean ± SD for triplicate trials and the statistical significance is measured as *p<0.05 against control.

Figure 4. ETC instigated apoptosis of SKOV3 cells. Scale bar = 50 μm. The human OC cells SKOV3 were treated with ETC (Control and 10μM) for one day. The apoptosis was assessed by using the staining with DAPI and observed below a fluorescence microscope. Data were displayed as mean ± SD for triplicate trials and the statistical significance is measured as *p<0.05 against control.

Figure 5. The stimulus of ETC on cell apoptosis activity of SKOV3 cells. Scale bar = 50 μm. The human OC cells SKOV3 were administered with ETC (Control and 10μM) for one day. The apoptosis was evaluated by using the staining with PI and viewed below a fluorescence microscope. Data were displayed as mean ± SD for triplicate trials and the statistical significance is measured as *p<0.05 against control.

Figure 6. The quantification of early/late apoptotic cells treated with the indicated concentration of ETC (IC_{50}: 10 μM) for 48 h by flow cytometry. The SKOV3 cells were stained with Annexin V/FITC and propidium iodide. Q1: Necrosis, Q2: late apoptosis, Q3: viability, Q4: early apoptosis. At least 10,000 cells were analyzed per sample, and quadrant analysis was performed.

Figure 7. Influence of ETC on SKOV3 cell apoptotic markers mRNA levels. The human OC cells were SKOV3 treated with 10μM of ETC for 24 hours. The mRNA level of cyclin-D1, Bcl-2**,** Bax, Caspase-3, myc, and survivin was analyzed by RT-PCR. Data were displayed as mean ± SD for triplicate trials and the statistical significance is measured as *p<0.05 against control.

Figure 8. ETC elevated the Cleaved-caspase-3 and Cleaved-PARP pathway. Human OC cells SKOV3 were administered with 10μM of ETC for one day. The protein expression of Cleaved-caspase-3 and Cleaved-PARP were measured by western blot. Data were displayed as mean ± SD for triplicate trials and the statistical significance is measured as *p<0.05 against control.

Figure 9. ETC relieves NF-κB-facilitated inflammation. Human OC cells SKOV3 were administered with 10μM of ETC for one day. The mRNA expression of pro-inflammatory mediators such as COX-2, IL-6, TNF-α, and NF-κB were measured by RT-PCR. Data were displayed as mean ± SD for triplicate trials and the statistical significance is measured as *p<0.05 against control.

Figure 10. ETC suppressed the JAK2/STAT3/ERK signaling pathway. Human OC cells SKOV3 were administered with 10μM of ETC for one day. The protein expression of JAK2, p-JAK2, STAT3, p-STAT3, ERK, and p-ERK were measured by western blot. Data were displayed as mean ± SD for triplicate trials and the statistical significance is measured as *p<0.05 against control.

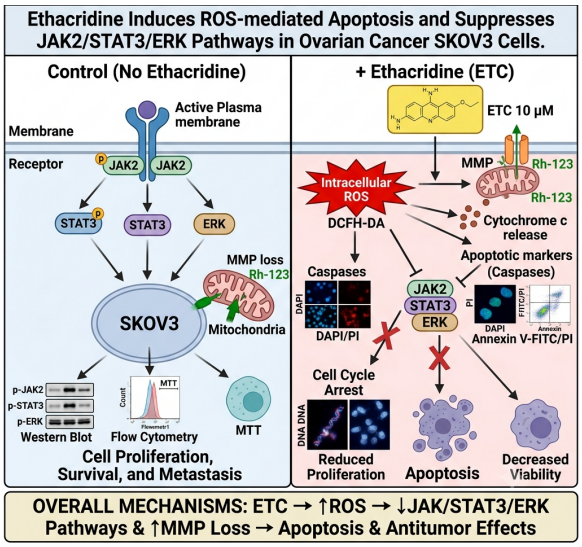

Figure 11. Ethacridine effectively reduces the viability of human ovarian cancer SKOV3 cells in a dose-dependent manner. This compound induces apoptosis by enhancing intracellular reactive oxygen species (ROS) and promoting mitochondrial membrane potential (MMP) loss, while simultaneously suppressing the JAK2/STAT3/ERK signaling pathways. These results identify ethacridine as a potential therapeutic agent for managing aggressive ovarian cancer.