PBR
Pharmaceutical & Biomedical Research

2026. Volume 12 Issue 3

Original Article

In Vitro and In Vivo Effects of *Quillaja saponaria* Bark Saponin Against Ovarian Cancer Cells

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Article info:
Received: 19 Mar 2026
Accepted: 27 Jul 2026

Keywords:
Ovarian cancer, Migration, *Quillaja saponaria* bark saponin (QBS), *ITGA5, MMP2, Zeb1, CDH1*

ABSTRACT

Background: Ovarian cancer is one of the most lethal gynecologic cancers in the world. It has also characterized by late diagnosis, high recurrence rate, and poor prognosis. The advanced stage at diagnosis and the development of chemoresistance are major challenges in its treatment, leading to limited therapeutic options and decreased survival rates. Natural products have emerged as a promising source of novel anticancer agents due to their diverse bioactive compounds and potential to target multiple signaling pathways involved in cancer initiation and progression.

Objectives: Ovarian cancer metastasis is the leading cause of cancer-related deaths in women worldwide. The effects of *Quillaja saponaria* bark saponin (QBS) on the viability and epithelial-mesenchymal transition (EMT) potential of human ovarian epithelial SKOV-3 and A2780 cells, and the cytotoxicity effect of QBS in the rat ovarian cancer model have been investigated.

Methods: The cytotoxicity of QBS against SKOV3 and A2780 cells was evaluated using the 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay to calculate IC_{50} values. The scratch assay assessed cell migration. To explore molecular changes in metastasis, the expression of key EMT-related genes, including *ITGA5, MMP2, Zeb1,* and *CDH1* was measured by real-time polymerase chain reaction. For the in vivo assay, the 7,12-dimethylbenz[a]anthracene (DMBA)-induced ovarian cancer rat model was used.

Results: The MTT assay demonstrated that QBS did not exert significant toxicity on NIH3T3 as normal cells; nevertheless, it induced cytotoxicity against A2780 and SKOV3 cells in a dose and time-dependent manner; IC_{50} was determined as 82 and 77 µg/mL for SKOV3 and A2780 cells, respectively. The scratch assay revealed that QBS significantly attenuated cell invasion at sub-IC_{50} concentrations (20 and 30 µg/mL). Gene expression indicated down-regulation of *Zeb1, ITGA5*, and *MMP2* at the IC_{50} value of both SKOV3 and A2780 cells ($P \leq 0.001$). Also, up-regulation of *CDH1* in A2780 cells at the IC_{50} value was significant ($P \leq 0.001$). The in vivo assay showed that QBS significantly reduced tumor growth ($P \leq 0.01$) and protected rats against 7,12- DMBA-induced ovarian cancer, with optimal effect of 16 mg/kg.

Conclusion: Overall, QBS can offer a potent cytotoxic agent against ovarian cancer cells in vitro and in vivo. However, this hypothesis requires further investigation to discover the molecular signaling pathway in human ovarian cancer cells.

Citation Koochaki R, Nabiuni M, Amini E. In Vitro and In Vivo Effects of *Quillaja saponaria* Bark Saponin Against Ovarian Cancer Cells. Pharmaceutical and Biomedical Research. 2026; 12(3):195-206. http://dx.doi.org/10.22034/PBR.12.3.1469.2

http://dx.doi.org/10.22034/PBR.12.3.1469.2

Introduction

Ovarian cancer is a fatal gynecological cancer ranking eighth among the leading causes of ca)ncer–related mortality in women worldwide. Epithelial ovarian cancer (EOC) is the most widespread subtype, including more than 95% of all ovarian cancer cases [1]. Based on surveys, ovarian cancer cells are very invasive; therefore, suppression of their invasion is considered a therapeutic approach for oncological research. In this regard, , epithelial cells must undergo epithelial–mesenchymal transition (EMT) to gain metastatic potential, a crucial step in cancer progression during which cancer cells acquire the invasive abilities necessary to complete all steps of the metastatic cascade [2, 3]. Related to EMT, metastasis of malignant epithelial cells requires lysis of the ECM by matrix metalloproteinase (MMP) enzymes. MMPs are zinc-dependent enzymes that play an essential role in degrading the basement membrane and ECM proteins. *MMP2* overexpression in malignant ovarian cancer is related to the metastasis of ovarian cancer [4]. *MMP2*, a key member of this family, digests type IV collagen and is overexpressed in EOC, which correlates with poor survival [5-7]. Integrins are adhesion molecules in the cell surface containing α and β subunits, which have different functions in cells. Notably, tumor cells can resist anoikis by expressing *ITGA5*. In addition, it was reported that high expression of *ITGA5* in tumor cells is associated with cell survival attenuation [8-10]. Studies showed that *ITGA5* overexpression initiates transformed cells towards a more malignant phenotype and metastasis in malignant ovarian cells [11]. On the other hand, *CDH1*, as an epithelial marker and Ca-dependent glycoprotein, is involved in cellular connections and controls metastatic progression. Studies showed that when *CDH1* is overexpressed, cancer cells are less likely to invade [12, 13]. In this regard, zinc finger E-box binding homeobox 1 (*ZEB1*) is a major transcription factor that plays a central role in EOC progression and stimulates EMT by downregulating epithelial genes, including *CDH1*, and increasing the metastatic ability of EOC [14, 15].

With the progression of ovarian cancer stage, the conventional therapeutic strategies, including surgery, chemotherapy, immunotherapy, and radiotherapy have indicated minimal success [16]. Meanwhile, natural compounds are considered promising sources of anticancer agents [17].

Saponins are triterpenoids found in many plant species, such as sunflower, chickpea, soybean, sugar beet, ginseng, and oats, and exhibit cytotoxic activity and play a protective role in plants by defending against predators. Many biological effects of saponins, such as anti-inflammatory and anti-microbial effects, cell membrane permeabilization, immune system stimulation, and cytotoxic attributes, have also been reported [18-20].

According to the literature, saponins have exhibited many pharmacological effects; however, their application is limited due to their in vitro hemolytic activity. Nevertheless, this hemolytic property is not the same in all saponins. *Quillaja saponaria* bark saponin (QBS), in particular, exhibits minimal hemolytic effects, so it was previously approved as a vaccine adjuvant [21]. Previous studies have demonstrated that saponin compounds can exert anti-cancer effects via modulation of EMT and invasion of ovarian and other cancer cells [19, 20]. Despite extensive evidence of the anti-proliferation and anti-invasion effects of saponins, the anti-migratory effects of QBS in ovarian cancer have not been fully elucidated. In the present study, SKOV3 and A2780 cell lines were used as ovarian cancer cell lines. A2780 cells are more sensitive than SKOV3 cells to treatment with fluorouracil, oxaliplatin, paclitaxel, etc. Furthermore, to provide more evidence supporting the potential anticancer properties of QBS, its effects were also tested in the Wistar rat model, which induced ovarian cancer with 7,12-Dimethylbenz[a]anthracene (DMBA) as carcinogen [19-22]. The DMBA is commonly used in animal models to induce breast cancer, skin cancer, and ovarian cancer. Studies have shown that DMBA-induced ovarian cancer resembles human ovarian cancer in terms of histology, molecular, and dissemination patterns. Thus, a DMBA-induced ovarian cancer model can be useful in human ovarian cancer studies [23, 24]. The SKOV3 cell line is a human ovarian adenocarcinoma derived from ascitic fluid, and the A2780 cell line is known as a human ovarian carcinoma isolated from tumor tissue. Besides, A2780 cells are more sensitive than SKOV3 cells to oxaliplatin, fluorouracil, gemcitabine, paclitaxel, etc. [25]. Furthermore, *ITGA5, MMP2, ZEB1*, and *CDH1* genes were selected because of their well-documented involvement in EMT and their recognized roles in tumor progression and metastatic dissemination. In this study, we aimed to evaluate the effects of QBS on EMT-related genes in A2780 and SKOV3 human ovarian cancer cell lines.

Materials and Methods

Chemicals and reagents

QBS powder (Cat No: S4521) was purchased from Sigma-Aldrich (USA). According to the manufacturer´s specifications, the product contained 25-35% sapogenins. A2780 and SKOV3 human ovarian cancer cell lines were obtained from the Gene-Iran laboratory (Tehran, Iran), and the NIH3T3 normal fibroblast cell line was obtained from National Cell Bank of Iran (NCBI). Roswell Park Memorial Institute (RPMI)-1640 and DMEM, fetal bovine serum (FBS), penicillin/streptomycin, and trypsin/EDTA were purchased from Bioidea (Tehran, Iran). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and DMBA solutions were prepared from Merck (Germany). RNA isolation (Cat No: A101231), complementary DNA (cDNA) synthesis (Cat No: A101161), and SYBR Green master mix (Cat No: C101051, C101052) were purchased from Parstous (Tehran, Iran). The abbreviations used in this study are presented in Table 1.

Preparation of *Q. saponaria* bark saponin stock and working solutions

A stock solution was prepared by dissolving QBS in sterile phosphate-buffered saline. The solution was sterilized by filtration through a 0.22-µm syringe filter and stored at 4 ˚C until use. Immediately before each experiment, the stock solution was diluted with complete culture medium to prepare working concentrations of 20, 30, and 80 µg/mL.

Cell culture

The study selected two ovarian cancer cell lines (A2780 and SKOV3) to assess the effects of QBS. Also, NIH3T3, a normal fibroblast cell line, was also cultured to assess the selectivity of QBS against ovarian cancer cells. A2780 and SKOV3 human ovarian cancer cells were cultured in RPMI-1640 medium with 1% antibiotics and 10% FBS, and NIH3T3 cells were cultured in DMEM under standard conditions (37 ˚C, 5% CO_2 incubator).

Cell viability assay

The MTT assay relies on the capacity of live cells to convert the yellow MTT tetrazolium salt into purple formazan crystals. MTT passes through the cell membrane and the mitochondrial inner membrane of viable cells, and it is reduced to purple formazan crystals that produce a quantifiable colorimetric signal [26]. A2780, SKOV3, and NIH3T3 cells were cultured (10^4 cells/well) in a 96-well plate to determine the effect of QBS on cell survival. After 24 h, NIH3T3, SKOV3, and A2780 cells were treated with QBS at 20, 30, and 80 µg/mL, or without QBS (control), for 24 hours, then 40 µL of MTT solution (5 mg/mL) was added to each well. Following 3 hours of incubation, 80 of DMSO was added to each well. Cell viability was determined. Four hours later, DMSO was added to each well to dissolve the formazan crystals, and then the absorbance of each well was recorded at 570 nm using an ELISA reader, a microplate reader that measures the absorbance of solubilized formazan, indicating cell viability. The optimal wavelength for measuring formazan is 570 nm. The MTT assay was performed in three independent biological experiments, with each experimental concentration analyzed in triplicate.

Table 1. List of abbreviations used in the manuscript

Abbreviation	Definition	Abbreviation	Definition
DMBA	7,12-Dimethylbenz[a]anthracene	ILK	Integrin-linked kinase
ECM	Extracellular matrix	MMP2	Matrix metalloproteinase-2
EMT	Epithelial–mesenchymal transition	PBS	Phosphate-buffered saline
EOC	Epithelial ovarian cancer	QBS	*Quillaja saponaria* bark saponin
FAK	Focal adhesion molecule	ZEB1	Zinc finger E-box-binding homeobox 1
FASN	Fatty acid synthase	*CDH1*	Gene encoding E-cadherin
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase	*ITGA5*	Gene encoding α5-integrin

Table 2. Sequences and Tm of PCR primers

Target Gene	Primer Sequences	TM
CDH1	F: 5′- ATTTTTCCCTCGACACCCGAT-3′ R:5′- TCCCAGGCGTAGACCAAGA-3′	59 ˚C
α5- Integrin	F: 5′- GCCGATTCACATCCTCTCAAC-3′ R: 5′- GTCTTCTCCAAGTCCAGCAAG-3′	59 ˚C
MMP2	F: 5′- GATACCCCTTTGACGGTAAGGA-3′ R: 5′- CCTTCTCCCAAGGTCCATAGC-3′	60 ˚C
ZEB1	F:5′- CAGCTTGATACCTGTGAATGGG-3′ R: 5′- TATCTGTGGTCGTGTGGGACT-3′	60 ˚C
GAPDH	F: 5′- GTCAACGGATTTGGTCGTATTG-3′ R: 5′- CTCCTGGAAGATGGTGATGGG-3′	60 ˚C

In vitro scratch wound assay

Cell migration ability was assessed using a scratch wound-healing assay. A2780 and SKOV3 cells (2×10^5) were cultured in a 6-well plate in RPMI-1640 medium with 1% FBS; when the cell density reached 70%, a wound was created with a sterile pipette tip. Detached cells were removed, and the wound was checked after 24 h of treatment with IC_{50} and sub-IC_{50} values of 20 and 30 µg/mL. Finally, cell invasion into the wound area was assessed by inverted microscopy, and images were captured. Then, the distance of cells from the scratch area was measured using ImageJ. The average scraped width per well was measured and subtracted from the pre-treatment measurement. The change in the width for each experimental condition was compared with the control.

RNA extraction and quantitative real-time polymerase chain reaction

Total RNA was extracted from treated and untreated A2780 and SKOV3 cells using a HighPure RNA isolation kit (ParsTous, Iran) according to the manufacturer's protocol. RNA concentration and purity were measured with a Nanodrop spectrophotometer, and RNA integrity was verified by assessing the A260/A280 and A260/A230 ratios. One µg of high-quality RNA was used for cDNA synthesis with the Easy cDNA Synthesis Kit (ParsTous, Iran). Quantitative real-time polymerase chain reaction (qRT-PCR) amplification was performed using RealQ Plus 2x Master Mix Green (Ampliqon, Denmark) on an Eppendorf Mastercycler EP Realplex instrument. Primer sequences used for amplification are listed in Table 2. Cycling conditions were as follows: initial denaturation at 95 ˚C for 10 min, followed by 40 cycles of denaturation at 95 ˚C for 15s, annealing at 56 ˚C for 30s, and extension at 72 ˚C for 30s. Specificity of amplification confirmed by melting curve analysis. Relative expression levels of target genes were normalized against GAPDH as an internal control and calculated using the $2^{-\Delta\Delta Ct}$ method. Primer efficiencies confirmed to fall within the acceptable range (90–110%), ensuring reliable quantification.

Induction of ovarian cancer and in vivo study design

Female Wistar rats (180-220 g, 6-8 weeks old) were obtained from the Animal Center Resources and Research of Royan Institute. Animals were housed in polypropylene cages under controlled conditions (20-22 ˚C temperature, 12 hours light/dark cycle, 40-50% humidity, and no noise pollution). The animals were acclimated for 10 days and fed a standard pellet diet and water. Ethical principles regarding the treatment of laboratory animals were observed. For tumor induction, rats were anesthetized with an intraperitoneal injection of 10% ketamine and 2% xylazine. Then, a single dose of DMBA (4 mg in 0.02 mL of sesame oil) was surgically delivered to the right ovary, and the ovaries were returned intact to the abdominal cavity. The muscle and skin layers were closed using a silk suture, and a prophylactic antibiotic was administered for a week post-surgery. Then, 33 rats were randomly assigned to three groups (n=11 in each group). The complete in vivo study was described below:

Group I (control group): No DMBA injection. Group II (DMBA+saline): Received an intraperitoneal injection of normal 0.9% saline (vehicle of saponin). Group III (DMBA+QBS): Treated with QBS administered intraperitoneally (at a concentration of 16 mg/kg; based on the dose-response assay).

Treatment was initiated 10 days after DMBA administration. QBS was administered at 16 mg/kg every 12 days for three consecutive cycles, starting 10 days after DMBA administration (days 10, 22, and 34). All rats were euthanized 7 days after the final injection, and the ovaries were collected for histological analysis [27].

Histomorphological assessment

Ovarian tissue was fixed in Bouin's solution for 24 h, dehydrated in a series of ethanol (70-100%), embedded in paraffin, and sectioned into 5 µm sesctions. After rehydration and hematoxylin and eosin staining, the changes in ovarian tissue structure were observed by an optical microscope.

Statistical analysis

All results were obtained from three separate experiments and performed in quadruplicate (n=12). The statistical significance was evaluated using SPSS software, version 22 with one-way analysis of variance, and the MTT assay was evaluated using Duncan's test for cell cytotoxicity, metabolic activity, and cell viability analysis. Data are presented as the Mean±SD. $P<0.05$ are considered significant. The experimental means were compared with those of untreated cells harvested in parallel (Figure 1).

Results

Effects of *Q. saponaria* bark saponin on viability of SKOV3 and A2780 cells in a dose-dependent manner

In SKOV3 cells, QBS at concentrations of 20, 30, and 80 µg/mL reduced cell survival percentage to 78.9±0.4, 61.4±0.2, and 52.6±0.1, respectively. In A2780 cells, cell viability under exposure to QBS at concentrations of 20, 30, and 80 µg/mL was 75.1±0.09, 64.4±0.06, and 45.9±0.1 (Figure 2). The QBS also did not significantly reduce the viability of NIH3T3 fibroblast cells as compared with untreated ovarian cancer cells. According to the MTT assay, the IC_{50} value were 82 µg/mL for SKOV3 cells and 77 µg/mL for A2780 cells. IC_{50} is the most commonly used and informative measure of a drug's efficacy. It shows how much drug is needed to reduce a biological process by half, helping measure the potency of an antagonist drug in pharmacological research. A nonlinear regression model in GraphPad Prism software, version 10.1.1 calculated IC_{50} values.

Q. saponaria bark saponin exerts the anti-migratory effect

The results showed that QBS reduced cell migration in a dose-dependent manner at concentrations below IC_{50} (20 and 30 µg/mL). As shown in Figure 3, untreated ovarian cancer cells migrated into the scratched area and substantially closed the wound after 24 h (97%, 98%; A2780 and SKOV3 cells, respectively). In contrast,

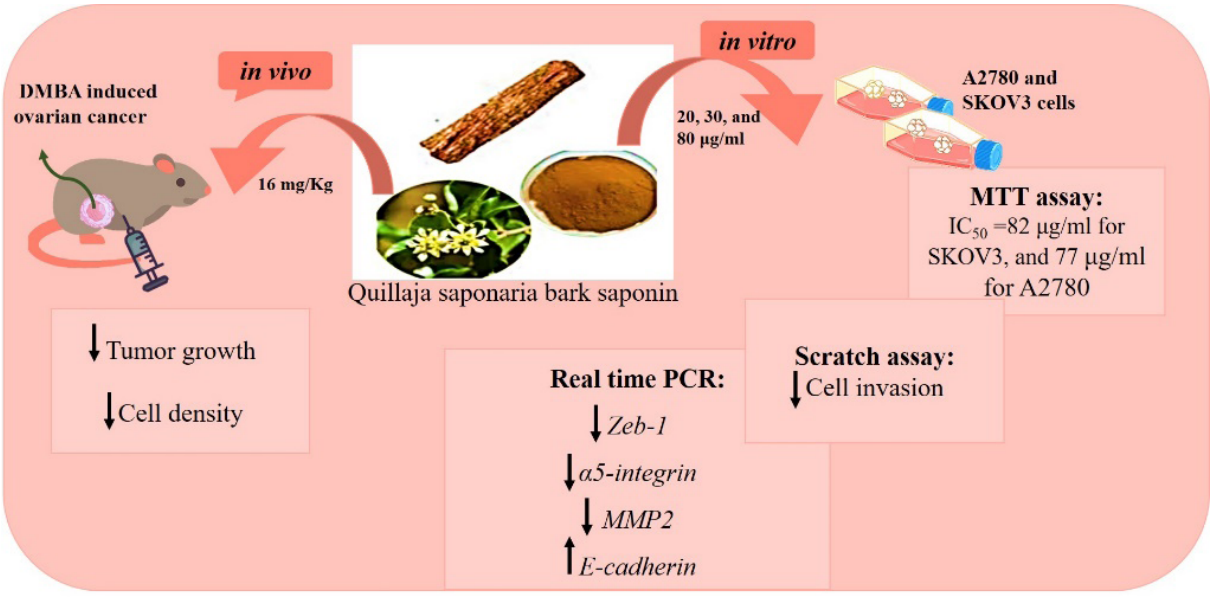


Figure 1. Graphical abstract

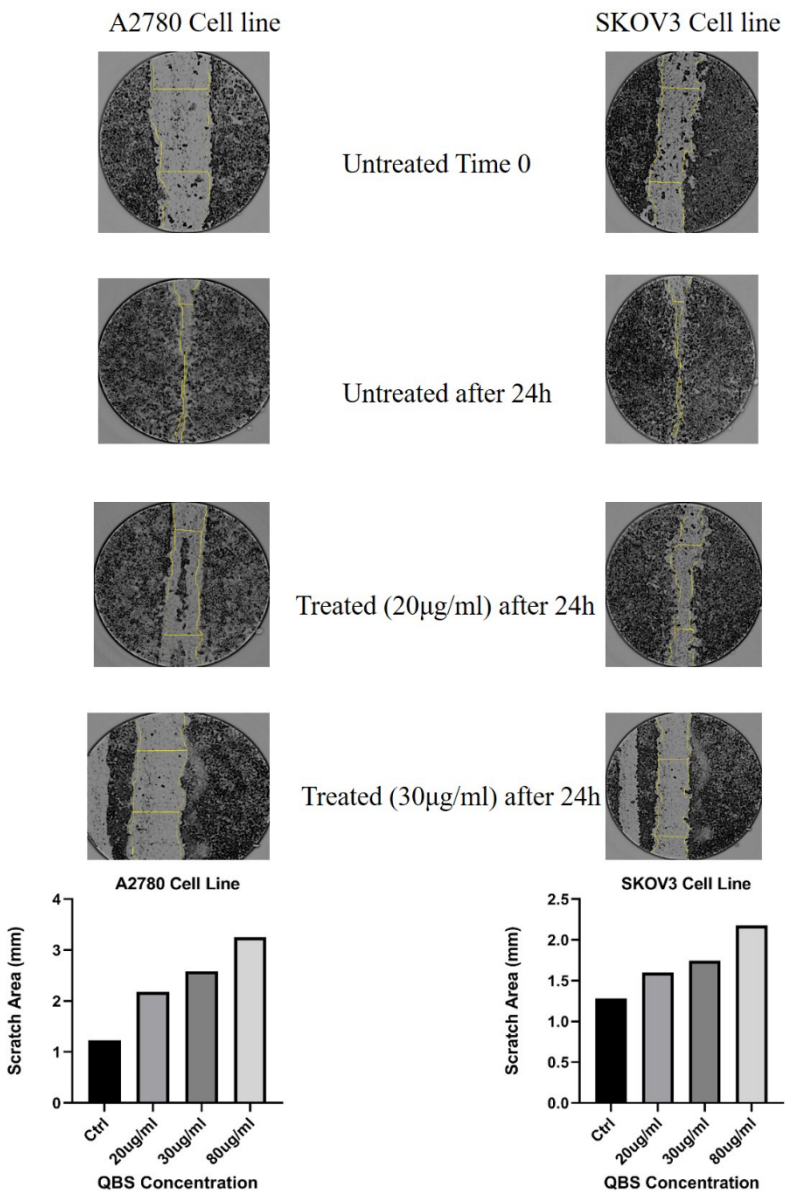


Figure 2. Cell viability assessment by MTT assay after 24h treatment with various concentrations of QBS (20, 30, and 80 µg/mL) on ovarian cancer cell lines (SKOV3, A2780), and NIH3T3 Cells

The data are expressed as Mean±SD (***P≤ 0.001).

QBS treatment reduced wound closure relative to the control, indicating decreased cell migration. This effect was more pronounced after exposure with 30 µg/mL of QBS (19%, 24%) than 20 µg/mL (33%, 42%) in both A2780 and SKOV3 cells, suggesting that QBS at 30 µg/mL may reduce the migratory potential of both ovarian cancer cell lines only after 24 h of treatment.

Gene expression analysis by quantitative real-time polymerase chain reaction

To identify the anti-EMT molecular mechanism of QBS, the messenger RNA (mRNA) expressions of factors associated with the regulation of cell adhesion were evaluated by RT-PCR (Figure 4). In this regard, A2780 and SKOV3 ovarian cancer cells were treated with IC_{50} and sub-IC_{50} values of QBS. Statistical analysis showed that in the SKOV3 cell line, *ZEB1* expression was significantly decreased under exposure to IC_{50} and sub-IC_{50}

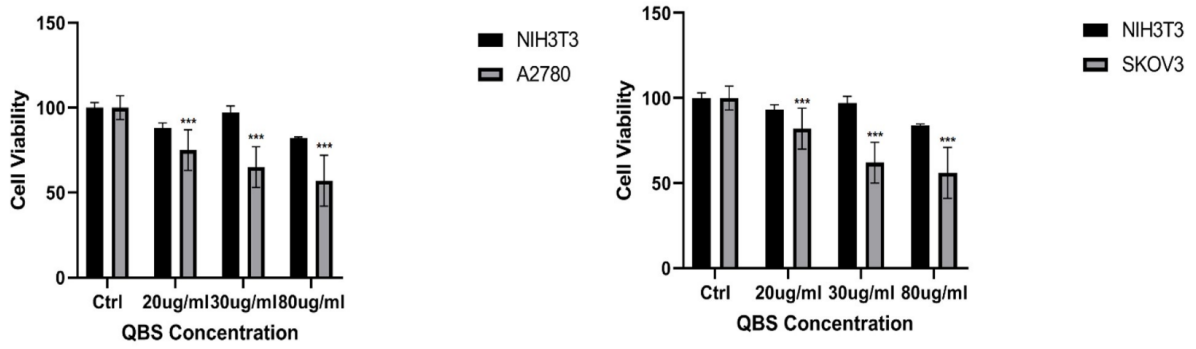


Figure 3. Scratch width changes in invasion assay for SKOV3 and A2780 cells during QBS treatment (inverted microscope ×100)

Note: (A2780 cells and SKOV3 cells, before treatment, the control after 24h, and treatment with IC_{50} sub-IC_{50} value. Images were transferred and analyzed with the ImageJ 1.3 software. Graphs quantitatively indicated the relative diameter of the scratch area with increased concentration of QBS.

values of QBS (***P≤0.001). The expression of *MMP2* was attenuated at IC_{50} and sub-IC_{50} values of QBS (***P≤0.001 and **P≤0.01). Data analysis showed that the *ITGA5* expression was significantly down-regulated at the IC_{50} value of QBS (*P≤0.05). *CDH1* expression analysis indicated non-significant down-regulation at a sub-IC_{50} value of QBS, and up-regulation at the IC_{50} value of QBS, but was not considered statistically significant. In the A2780 ovarian cancer cell line, statistical analysis revealed that *ZEB1* and *MMP2* expression

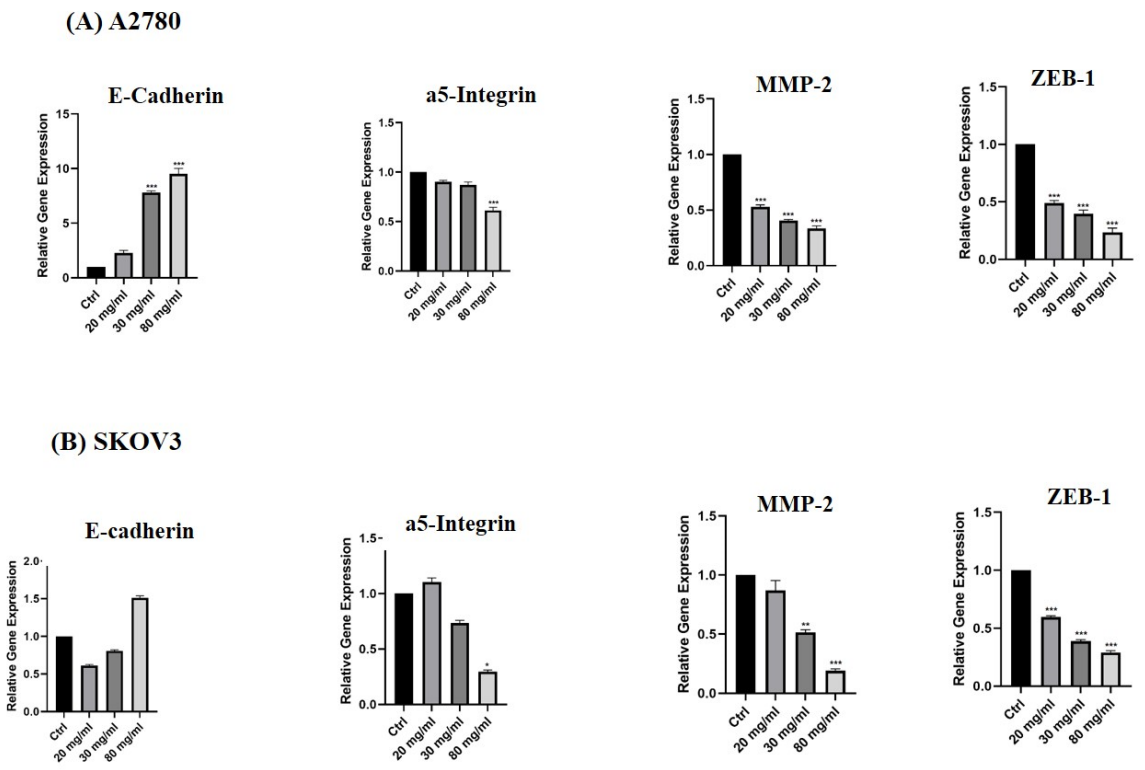
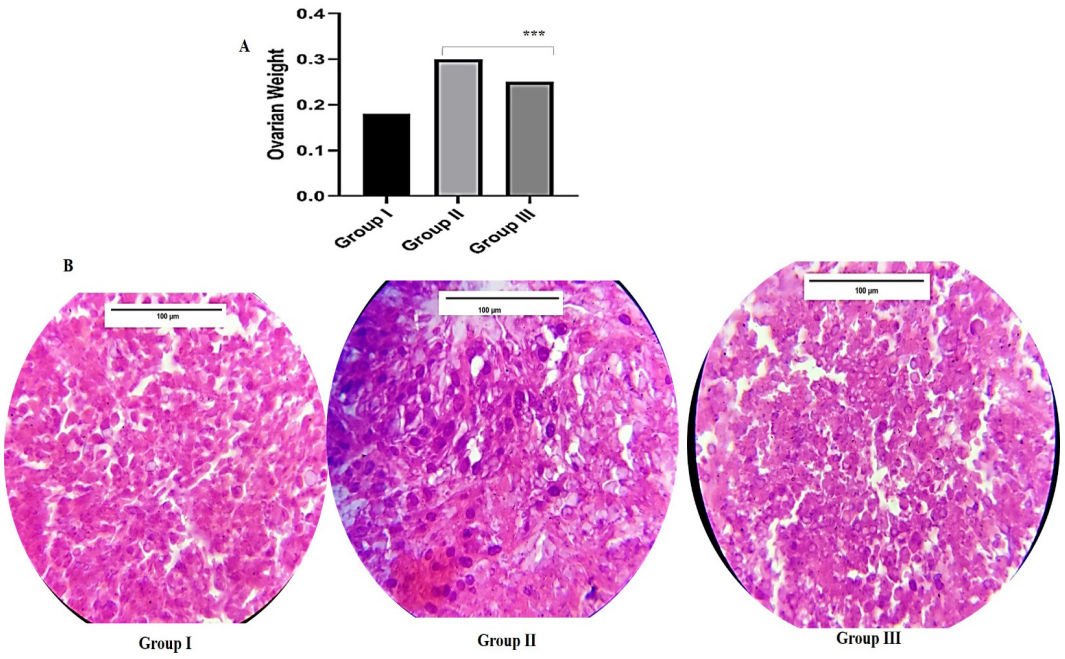


Figure 4 Gene expression analysis under exposure to QBS by real-time PCR in (A) A2780 and (B) SKOV3 cells

Note: *GAPDH* was considered an internal control. Relative expression of *CDH1, ZEB1, MMP2*, and *ITGA5* was calculated using the $2^{-\Delta\Delta ct}$ method, and data are represented as Mean±SD (*P≤0.05, ** P≤0.01, *** P≤0.001).



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Figure 5. A) The chart represents the effects of QBS treatments on the weight of the ovaries compared with the control group. Tumor weight in group III was significantly decreased compared with tumor weight in the untreated Group II. Tumor data are represented in B) Microphotographs of H&E staining. Group I (control): Rats did not receive DMBA. Group II: Rats only received DMBA. Group III: Rats received DMBA and then 16 mg/kg of QBS every 12 days.

were significantly attenuated at the IC_{50} value and sub-IC_{50} values of QBS ($^{***}P \leq 0.001$). *ITGA5* expression was slightly decreased at the sub-IC_{50} value of QBS, which was not statistically significant. In addition, the expression of *ITGA5* showed significant down-regulation at the IC_{50} value of QBS ($^{***}P \leq 0.001$). *CDH1* expression was non-significantly overexpressed at sub-IC_{50} values of QBS, and at the IC_{50} value of QBS, this overexpression was noticeable ($^{***}P \leq 0.001$).

Histomorphological changes

The mean ovarian weight was 0.19±0.01 in group I, 0.3±0.1 in group II, and 0.21±0.08 in group III. Ovary' weight analysis indicated a significant increase in mean ovarian weight in the DMBA-exposed group (group II) compared to the control group (group I) ($^{***}P \leq 0.001$), indicating successful induction of ovarian tumor formation. The results also showed that the tumor weight was significantly decreased in the QBS-administered experimental group (Group III) compared with group II ($^{***}P \leq 0.001$), suggesting a suppressive effect of QBS on tumor growth, while there was no significant difference between the mean tumor weight in the QBS-administered group (group III) and ovarian weight in the control group (group I) ($P > 0.05$) (Figure 5A). As shown in Figure 5B, histopathological evaluation of ovarian tissues revealed that the control group (group I) preserved cell structure with normal stromal organization without neoplastic alterations. In the DMBA-exposed group (group II), ovarian tissues exhibited significant histopathological abnormalities characterized by dense cellular proliferation, nuclear hyperchromasia, and cellular pleomorphism, indicating tumor progression. In contrast, ovarian tissues from the QBS-administered group (group III) showed fewer histopathological changes compared to group II. QBS treatment reduced cellular density and partially improved tissue structure.

Discussion

Ovarian cancer is the most lethal gynecologic cancer, with malignant metastasis and poor prognosis. Effective components that can block metastasis and EMT in ovarian cancer may be used as a new alternative drug for the suppression of tumor invasion and metastasis [28]. Blocking or reversing EMT in tumor cells can reduce resistance to chemotherapy, radiotherapy, and immunotherapy [29].

In this study, we found that QBS has cytotoxic effects against SKOV3 and A2780 ovarian cancer cells with minimum cytotoxicity in normal fibroblast cells. Based on these findings, administration of 16 mg/kg QBS to the rat´s ovarian tumor reduced tumor cell burden and ovarian weight compared with untreated DMBA-induced ovaries, suggesting a potential inhibitory effect on DMBA-induced ovarian tumor development. In addition, the scratch wound assay indicated the inhibitory effect of QBS on ovarian cancer cell migration in the IC_{50} value of QBS. To confirm QBS's inhibitory effect on cell migration, the expression of ovarian cancer EMT-related genes has been evaluated. RT-PCR analysis exhibited that QBS influenced EMT and migration in both SKOV3 and A2780 cell lines by up-regulation of *CDH1* and down-regulation of *ZEB1, MMP2*, and *ITGA5*.

CDH1 is one of the most frequently expressed epithelial adhesion molecules in transformed ovarian epithelia, and decreased *CDH1* expression in ovarian cancer tissue may promote disease progression and an invasive phenotype [30]. Related to *CDH1*, down-regulation of *CDH1* causes free β-catenin to migrate the cell nucleus and promotes transcription of metastasis markers such as *ZEB1* and *MMPs* [31]. *ZEB1* is a transcriptional repressor that can inhibit the transcription of *CDH1* by binding to the *CDH1* promoter, and the enzymatic activity of *MMP2* increases with increasing *ZEB1* [32]. Further, *ZEB1* overexpression inhibited cell apoptosis in EOC [33]. Therefore, suppressing *ZEB1* can decrease EMT by reducing *MMP2*-mediated digestion and restoring *CDH1* expression, making it a valuable strategy against tumor development.

α5-integrin is a focal adhesion molecule that can remodel or stabilize adhesion junctions like *CDH1*, and it can play a role in *CDH1* endocytosis [34]. On the other hand, recent studies have shown that adhesive molecules, such as cadherins and integrins interact in many ways, including crosstalk with each other [35, 36]. It was shown that ovarian cancer cells isolated from the primary tumor started developing mesenchymal characteristics when floated in peritoneal fluid, and they could resist the anoikis by expressing factors, such as *ITGA5* [10]. In addition, *ITGA5* expression can induce tumor growth and migration; therefore, the connection between α5-integrin and fibronectin interaction triggers a signaling cascade to start up that induces focal adhesion molecule phosphorylation and activation via integrin-linked kinase and the integrin intracellular domain. Activation of the *PI3K/AKT* pathway and *AKT* phosphorylation inhibit apoptosis, which triggers EMT during cancer progression. On the other hand, activated *AKT* up-regulates *ZEB1* and promotes metastasis [10, 37].

In the present study, statistical analysis showed that *ZEB1* and *MMP2* expression were significantly decreased at all concentrations ($^{***}P\leq0.001$). Expression of *ITGA5* at the IC_{50} value significantly decreased ($^{***}P\leq0.001$). In addition, *CDH1* at sub-IC_{50} and IC_{50} values of QBS was significantly overexpressed in A2780($^{***}P\leq0.001$).

In recent years, many studies have examined the role of EMT and the molecules involved in this process, and the anti-metastatic function of naturally occurring triterpenoids, such as saponins. Zhang et al. indicated that *CDH1* downregulation may play an important role in gastric cancer progression and metastasis [38]. Mou et al. showed the inhibitory effect of Paris saponin VII on A2780 cell EMT by up-regulation of *CDH1* [39]. On the other hand, Chen et al. evaluated the effect of Paris saponin H on hepatocarcinoma cells in vitro and showed suppression of tumor growth and EMT under Paris saponin H treatment in vitro and in vivo [40]. Zhu et al. showed that pulsatilla saponin E suppressed A549 NSCLC cell invasion with inhibition of lipid rafts and negatively regulated the Akt/FASN pathway [41]. Kim et al. demonstrated that Panax notoginseng saponin affect induced mammary carcinogenesis in rats by decreasing tumor volume and reducing lipid peroxidation levels [42].

All of the above studies, consistent with our study, have confirmed the cytotoxic effects of saponins. Further, the special role of *ZEB1, ITGA5, MMP2*, and *CDH1* on the EOC metastasis pathway, suppression level of *MMP2, ZEB1*, and *ITGA5*, and overexpression of *CDH1* level by QBS has been considered this herbal product an essential component against some of the EMT pathways in SKOV3 and A2780 EOC cells.

Accordingly, the results of this study indicated that QBS could be an EMT suppressor agent for ovarian cancer in vitro. Nevertheless, a Western blot to detect proteins would be necessary to confirm the proposed molecular mechanisms, using another human epithelial ovarian cell line, such as human ovarian surface epithelium, and further studies on the metastasis pathways regulated by QBS seem obligatory in the rat model.

Conclusion

The current study provides data on the cytotoxicity of QBS against sensitive and resistant ovarian cancer cells and in the ovarian tumor rat model. Further, we provide

valuable data associated with the involvement of EMT signaling pathway genes, such as *MMP2, ZEB1, CDH1*, and *ITGA5*, in QBS's potential against ovarian cancer in vitro. Preclinical and clinical research are required to confirm the anti-cancer mechanism of QBS as an EMTmodulating therapeutic agent for clinical applications.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of Kharazmi University, Tehran, Iran (Code: IR.KHU.REC.1401.015).

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions

Conceptualization, methodology, investigation, data curation, formal analysis, writing the original draft: Raoufeh Koochaki; Supervision, study design and data interpretation: Mohammad Nabiuni; Scientific consultation, data interpretation, review & editing, critical revision: Elaheh Amini; Final approval: All authors.

Conflict of interest

The authors declared no conflicts of interest.

Acknowledgments

The authors thank the Faculty of Biological Sciences, Kharazmi University, Tehran, Iran.

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