AMERICAN UNIVERSITY OF BEIRUT

ASSESSING THE EFFECTS OF THYMOQUINONE (TQ) ALONE AND IN COMBINATION WITH CISPLATIN (CDDP) ON OVARIAN CANCER CELLS

by
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ABSTRACT

OF THE THESIS OF

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Title: Assessing the Effects of Thymoquinone (TQ) alone and in Combination with Cisplatin (CDDP) on Ovarian Cancer Cells

Background: Ovarian cancer, the most lethal gynecologic malignancy, ranks among the top five causes of cancer-related deaths in women. The high mortality rate is attributed to late-stage diagnosis, the absence of effective public screening methods, and resistance to platinum-based treatments. Consequently, combination drug therapy emerges as a promising avenue in ovarian cancer management, aiming to augment therapeutic efficacy and circumvent resistance to conventional therapies. Thymoquinone (TQ), a phytochemical compound derived from the Nigella sativa herb, has demonstrated anti-tumorigenic and anti-proliferative properties across various cancer types on ovarian cancer specifically when combined with platinum agents such as cisplatin (CDDP). Therefore, this study seeks to explore the impact of TQ on ovarian cancer cells, both alone and in combination with CDDP.

Methods: Two ovarian cancer cell lines OVCAR-420 and SKOV-3 were used in this study. A range of two-dimensional (2D) in vitro assays, including MTT, trypan blue assay, and wound healing, were used to assess cell proliferation, viability, and migration respectively. Additionally, a three-dimensional (3D) MatrigelTM-based cell culture assay was employed to evaluate stemness. Both TQ and CDDP were administered as monotherapies and in combination to ascertain their effectiveness.

Results: Our results revealed that TQ, both alone and in combination with cisplatin, significantly and synergistically reduced the proliferation, viability, migration, and sphere growth of ovarian cancer cells. TQ specifically at 25μM for OVCAR-420 and 10μM for SKOV-3, significantly lowered the concentration of CDDP when used in combination enhancing the susceptibility of cancer cells to treatment.

Conclusion: Our findings show a strong synergistic effect between TQ and CDDP on ovarian cancer cells. Further validation of these findings could pave the way for a more potent therapeutic strategy for women diagnosed with ovarian cancer.

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ABBREVIATIONS

2D: Two-Dimensional

3D: Three-Dimensional

IARC: International Agency for Research on Cancer

NCI: National Cancer Institute

FIGO: Federation of Gynecology and Obstetrics

OC: Ovarian Cancer

BMI: Body Mass Index

CA-125: Cancer Antigen-125

BRCA: BReast CAncer gene

EOC: Epithelial Ovarian Cancer

SC: Serous Carcinoma

HGSC: High-Grade Serous Carcinoma

LGSC: Low-Grade Serous Carcinoma

TVU: Transvaginal Ultrasound

RRSO: Risk Reducing Salpingo-Oophorectomy

MRI: Magnetic Resonance Imaging

CT scan: Computed Tomography Scan

PARP: Poly (ADP-ribose) polymerase

TKI: Tyrosine Kinase Inhibitors

VEGP: Vascular Endothelial Growth Factor

CSCs: Cancer Stem Cells

IL-1β: Interleukin-1 beta

EpCAM: Epithelial Cellular Adhesion Molecule

ROR1: Receptor Tyrosine Kinase-like Orphan Receptor

CDDP: Cisplatin

TQ: Thymoquinone

FAK: Focal Adhesion Kinase

ERK: Extracellular Signal–Regulated Kinase

MMP: Metalloproteinases

Akt : Protein Kinase B

DMSO: Dimethylsulfoxide

IC50: Half-Maximal Inhibitory Concentration

MTT: [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium

OD: Optical Density

PBS: Phosphate-Buffered Saline

ROS: Reactive Oxygen Species

SEM: Standard Error Mean

SFU: Sphere Formation Unit

TME: Tumor Microenvironment

TNF: Tumor Necrosis Factor

CI: Combination Index

CHAPTER I

INTRODUCTION

A. Ovarian Cancer Overview

1. Epidemiology

Cancer, a disease marked by uncontrolled cell growth, is one of the most feared health conditions of the 21st century. The organ or tissue of origin primarily classifies it, but increasingly, the molecular characteristics of cancer cells are also considered[1]. The incidence of cancer is strongly correlated with age, with the highest rates observed in populations aged 75 or above. According to the International Agency for Research on Cancer (IARC) GLOBOCAN 2022 report, there were approximately 20 million new cancer cases and 9.7 million cancer-related deaths worldwide in 2022. Changes in lifestyle and habits have led to a significant increase in the lifetime risk of developing cancer, affecting one in four individuals.[2].

Gynecological tumors, particularly ovarian cancer (OC), have become a significant health concern for women globally. OC is the 5th most common cause of gynecological cancer-related deaths, more widely present among whites than blacks in women aged between 55 to 64 years [3]. According to the National Cancer Institute (NCI), more than 200,000 women are estimated to develop OC every year and about 100,000 die from it [4].

Interestingly, the prevalence of OC varies with age, geography, and ethnicity. For instance, the disease is most common in Northern and Eastern Europe, while Asian and African populations have the lowest incidence rates. Even within the same country, disparities exist[5]. For example, recent studies have highlighted a higher risk of OC

among white women compared to African-American women in the United States (**Figure 1**)[6]. The precise cause of OC is unknown so far. However, several risks and contributing factors play a huge role in identifying the disease.

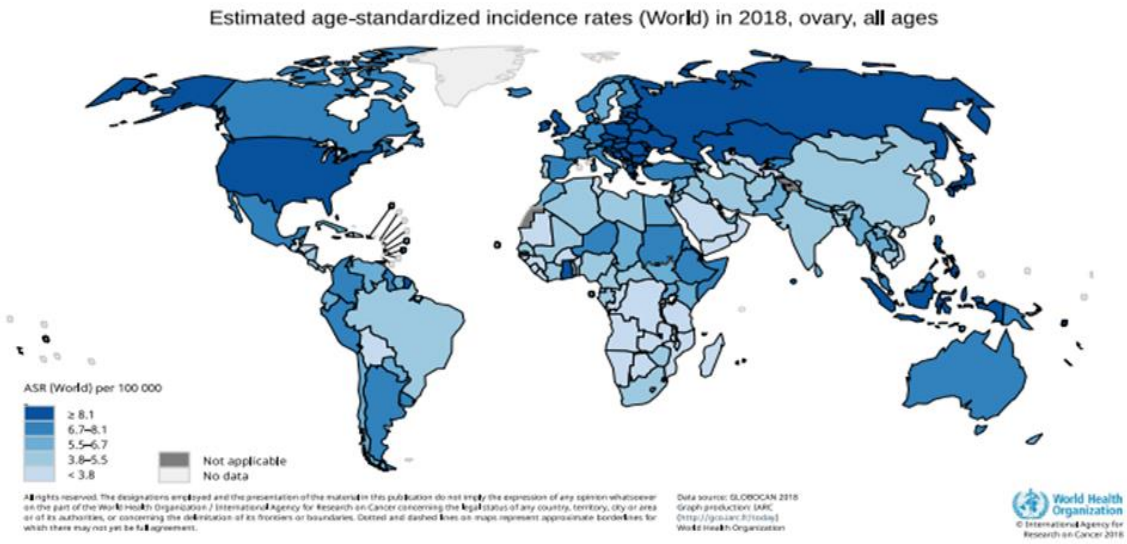

Figure 1. Global incidence of Ovarian Cancer in 2018.
Estimated age-standardized incidence rates (world) in 2018, ovarian cancer of all ages[6].

2. Risk Factors

Several factors may influence the risk of developing OC, with genetics being one of the most significant and beyond an individual's control. Women who have a family history of OC are at a greater risk of developing the disease compared to those without such a history [7]. Hereditary OC is strongly associated with mutations in the BRCA1 and BRCA2 genes. Women with these mutations have a higher risk of developing ovarian cancer, with BRCA1 mutations conferring a greater increase in risk compared to BRCA2 mutations. These genetic alterations are inherited and can be passed down through families, contributing to the development of OC [8]. Besides genetic mutations,

many risk factors are associated with OC including age, weight/BMI, hormonal therapy, pregnancy/breastfeeding, and diet.

Studies have shown conflicting results, regarding the association between age and OC risk. Some studies indicate a direct correlation, while others do not find a significant association [9, 10]. Early menarche and late menopause may increase the risk due to increased ovulatory cycles, but further research is needed [11].

Higher weight and BMI are associated with an increased risk of ovarian cancer, potentially due to the conversion of androstenedione to estrone and elevated levels of estradiol [12].

Long-term use of hormonal therapy, especially estrogen therapy used among menopausal women, is associated with an increased risk by 20% every 5 years of use and continues to increase with longer duration [13]. Notably, after the cessation of therapy, a more than 40% increase in OC risk is observed. This finding aligns with the literature, showing that the use of contraceptives which reduce ovulation, is inversely related to OC risk [5, 14].

Breastfeeding may decrease the risk by 20-25% according to some analyses [15]. In contrast, a recent statistical study showed no significant decrease in the risk as opposed to breastfeeding duration[16].

The relationship between diet and OC risk is still unclear. However, a higher vegetable intake appears to be associated with a lower risk, while a lower vegetable intake may elevate the risk[17]. Fat intake has also been correlated with a higher risk, and lactose-intolerant women may have a reduced risk [18, 19]. It is important to note that these risk factors are not definitive causes of OC but, understanding them can aid in risk assessment and preventive strategies.

3. Classification of Ovarian Cancer

OC encompasses a diverse range of subtypes, each with its unique histological and molecular characteristics. Understanding these different types of OC is crucial for accurate diagnosis, prognosis, and treatment selection. The most common type of OC is epithelial ovarian cancer (EOC), which accounts for approximately 90% of all cases. EOC is further classified into several subtypes based on histological features, including serous, endometrioid, clear cell, mucinous, and mixed subtypes. Each subtype exhibits distinct histopathological characteristics and is associated with specific molecular alterations (**Figure 2**) [20].

- Serous OC accounts for 75-80%, is the most prevalent subtype of EOC and is characterized by the presence of papillary structures and psammoma bodies. It is further categorized into High-grade Serous Carcinoma(HGSC), representing 85-90%, and Low-grade Serous Carcinoma(LGSC) representing 10-15% of all SC [21]. HGSC usually occurs in elderly patients. They are detected at an advanced stage and are responsible for the majority of cancer deaths and are associated with TP53 mutations and alterations in DNA repair pathways. LGSC is less common and is typically driven by mutations in KRAS or BRAF genes [20].
- Endometrioid OC accounts for 10% of all ovarian carcinomas. It is a unilateral solid mass characterized by the presence of glandular structures resembling endometrial tissue. It is often associated with endometriosis and is frequently estrogen-dependent. There are 3 grades according to the FIGO (Federation of Gynecology and Obstetrics) system, based on the presence of solid areas and nuclear atypia. This subtype is the most frequently seen in patients with Lynch syndrome[21].

- Clear-cell OC, on the other hand, is rare, accounting for 3.7-10% of all characterized by clear cytoplasm, and is associated with endometriosis and mutations in the ARID1A gene [22] .
- Mucinous OC accounts for only 2-3% of ovarian carcinomas. Characterized by the presence of mucin-secreting cells and typically presents as large cystic masses. This subtype of OC is often bilateral and is frequently associated with pseudomyxoma peritonei [23].

Apart from epithelial OC, there are also other less common types, of non-epithelial OC which account for 5% including, germ cell tumors, sex cord-stromal tumors, and metastatic tumors[24]. Germ cell tumors arise from the germ cells of the ovary and include subtypes such as dysgerminoma, yolk sac tumor, and teratoma [21]. Sex cord-stromal tumors arise from the ovarian stroma and include subtypes such as granulosa cell tumors and Sertoli-Leydig cell tumors. While metastatic tumors originate from primary sites and spread to the ovaries [20].Further research into the molecular mechanisms underlying these subtypes is necessary to develop more effective targeted therapies for OC patients [25].

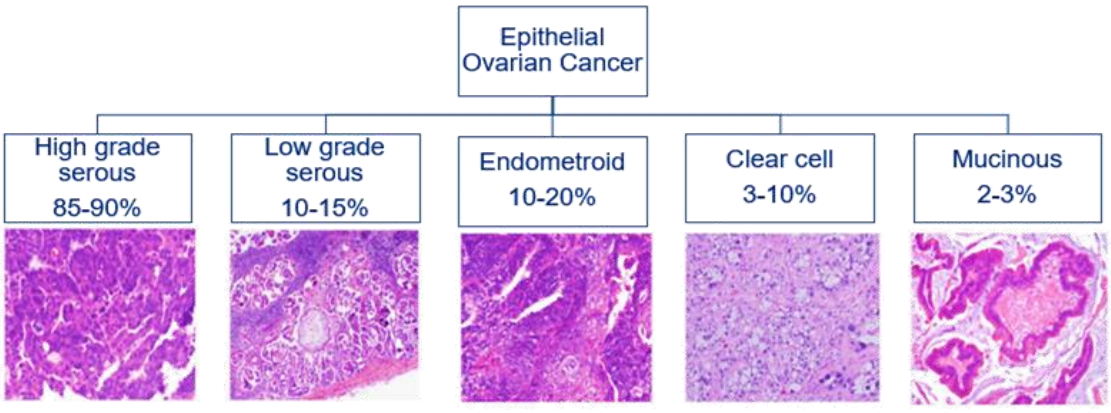

Figure 2.Different types of epithelial ovarian cancer and their percentages.
High-grade serous, low-grade serous, endometrioid, clear cell, and mucinous are types of EOC with their percentages [21].(Created with PowerPoint)

4. Screening

Screening aims to detect the disease at an early stage before symptoms manifest. However, OC is often asymptomatic in its early stages, and by the time symptoms become noticeable, the cancer may have already progressed to advanced stages, making it more challenging to treat effectively [26].

The pelvic exam once considered a standard preliminary screening method, involves a physician palpating the ovaries for irregularities, but its efficacy in reducing OC mortality is unproven. Transvaginal ultrasound (TVU) utilizes sound waves to identify abnormalities, but its utility is hampered by high false-positive rates, which have not translated into a decrease in OC deaths[27]. The CA-125 blood test measures a protein that can be elevated in OC. Despite its potential, the test's specificity is limited, as elevated CA-125 levels can also arise from benign conditions, leading to false-positive results[28].

For women with a high genetic risk, such as those with BRCA mutations, more intensive surveillance measures like risk-reducing salpingo-oophorectomy (RRSO) have shown effectiveness in decreasing incidence and mortality rates, although regular screening has not conclusively improved survival [29].

Recent advances such as genetic testing for cancer susceptibility genes is an emerging revolutionary strategy to identify high-risk individuals for targeted screening [30]. Currently, there is no effective screening program for OC primarily due to its insufficient impact on mortality and lack of specific biomarkers, identifying women at increased risk can help allocate resources more effectively and implement personalized prevention strategies [26].

5. Symptoms and Diagnosis

More than 75% of affected women are diagnosed at an advanced stage because symptoms are vague and nonspecific [31]. Common symptoms include persistent bloating, abdominal or pelvic pain, difficulty eating or feeling full quickly, and urinary symptoms such as urgency or frequency. Other symptoms may include fatigue, unexplained weight loss, changes in bowel habits, and abnormal vaginal bleeding[32].

Diagnostic procedures typically begin with a pelvic exam to check for lumps or abnormalities in the ovaries. Imaging tests such as TVU, CT scans, and MRIs are commonly used to visualize the ovaries and surrounding tissues [31].

Staging of OC is crucial for determining the extent of the disease and planning treatment. The stages range from Stage 1, where cancer is confined to the ovaries, to Stage 4, where it has spread to distant organs. Early detection improves prognosis, but unfortunately, most cases are diagnosed at a later stage when cancer has already spread to distant body regions(

Figure 3)[33].

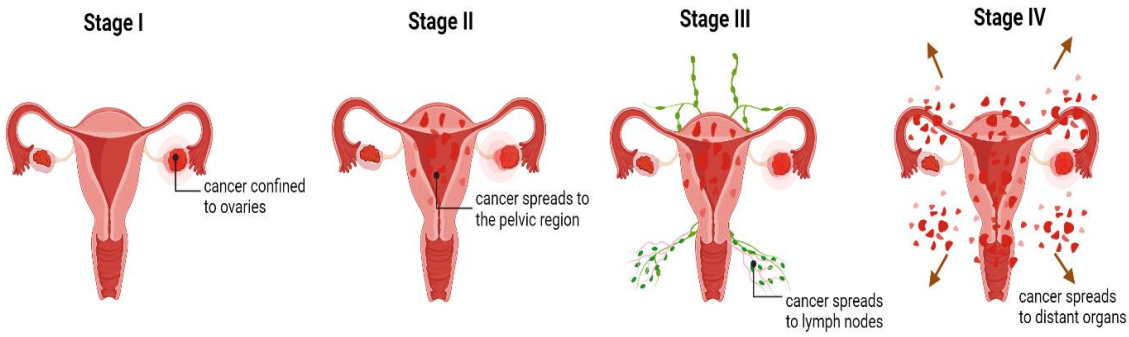

Figure 3. Stages of Ovarian Cancer.
Stage I is confined to one or both ovaries, stageII spreads to the pelvic region, stageIII spreads to the lymph nodes in the back of the abdomen, and stage metastasizes to distant organs. (Created with BioRender.com)

B. Ovarian Cancer Management Strategies

OC management involves a combination of surgical intervention and chemotherapy. It typically begins with surgery, which aims to confirm the diagnosis, determine the extent of the disease, and remove as much of the tumor as possible then followed by the traditional platinum-based compounds such as carboplatin and cisplatin which play a central role due to their DNA damaging effects[34]. However, resistance to platinum-based chemotherapy is a significant challenge, often due to enhanced DNA repair mechanisms within the cancer cells[35].

1. Standard Therapeutics

Cisplatin and its analogue, carboplatin are often used in combination with taxane derivatives such as paclitaxel and docetaxel, to target OC cells by inducing DNA damage[36]. These emerging treatments have shown promise in impeding cancer progression and attenuating chemoresistance whether used as monotherapies or in combination with traditional chemotherapy[37].

PARP inhibitors are a class of targeted cancer drugs that have shown promising outcomes in clinical applications. They were developed based on synthetic lethality, which exploits specific vulnerabilities in cancer cells. Poly (ADP-ribose) polymerase (PARP) is an enzyme that repairs DNA damage. PARP inhibitors work by blocking the activity of PARP, preventing cancer cells from repairing damaged DNA. This leads to the accumulation of DNA damage and ultimately causes cancer cell death[38]. PARP inhibitors have shown effectiveness in tumors with deficiencies in DNA repair pathways, particularly in cancers with mutations in the BRCA1 and BRCA2 genes. These genes play a crucial role in repairing DNA damage. Cancer cells with faulty

BRCA genes already have a compromised repair system, and blocking PARP further impairs their ability to repair themselves. Olaparib, Niraparib, and Rucaparib are already approved for treating recurrent EOC and their indications partially overlap. Studies are evaluating PARP in monotherapy or associations, in a wide range of settings[39].

Tyrosine kinase inhibitors (TKIs) are targeted therapy used to treat cancer. They work by inhibiting the activity of tyrosine kinases, which are enzymes involved in cell signaling and growth. They play a role in tumorigenesis and progression, making them attractive targets for drug development. TKIs are often used in the treatment of OC. They are considered multi-target inhibitors because they have a variety of downstream effects caused by tyrosine kinases[40]. However, clinical trials for some types of TKIs have not shown significant results compared to in vitro trials. To overcome this, TKIs are being tested in combination with other therapeutic drugs to enhance their synergistic effect. For example, the TKI Nintedanib, has demonstrated an increase in response rate of 18-28% in clinical trials and maintained a tolerable level of toxicity when administered in combination with the standard first-line treatment for OC[41]. Other TKIs, such as lapatinib, gefitinib, and erlotinib, are currently under clinical trials and have shown a significant increase in response rate when used as part of first-line therapy for treating OC[42].

Aromatase inhibitors are a novel approach to cancer treatment, particularly for estrogen-dependent cancers. They target the aromatase enzyme, which is involved in estrogen production[43]. By inhibiting aromatase, these inhibitors reduce estrogen levels in the body, which can help prevent the growth and progression of hormone receptor-positive breast cancer. In the context of OC, there is ongoing research and

clinical trials to explore the potential of aromatase inhibitors. A phase II clinical trial on patients with persistent OC showed that the majority of patients experienced a tolerant condition of the disease when treated with an aromatase inhibitor called Anastrozole[44].

Vascular endothelial growth factor (VEGF) is a critical component of the process of forming new blood vessels (angiogenesis). It plays a significant role in tissue vascularization and angiogenesis and is known to be involved in OC progression and tumor development[45]. In cancer research, VEGF is a major target due to its role in promoting angiogenesis and tumor growth. One promising approach is the use of bevacizumab, a monoclonal antibody that specifically targets VEGF. In clinical trials, bevacizumab inhibited the binding of VEGF to its receptor, preventing downstream activation of signaling pathways that promote vascularization, cell proliferation, and metastasis[46]. Importantly, bevacizumab does not show associated cytotoxic effects, making it a valuable therapeutic option that prolongs survival by 4 months. Phase III clinical trials have confirmed its efficacy and safety profile. Currently, bevacizumab is being studied in combination with other cancer therapeutics, to treat OC[47].

2. Challenges in therapy: Stem Cells

Studying stem cell biology and its role in tumorigenesis is crucial for effective tumor targeting and treatment. Cancer stem cells (CSCs) are a subpopulation of tumor cells that can drive tumor initiation, disease progression, and subsequent post-therapeutic relapse [48].

Stem cells possess various characteristics that make them highly resistant to chemotherapeutic agents, including the expression of anti-apoptotic proteins, and DNA

repair mechanisms. Chemotherapy and radiotherapeutics change the tumor microenvironment which alters the CSC phenotypic and functional ability proving their high adaptability and resilience, which can contribute to therapy resistance and tumor recurrence. The "seed and soil hypothesis" in cancer metastasis, first proposed by Steven Paget in 1889, suggests that cancer cells within a tumor can spread to distant organs that provide favorable conditions for secondary tumor growth[49]. This hypothesis emphasizes the interaction between the tumor cells (the "seed") and the specialized microenvironment of the target organ (the "soil"), which highlights the importance of tumor-stroma interactions that contribute to cancer metastasis [49, 50].

Chemotherapeutics targets active cells in the S phase of the cell cycle, while cells in the quiescent state are typically present in the G0 phase. Therefore, a tumor occurs when previously dormant inactive cells become more resistant to therapy than the primary cell tumors[51]. CD133 is the most common CSC surface biomarker associated with OC stem cell tumorigenesis, chemoresistance, and tumor recurrence [53]. Other surface markers include CD44, CD24, EpCAM, and ROR1[52]. The expression of these markers varies among patients and tumor cell lines. Combining these markers and assessing other factors may provide a more precise understanding of ovarian CSCs and their role in cancer progression (**Figure 4**)[53].

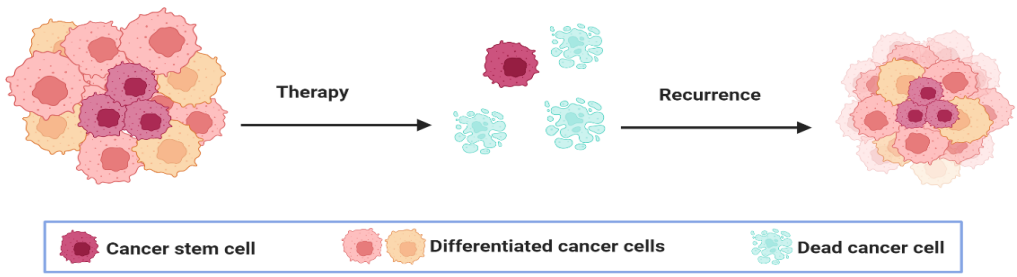

Figure 4.Cancer stem cells model.
CSC initiates, drives, and re-establishes tumor growth. (Created with BioRender.com)

C. Novel Therapeutic Strategies: Herbal Therapy

The use of natural products extracted from herbs has been a cornerstone of traditional Chinese medicine representing one of the oldest forms of therapy due to their nourishing and synergistic actions making them an excellent choice for treating disease[54]. Herbal therapy demonstrated antioxidant, anti-inflammatory, antitumorigenic, anticarcinogenic, glucose, and cholesterol-lowering activities. Overall, 45% of studies reported positive findings of herbal medicine with fewer adverse effects compared with conventional medications [55]. Plants have eminent bioactive phytochemical constituents including alkaloids, glycosides, taxanes (paclitaxel, docetaxel), flavonoids, curcumin, lectins, etoposides, phenolic acids, and anthracyclines (doxorubicin, daunorubicin). These compounds are being investigated for their potential use in targeting cancer signaling pathways, proliferation, and chemoresistance prevention which is a major hurdle in case of therapy[56, 57].

Quinones, a class of organic compounds, obtained from plant secondary metabolites have shown potential as an anti-cancer agent that enhances treatment efficacy and reduces the damage caused by conventional therapies.

1. Quinones

Quinones derived from plant secondary metabolites have shown promise as potential anti-cancer agents. These compounds, sourced from natural sources, exhibit significant biological activities that can inhibit cancer cell growth and proliferation. Quinones are found as biological pigments, in various organisms like bacteria, certain fungi, and higher plant forms. However, they are found in only a few animal species,

such as sea urchins and aphids[58]. The quinone family includes benzoquinones, naphthoquinones, anthraquinones, and phenanthraquinones (**Figure 5**) [59].

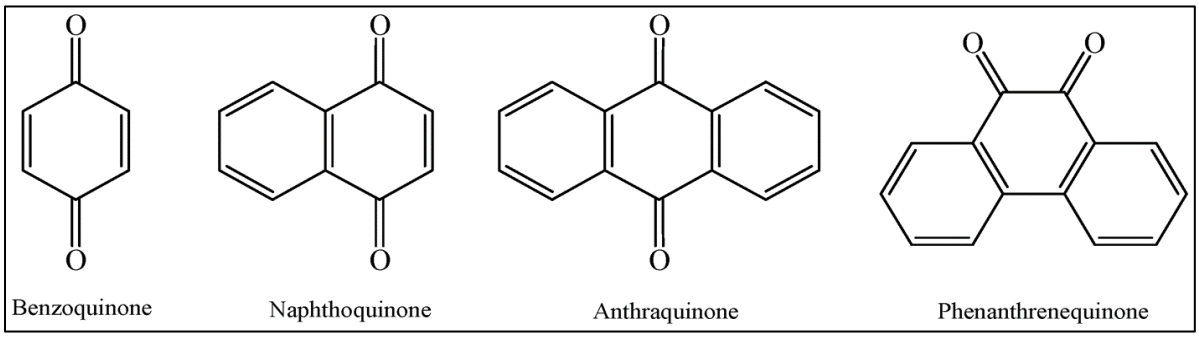

Figure 5.Structures of quinones.
Includes Benzoquinone, naphthoquinone, and phenanthrenequinone. Adapted from Scholarly Community Encyclopedia.

2. Thymoquinone Overview

Nigella Sativa plant known as the black seed contains the active compounds thymoquinone and monoterpenes (such as P-cymene and α-piene), known for their pharmacological properties including antioxidant, anti-inflammatory, and anti-cancer effects Thymoquinone (TQ), belonging to the benzoquinone subfamily, has the chemical formula thymoquinone 2-isopropyl-5-methyl-1,4-benzoquinone (**Figure 6**) [60]. TQ shows promising effects in treating a variety of acute/chronic diseases such as cancer, asthma, bronchitis, rheumatism, headache, back pain, anorexia, amenorrhea, inflammation, eczema, obesity, infections, depression, hypertension, gastrointestinal, cardiovascular, hepatic, and renal disorders (**Figure 7**) [61-64].

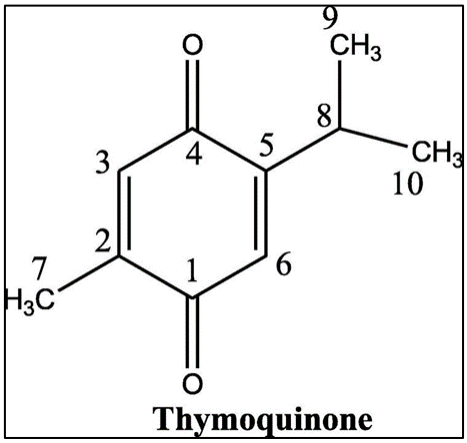

Figure 6.Chemical structure of TQ [63].

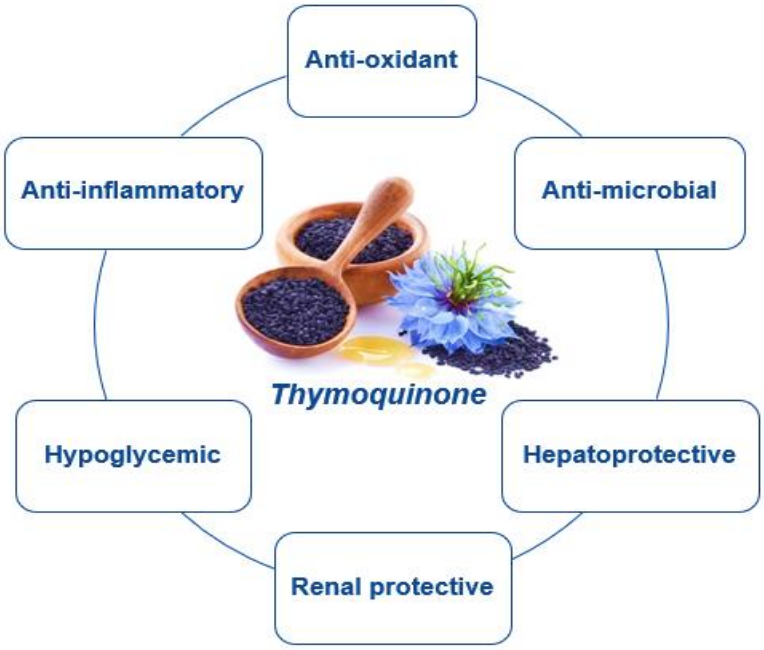

Figure 7. TQ therapeutic actions.
TQ compound includes antioxidant, anti-inflammatory, antimicrobial, renal, and hepatic protective properties. (Created with PowerPoint).

3. Thymoquinone and Cancer

TQ demonstrates a broad spectrum of anti-cancer activity by disrupting cancer progression through numerous pathways across various cancer types, including ovarian, breast, colon, and leukemia [60, 65, 66]. It inhibits cell proliferation, migration,

invasion/metastasis, angiogenesis, colony formation, and epithelial-mesenchymal transition (EMT), impeding autophagy and cancer stem cell development[65]. Additionally, TQ enhances tumor cell cytotoxicity, induces cell cycle arrest at stages like G2/M, G1/S, S, and G0/G1, and promotes apoptosis and necroptosis[67]. TQ can sensitize tumor cells to conventional treatments such as chemotherapy, radiotherapy, and immunotherapy, enhancing their effectiveness. TQ is also noted for protecting healthy cells from oxidative damage induced by free radicals and chemotherapy agents like doxorubicin, thus potentially reducing side effects.

TQ interferes with several oncogenic signaling pathways, including JAK2/STAT3, PI3K/Akt/mTOR, and NF-κB, crucial for cancer cell survival and proliferation[68]. Studies have shown the effect of TQ effect in specific cancers:

In breast cancer, TQ has shown promising results in doxorubicin-resistant human breast cancer cells by enhancing the chemo potentiation of doxorubicin. It also stimulates the expression of miR-603, which is associated with tumor suppression[69]

In melanoma, TQ increases survival rates in mice with intracerebral B16-F10 melanoma cells and inhibits the JAK2/STAT3 pathway, which is crucial for tumor growth and survival[70].

In colorectal cancer, TQ enhances the efficacy of 5-fluorouracil (5-FU) and vitamin D3 in colorectal cancer (CRC) cells[71]. It inhibits the PI3K/Akt/mTOR pathway and increases the levels of activated JNK1/2 and ERK1/2, likely through reactive oxygen species (ROS) production[72, 73]

As for pancreatic cancer, TQ inhibits cell proliferation, invasion, and metastasis in pancreatic cancer. It enhances the sensitivity of cancer cells to gemcitabine (GEM)

and reduces the synthesis of inflammatory cytokines, thereby exerting anti-inflammatory effects [74].

For glioblastoma cells, treatment with TQ resulted in a reduction in the expression of focal adhesion kinase (FAK). This decrease in FAK expression led to a decrease in the phosphorylation of extracellular signal-related kinase (ERK), a signaling pathway. Consequently, the secretion of metalloproteinases (MMP-2 and MMP-9) was reduced [75]. Additionally, TQ treatment inhibited the activity of the telomerase enzyme, resulting in DNA damage and inhibiting the growth of glioblastoma cancer cells [76].

TQ interferes with the PI3K/Akt signaling pathway in OC, promoting G1 cell cycle arrest. It also inhibits STAT3 phosphorylation, which is associated with the reduction of JAK2 and c-Src activity, as well as the downregulation of Bcl-2, cyclin D, surviving, and VEGF[65]. Moreover, the mechanism of action of TQ induces apoptosis in OC cells by targeting signaling molecules and pathways [77]. This dual action makes it a potent candidate for OC treatment [78].

D. Aim of the study

Despite significant strides in the treatment of OC, the disease develops drug resistance with a poor prognosis. This study aims to assess the effect of TQ on human epithelial ovarian carcinoma cell lines: OVCAR-420 and SKOV-3. Specifically, the study will investigate the ability of TQ, both alone and in combination with cisplatin (CDDP), to suppress cell viability, proliferation, and migration using two-dimensional (2D) in vitro models. Furthermore, the study will evaluate the potency and synergy of this combination in targeting the cancer stem cell (CSC) population using a three-

dimensional (3D) sphere formation assay [79]. These efforts are intended to enhance our understanding of OC treatment and identify optimal drug combinations to improve patient outcomes.

CHAPTER II

MATERIALS AND METHODS

A. Cell Culture

Two ovarian cancer cell lines OVCAR-420(high-grade serous carcinoma) and SKOV-3(non-serous adenocarcinoma), were maintained and cultured in RPMI-1640 media (Sigma-Aldrich) supplemented with 10% of fetal bovine serum (FBS) (Sigma-Aldrich), 1% Penicillin/Streptomycin (Sigma-Aldrich) and Plasmocin™ prophylactic (Invivogen). Cells were incubated at 37℃ in a humidified incubator containing 5% CO2.

B. Drug Preparations and Treatment

TQ was reconstituted in pure dimethylsulfoxide (DMSO; Sigma Aldrich) prepared per manufacturer's instructions into a stock solution of 50mM stored at -20 ℃. The stock solutions were then dissolved in a cell culture medium to obtain daughter solutions of different concentrations.

CDDP was reconstituted in filtered sodium chloride (NaCl) to reach a stock concentration of 1.7mM stored at -20 ℃. The stock solutions were then dissolved in a cell culture medium to obtain daughter solutions of different concentrations.

C. MTT Cell Proliferation Assay

The antiproliferative effects of TQ and CDDP alone and in combination were assessed using the MTT assay ([3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide]) (Sigma-Aldrich). This assay relies on the ability of metabolically active cells

to reduce the yellow tetrazolium MTT salt into purple formazan crystals via mitochondrial NAD(P)H-dependent oxidoreductase enzymes. The higher the activity of the mitochondrial enzymes, the greater the amount of formazan dye formed, which reflects the number of metabolically active cells. Thus, this assay provides an estimation of cellular proliferation. OVCAR-420 and SKOV-3 cells were seeded in triplicates in 100 µL complete media in 96-well plates at a seeding density of $8x10^3$ cells/well for both cell lines. The cells were treated with increasing concentrations of TQ (5, 10, and 20 µM), CDDP (2.5,5 and 10 µM), and their combinations, while the control wells contained growth media and the vehicle consisted of pure DMSO for TQ and NaCl for CDDP corresponding to the highest drug concentration. The cells were then incubated for 24,48, and 72 hours for optimization and 48 hours post-optimization. After that, the media containing treatment was discarded, fresh media supplemented with 5 mg/ml MTT reagent (dissolved in 1X phosphate-buffered saline (PBS) was added to each well and the plate was incubated at 37 °C for 4 hours. After incubation, the reagent was removed and 100µL of absolute isopropanol (Sigma-Aldrich) was used to solubilize the formazan crystals. The plate was covered by foil and incubated for 15-60 minutes hour at room temperature. MTT optical density (OD) was measured at 595 nm by an ELISA reader (Multiskan Ex). The blank well was used for the baseline zero and the percentage of cell proliferation for control was determined for each drug dose. The data are derived from the mean of triplicate wells of at least three independent experiments.

D. Trypan Blue Assay

The trypan blue viability assay was used to determine the cytotoxic effects of TQ and CDDP, alone and in combination. This assay uses trypan blue as an exclusion

dye to discriminate between viable and dead cells. Live cells have intact cell membranes that prevent the dye from penetrating the cytoplasm and appear bright white, while dead cells lose the integrity of their plasma membrane allowing the dye to stain the cells in dark blue. OVCAR-420 and SKOV-3 cells were seeded in duplicates in 500 µL complete growth media in 24-well plates at a density of $50x10^3$ cells/well and $60x10^3$ cells/well respectively. On the following day, the cells were treated with different concentrations of TQ (10, 25, and 50 µM) and CDDP (5, 10, and 20 µM) for OVCAR-420. However, TQ (5,10, and 20 µM) and CDDP (2.5, 5, and, 10 µM) for SKOV-3. After 48 hours of incubation, the attached live cells were trypsinized, and harvested, and a mix of cell suspension and trypan blue dye at a ratio of 1:4 was prepared 10 µL of this mixture was added to the counting chamber of the Neubauer-improved Hemocytometer and visualized under an inverted optical microscope. Viable cells were counted in four quadrants of the two chambers of the hemocytometer and the average was calculated. The percentage of cell viability with respect to control was determined for each drug dose. The data are derived from the mean of duplicate wells of three independent experiments.

E. Wound Healing Assay

The wound healing or scratch assay was used to assess the effects of TQ, and CDDP, alone and in combination on the collective cellular migration potential of the cells. OVCAR-420 and SKOV-3 cell lines were seeded in duplicates in 500 µL complete growth media in 24-well plates with a density of $180x10^3$ cells/well and $300x10^3$ cells/well respectively and, incubated to reach a monolayer of 90-100% confluency. The cells were treated with 10µg/mL mitomycin C (Sigma) and incubated

for 5 minutes for OVCAR-420 and 20 seconds for SKOV-3 to block cellular proliferation. Using a sterile 200 µL tip, a scratch (wound) was made. The scraped cells, where the wound was performed, were washed twice with PBS. The OVCAR-420 cells were then cultured at a drug concentration of TQ (10,25 and, 50 µM), and CDDP (5, 10 and, 20 µM). SKOV-3 were cultured at different drug concentrations of TQ (5,10 and, 20 µM), and CDDP (2.5, 10 and,20 µM). After that scratch images were taken at (0,9,12,24 hours) for OVCAR-420 and at (0,6,9,12 hours) for SKOV-3. Images were acquired using bright-field microscopy directly after inducing the scratch (0,9,12, and 24 hours) post-treatment to compare the difference in the wound width. The wound width was measured and expressed as percentage of the relative width for each condition.

F. Sphere Formation Assay

The sphere formation 3D assay was performed to investigate the ability of TQ, and CDDP, separately and when combined in targeting the highly enriched self-renewing ability of OC stem cells pool residing within the OVCAR-420 and SKOV-3 cell lines. 1000 single cells were suspended in cold Matrigel^{TM}/serum-free medium (1:1 dilution) in a total volume of 10µL for OVCAR-420 and 20µL for SKOV-3. Cells were seeded in duplicates in a circular motion around the rim of the wells of a 96-well plate, with a seeding density of 1500 cells/well for both cell lines. Matrigel was then allowed to solidify for 20 minutes in the incubator at 37 °C. Afterward, treatment of 100µL was added for OVCAR-420, of drug concentrations of TQ (1 and 5µM), and CDDP (0.5 and 2.5 µM). While for SKOV-3 at concentrations of TQ (2.5 and 20 µM), CDDP (0.5 and 1 µM). These treatments were diluted in RPMI media with 5% FBS and gently added to

the middle of each well. Spheres were replenished with warm media every other day after the day of seeding. After 7-10 days, OC spheroids were counted and bright field images were acquired using an Axiovert microscope from Zeiss at 10× magnification. Images were analyzed by Carl Zeiss Zen 2012 image software to determine sphere sizes. Sphere-formation unit (SFU) was calculated as follows:

$$SFU = \frac{Number\ of\ spheres\ formed}{Number\ of\ cells\ origonally\ plated} x100$$

Results were represented as a percentage of the SFU of the treated spheres compared to the control ones. The data are derived from the mean of duplicate wells of three independent experiments.

G. Synergy Analysis

Synergy analysis was performed using CompuSyn software according to Chou and Talalay's method. These experiments were conducted in triplicate, and the Fraction affected (Fa) values, representing the fraction of cells inhibited by the drug, were determined. Synergism, additivity, or antagonism in the different combinations were calculated using the combination index (CI), where CI<1 indicated synergism, CI=1 indicated an additive effect, and CI >1 indicated antagonism.

H. Statistical Analysis

The means ± the standard error of the means (SEM) of all independent repeats were calculated and the statistical analysis was performed using GraphPad Prism 9 Software (version 9.5.1, GraphPad Software Inc., La Jolla, CA, USA) and ZEN. A two-way ANOVA test was used to analyze the results of the MTT, trypan blue, and wound healing assays, whereas a one-way ANOVA test was performed for the analysis of the

sphere-formation assay. Statistical significance was reported at p-values of $P < 0.05$(*), $P < 0.01$(**), $P < 0.001$(***)

CHAPTER III

RESULTS

A. TQ and CDDP reduce the proliferation of ovarian cancer cells.

An MTT assay was conducted to evaluate the effects of TQ and CDDP on cellular proliferation as monotherapies. TQ and CDDP demonstrated a significant reduction in cellular proliferation in a time- and dose-dependent manner. Determining the half-maximal inhibitory concentration (IC50) was essential to assess the amount of drug required to inhibit biological processes by 50%.

For OVCAR420 cells, the IC50 values for CDDP were calculated to be 16.58 µM, 10.92 µM, and 8.62 µM at 24, 48, and 72 hours, respectively (**Figure 8a**). Correspondingly, the IC50 values for TQ were 58 µM, 25 µM, and 18 µM (**Figure 8b**).

In SKOV-3 cells, CDDP exhibited IC50 values of 6.8 µM, 5.3 µM, and 5.2 µM (**Figure 8c**), while TQ demonstrated IC50 values of 20 µM, 10 µM, and 12.7 µM at the same time points (**Figure 8d**). These findings were instrumental in determining the concentrations to be used in subsequent experiments.

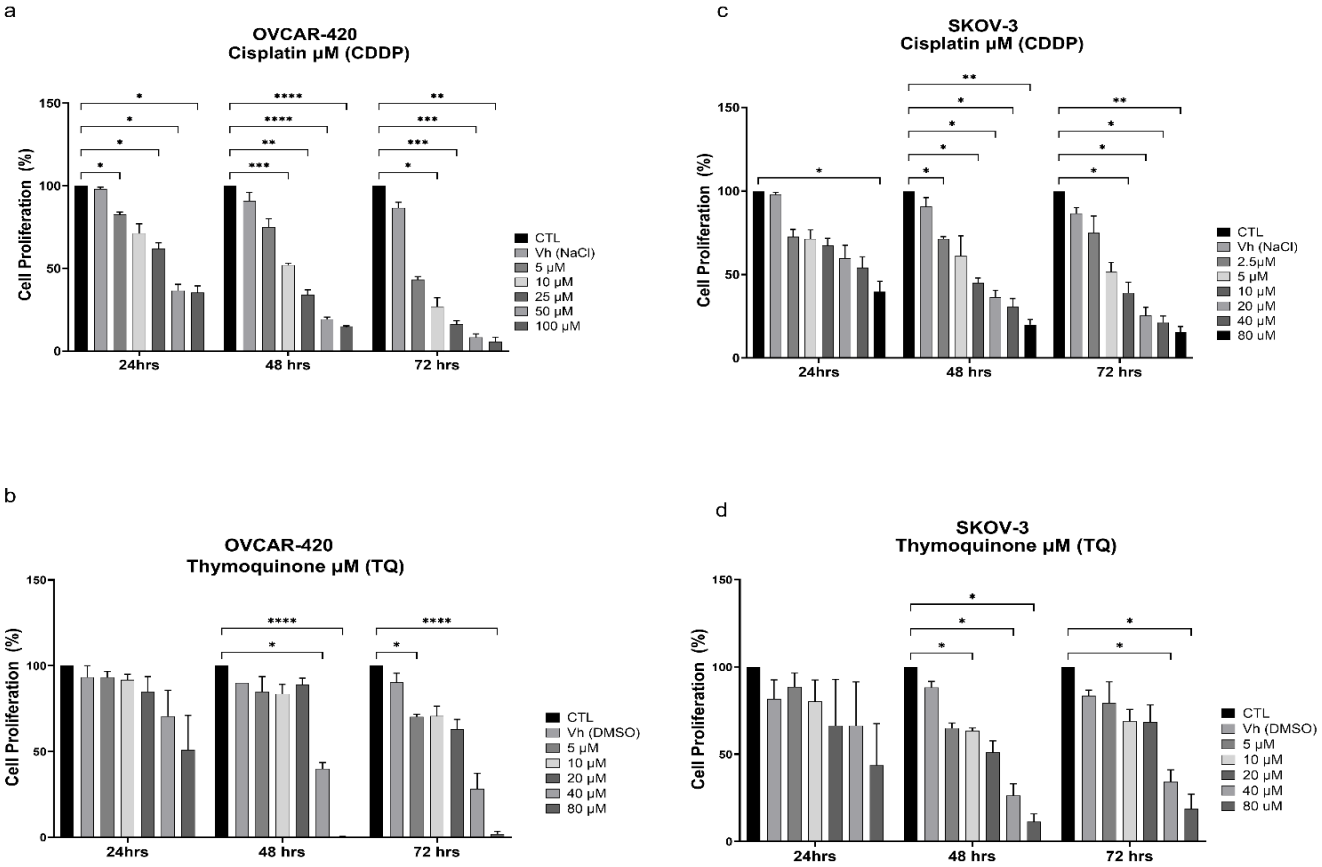

Figure 8. TQ and CDDP reduce cell proliferation of OVCAR420 and SKOV-3 in a time and dose-dependent manner.
Cell proliferation was determined using the MTT assay after incubation for 24, 48, and 72 hrs. Results are expressed as a percentage of the studied group compared to its control. Data represents an average of 3 independent experiments and are reported as mean ± SEM (SEM being represented by error bars; (*P<0.05, **P<0.01, ***P<0.001).

B. TQ reduces the proliferation of ovarian cancer cells alone and in combination with CDDP.

OVCAR-420 and SKOV-3 cells were treated with increasing concentrations of TQ: 10, 25, and 50 µM for OVCAR-420, and 5, 10, and 20 µM for SKOV-3 alone and in combination over 48 hours. The results demonstrated that TQ alone significantly inhibited the proliferation of both cell lines in a dose-dependent manner (**Figure 9**). For OVCAR-420 cells, treatment with TQ alone resulted in an approximately 50% reduction in cell growth. Notably, a significant decrease in cellular proliferation was observed when combining 5 µM of CDDP with 25 µM of TQ, as well as 10 µM of CDDP with 25 µM of TQ (**Figure 9b)**. The effects on proliferation were significantly greater with the combination of 5 µM and 25 µM TQ compared to 10 µM CDDP alone (**Figure 9b)**. Similarly, SKOV-3 cells exhibited a decrease CDDP in cellular proliferation with increasing concentrations of TQ (5, 10, and 20 µM). Adding 20 µM TQ to all concentrations of CDDP resulted in a significant antiproliferative effect **(Figure 9f).**

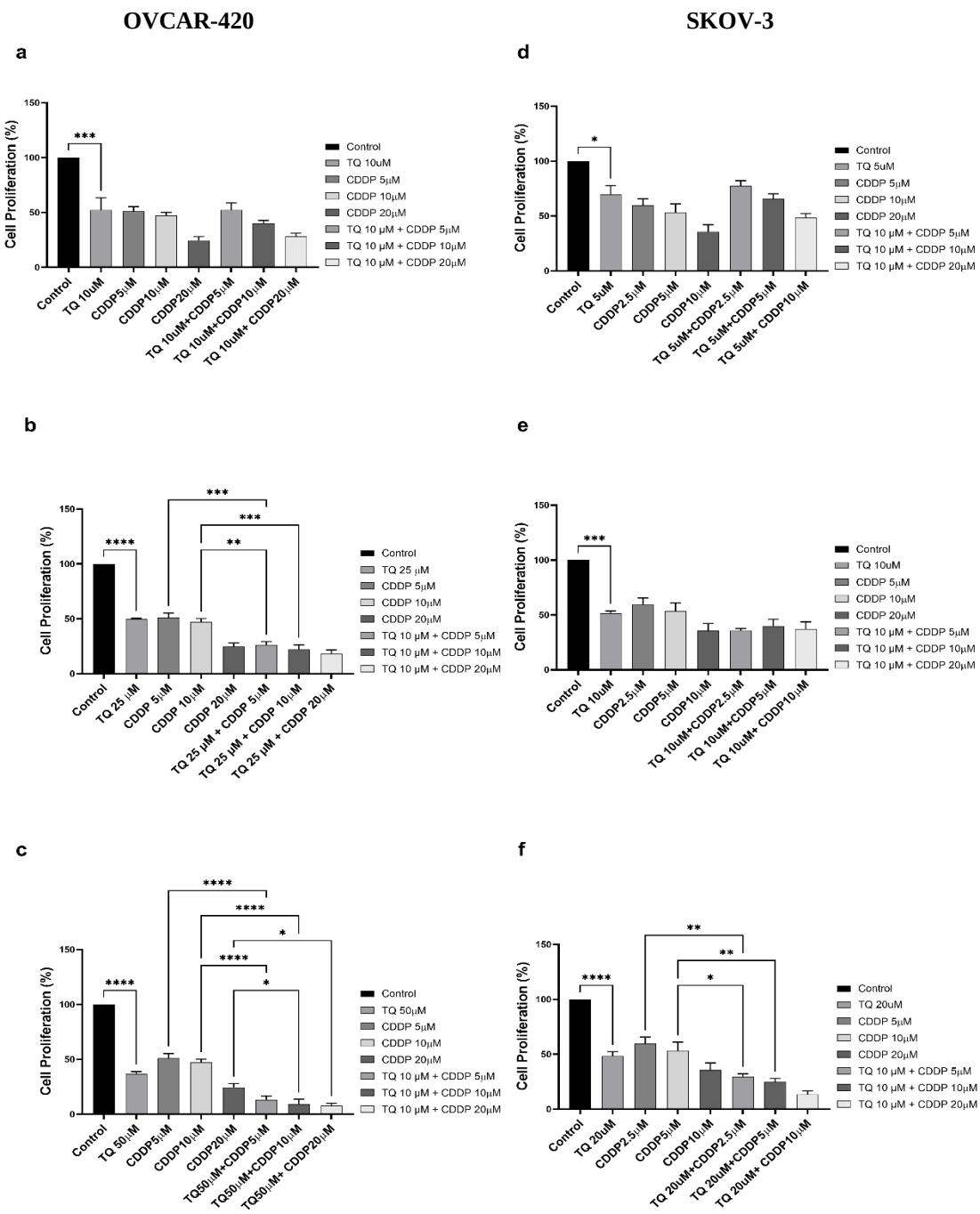

Figure 9.TQ reduces cell proliferation of OVCAR-420 and SKOV-3 alone and in combination with CDDP.

Cell proliferation was assessed using the MTT assay after 48 hours of treatment with TQ alone and in combination with CDDP. TQ at 50 µM reduced OVCAR-420 cell growth by approximately 50%. The combination of 5 µM CDDP with 25 µM TQ significantly enhanced anti-proliferative effects compared to CDDP alone. In SKOV-3 cells, TQ at 20 µM combined with all CDDP concentrations also demonstrated significant inhibition of proliferation. Results are expressed as a percentage of the control group, averaged from three independent experiments (*P<0.05, **P<0.01, ***P<0.001).

C. TQ decrease the cellular viability of ovarian cancer cells alone and in combination with CDDP.

Trypan blue staining was used to investigate the effect of TQ alone and combined with CDDP on the cell viability of OVCAR-420 and SKOV-3 cell lines. Our findings revealed a dose-dependent reduction in cell viability. In OVCAR-420 cells, 25µM TQ resulted in a noticeable reduction in viability, while a more pronounced effect was observed at 50 µM TQ **(Figure 10b, c)**. Notably, the combination of 25µM TQ and 10µM CDDP significantly decreased cell viability relative to the control and relative to CDDP alone **(Figure 10b).**

Similarly, in SKOV-3 cells, the combination of TQ at 10µM and 20µM with all concentrations of CDDP resulted in a significant reduction in viability compared to CDDP alone (**Figure 10e, f)**.

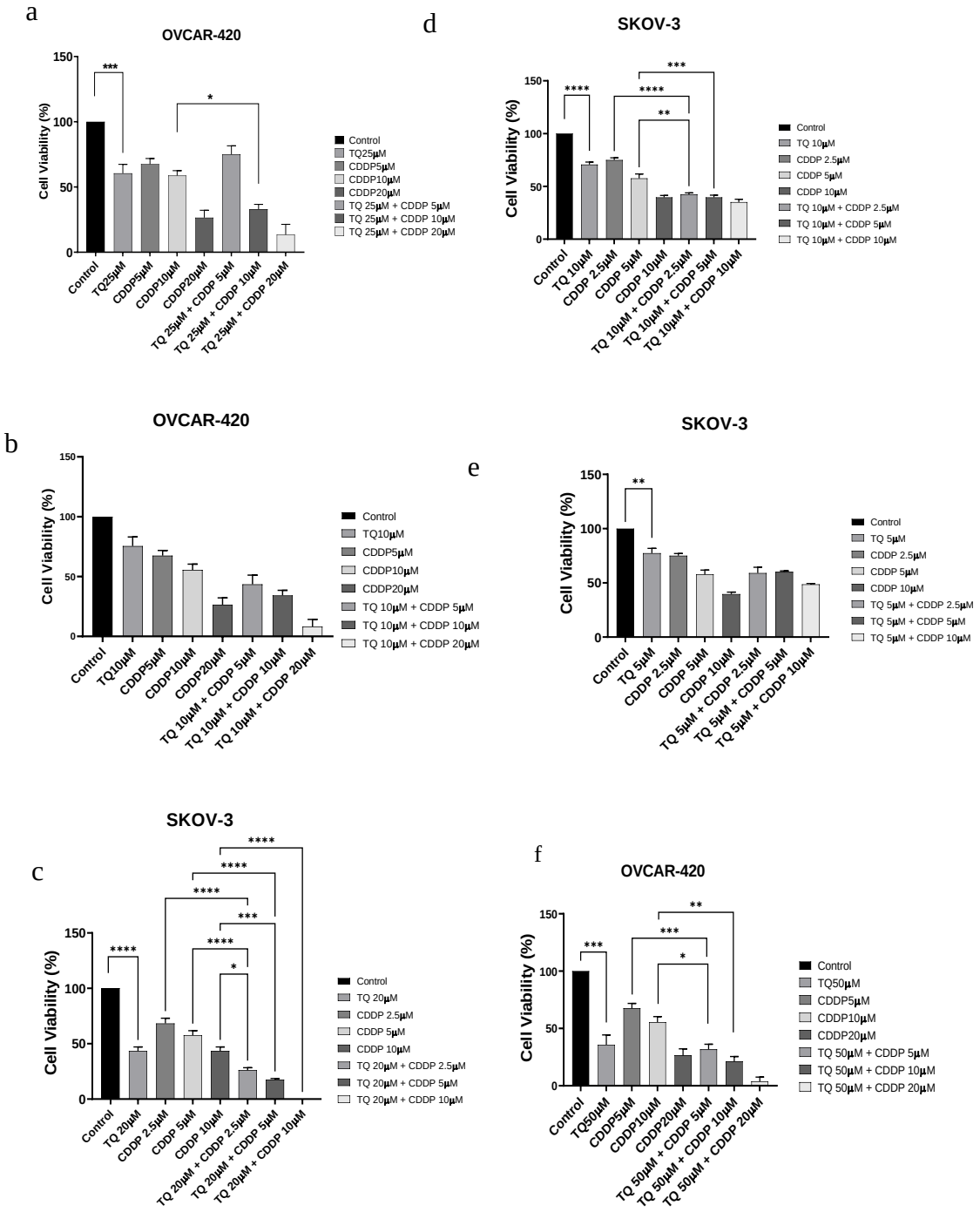

Figure 10.TQ reduces cell viability of OVCAR-420 and SKOV-3 alone and in combination with CDDP.

Cell viability was evaluated using the MTT assay after 48 hours of treatment with TQ alone and in combination with CDDP. In OVCAR-420 cells, 25 µM TQ reduced viability, with a more significant effect at 50 µM. The combination of 25 µM TQ and 10 µM CDDP further decreased cell viability. In SKOV-3 cells, TQ at 10 µM and 20 µM combined with all CDDP concentrations significantly reduced viability compared to CDDP alone. Results are presented as a percentage of the control group, averaged from three independent experiments (*P<0.05, **P<0.01, ***P<0.001).

D. TQ and CDDP exhibit synergistic effects on the cellular viability of ovarian cancer cells.

To confirm the potential synergistic effect of combining TQ with CDDP, we analyzed the trypan blue exclusion assay results using the computational Compusyn tool, which involves calculating the combination index (CI) via assessing the combined effect of different agents on cell viability. Remarkably, synergy was observed in all the conducted combinations with a combination index (CI) less than 1 (**Table 1**,**Table 2**). Very strong synergism (CI <1) was detected when 25μM (which is the IC- 50) of TQ was combined with 20μM of CDDP in OVCAR-420 (**Table 1**,**Figure 11a**,).SKOV-3 cells synergy was observed at 10uM of TQ (which is the IC-50) with 2.5 and 5uM CDDP while strong synergy qt 20uM CDDP (**Table 2 , Figure 11b**).

Table 1. Synergy analysis results for OVCAR-420
The OVCAR-420 data reveals a **synergistic effect** at concentrations shown in red, indicating the combination index values relative to the fraction affected (FA).

Dose TQ	Dose CDDP	Combination Index	Indication
10.0	5.0	2.64393	Antagonism
10.0	10.0	0.65288	Synergism
10.0	20.0	0.07894	Synergism
25.0	5.0	1.13998	Antagonism
25.0	**10.0**	**0.89695**	**Synergism**
25.0	20.0	0.04086	Strong Synergism
50.0	5.0	1.46206	Antagonism
50.0	10.0	0.69373	Synergism
50.0	**20.0**	**0.00153**	**Strong Synergism**

Table 2.Synergy analysis results for SKOV-3
The SKOV-3 data reveals a **synergistic effect** at concentrations shown in red, indicating the combination index values relative to the fraction affected (FA).

Dose TQ	Dose CDDP	Combination Index	Indication
5.0	2.5	2.31519	Antagonism
5.0	5.0	1.54589	Antagonism
5.0	10.0	0.86273	Synergism
10.0	2.5	0.38428	Synergism
10.0	**5.0**	**0.63459**	**Synergism**
10.0	10.0	0.74456	Synergism
20.0	2.5	0.49965	Synergism
20.0	5.0	0.35740	Synergism
20.0	**10.0**	**0.11297**	**Strong synergism**

Figure 11. Synergy analysis on OVACR-420 and SKOV-3 using CompuSyn showing data points.

In the provided scatter plot, each circle represents a specific combination of TQ and CDDP concentrations tested on OC cells. The x-axis (FA) typically represents the fraction affected or the effect level, and the y-axis (CI) is the combination index, a quantitative measure of the degree of drug interaction. Points below the line represent synergism, points at the line indicate additive effect and the above points show antagonism.

E. TQ reduces the migration ability of OVCAR-420 and SKOV-3 cells alone and in combination with CDDP.

A wound-healing assay was employed to assess the effect of TQ, alone or in combination with CDDP, on the migration of OC cells. Our results for the untreated control samples showed that the wounds were closed after 12 and 24 hours for OVCAR-420 and SKOV-3, respectively (**Figure 12**). Treatment with TQ alone significantly delayed the migration of OVCAR-420 and SKOV-3 cells compared to untreated cells (**Figure 12b, f, and Figure 13)**.

In OVCAR-420, increased obstruction was observed with combinatorial treatment of TQ 25µM plus 5µM and 10 µM CDDP. This was statistically significant relative to control and CDDP alone (**Figure 13a)**.

In SKOV-3 combining TQ10uM with 5µM of CDDP suppressed migratory ability by 60% (40% wound closure) 24 hours after treatment (**Figure 12 and Figure 13**).

However, a statistical significance was observed when TQ and CDDP were combined

(**Figure 12 and Figure 13**)

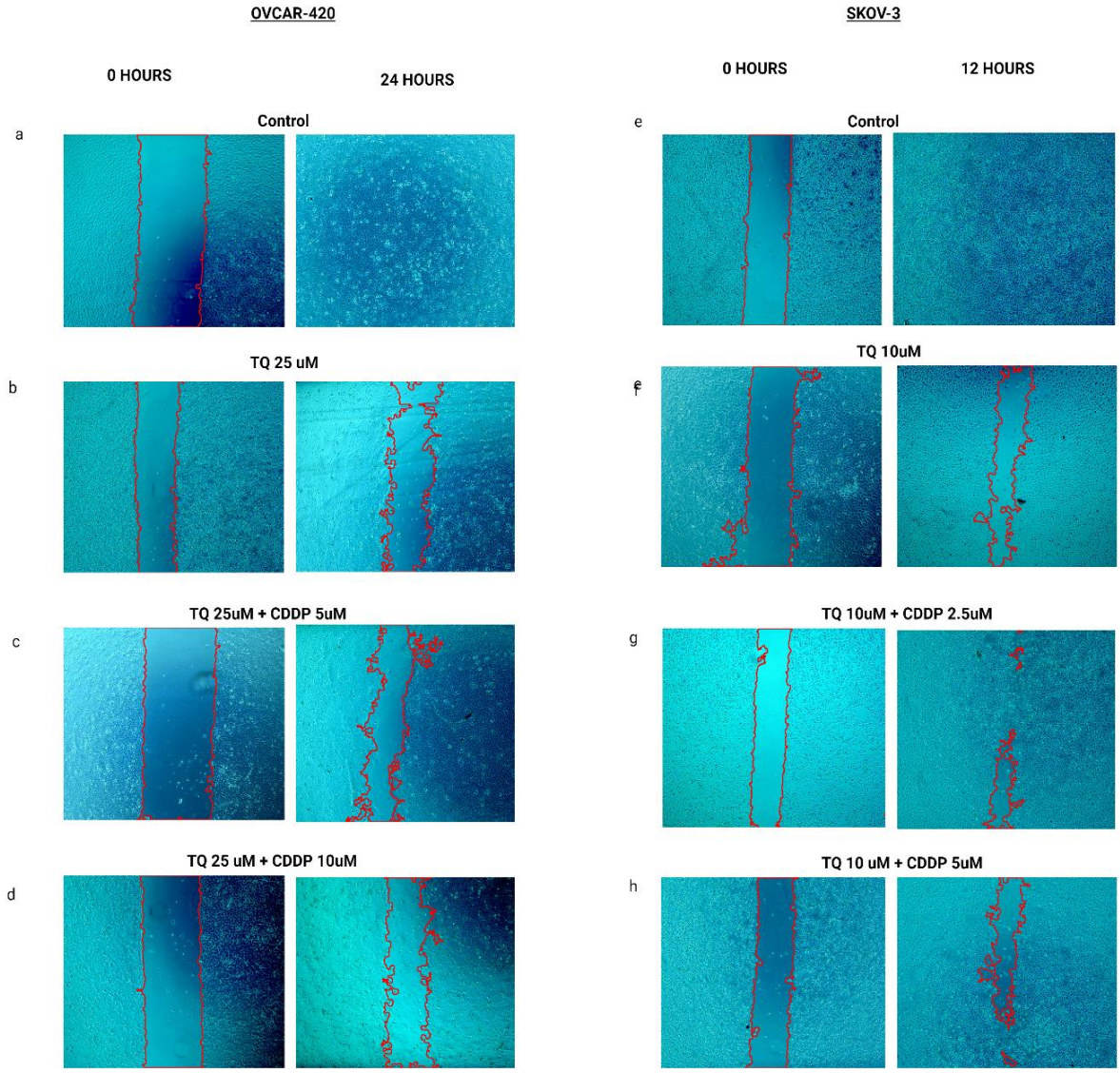

Figure 12.The effect of combined treatment on cell migration in OVCAR-420 and SKOV-3.
Representative images illustrate the impact of combined treatment after 24 hours. A scratch was made in the monolayer of confluent cells in 24-well plates using a 20 µL pipette tip. Cell migration was assessed at four time points: 0, 6, 9, and 12 hours for SKOV-3, and 0, 9, 12, and 24 hours for OVCAR-420. OVCAR-420 exhibited 20% greater inhibition of migration compared to SKOV-3 post-treatment. Scale bar = 100 µm.

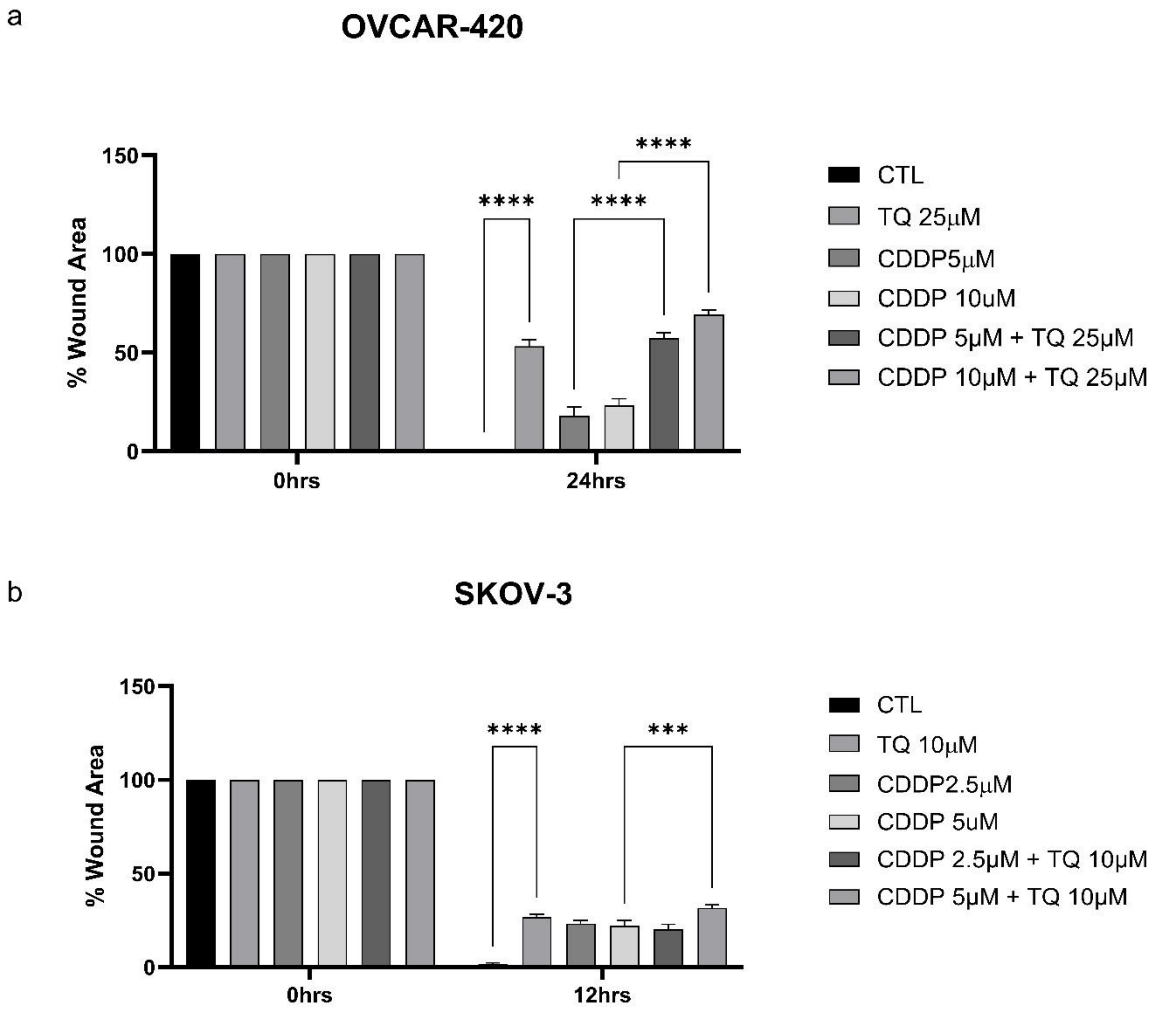

Figure 13. TQ hinders cell migration of OVCAR-420 and SKOV-3 cancer cells alone and in combination with CDDP.
Wound-healing assays demonstrated that TQ alone significantly delayed cell migration in both cell lines. Combining 25 µM TQ with 5 µM and 10 µM CDDP reduced OVCAR-420 migration by 80% at 12 hours (20% wound closure). For SKOV-3, TQ at 10 µM with 5 µM CDDP suppressed migration by 60% at 24 hours (40% wound closure). However, no significant inhibition was observed with TQ combined with other CDDP concentrations in SKOV-3.

F. TQ reduces the growth of OVCAR-420 and SKOV-3 cell-derived spheres alone and in combination with CDDP.

To evaluate the efficacy of TQ, both as monotherapy and in combination with CDDP, we employed an in vitro 3D sphere formation assay targeting stem/progenitor cells. OVCAR-420 and SKOV-3 cell lines were cultured in Matrigel™ under various treatment conditions for durations of up to 7 and 10 days, respectively. Both cell lines successfully formed spheres in the untreated groups, confirming the presence of a stem/progenitor sub-population.

Administered treatment of TQ, whether alone or in combination with CDDP, resulted in a dose-dependent decrease in both the count and size of the spheres compared to control groups.

Treatment of OVCAR-420 cells with TQ at concentrations of 1 µM and 5 µM significantly reduced the number of sphere-forming units (SFUs) and diameter. A higher statistically significant reduction was observed when the concentrations combined with CDDP at 0.5 µM and 2.5 µM, significantly exceeding the effects of CDDP alone **(Figure 14 and Figure 16 a, b)**

In SKOV-3 cells, treatment with TQ at a concentration of 5 µM resulted in a significant decrease in the number of SFU and size. A further reduction was observed when this concentration of TQ was combined with CDDP, with effects greater than those seen with TQ or CDDP used alone. In addition to a sharp decrease in size and count at TQ 20 µM especially when combined with 1µM of CDDP **(Figure 15 and Figure 16 c, d)**

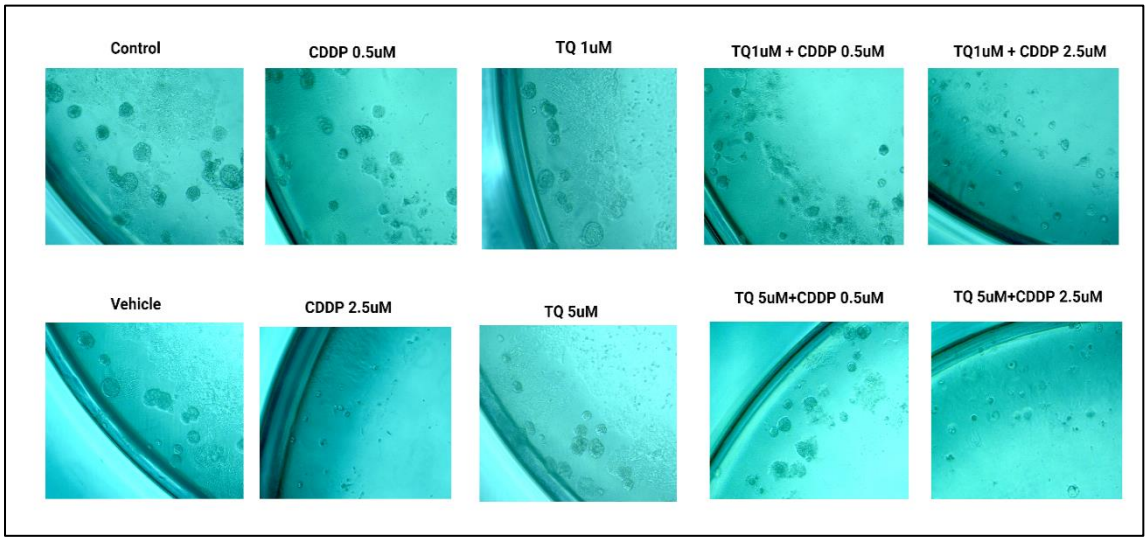

Figure 14. Effect of TQ, CDDP, and, their combinations on the sphere-forming ability and size of OVCAR-420 cells.
Representative images were taken for the spheres with and without treatment with TQ, CDDP, and both in combination. Images were visualized by Axiovert inverted light microscope and analyzed by ZEN image software. Scale bar = 100µm.

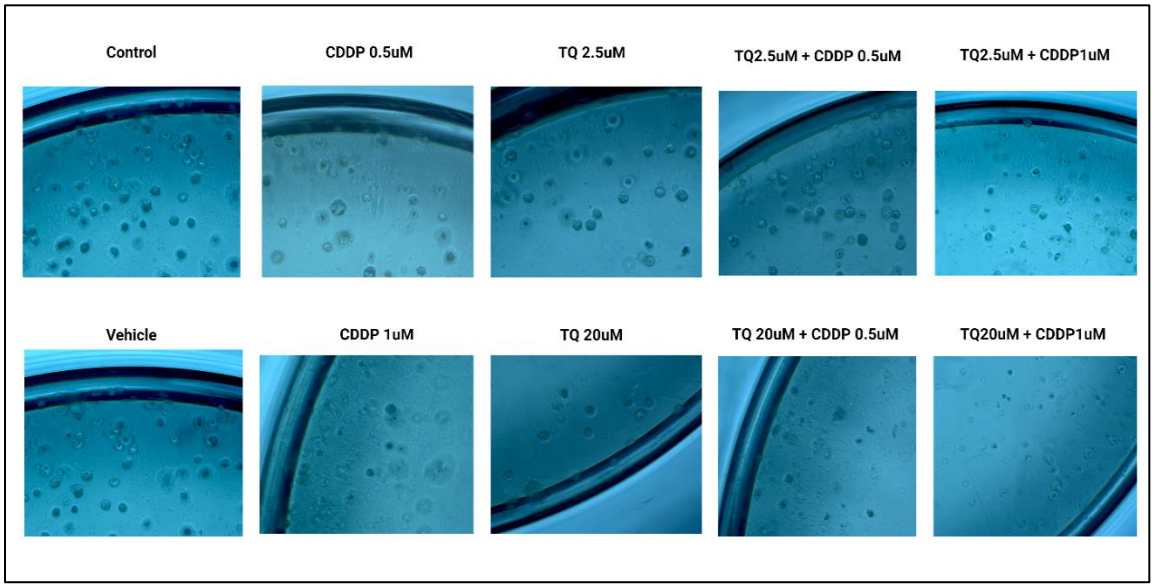

Figure 15.Effect of TQ, CDDP, and their combinations on the sphere-forming ability and size of SKOV-3 cells.
Representative images were taken for the spheres with and without treatment with TQ, CDDP, and both in combination. Images were visualized by Axiovert inverted light microscope and analyzed by ZEN image software. Scale bar = 100µm

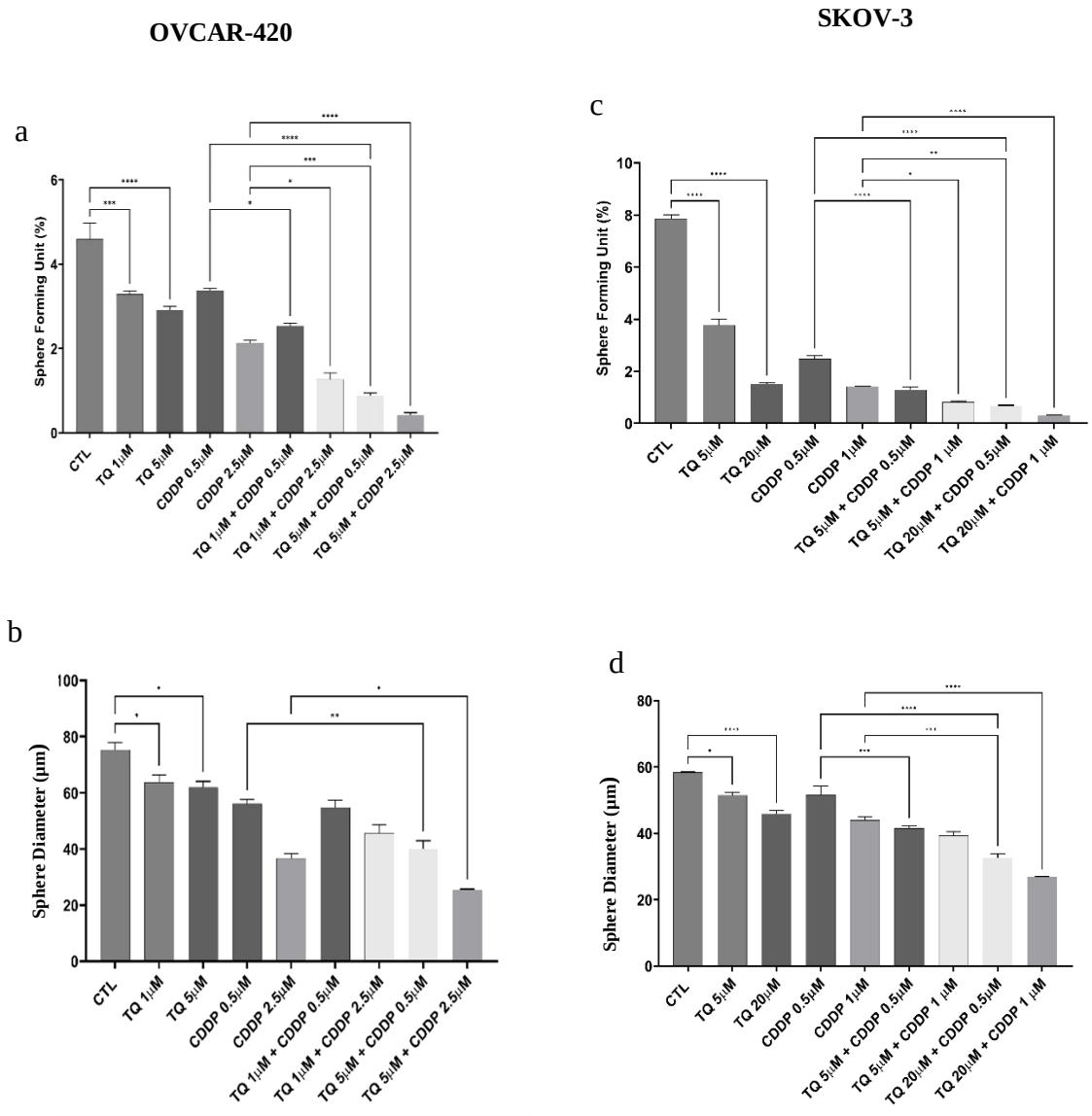

Figure 16. TQ reduces the sphere-forming ability and diameter of OVCAR-420 and SKOV-3 cultured cells alone and in combination with CDDP.
Cells were seeded in Matrigel™ and spheres were formed and counted for the OVCAR-420 and SKOV-3 cell lines at day 7 and day 10 respectively. Results are expressed as SFU which is calculated according to the formula: number of spheres counted \ Number of input cells) × 100.Using an inverted light microscope, images of the formed OVCAR-420 and SKOV-3 spheres were taken, and the images were analyzed using ZEN software. A greater inhibitory effect was observed on OVCAR-420 than SKOV-3 in TQ treatment solely or in combination. Results represent the average diameter of 60 and 120 of control fully formed spheres for OVCAR420 and SKOV-3 respectively. Values are presented as percentages concerning the size of spheres for the control. Data is reported as mean ± SEM (SEM represented by error bars; (*P<0.05 **P <0.01 ***P <0.001)

CHAPTER IV

DISCUSSION

OC remains the most lethal gynecological malignancy and often goes unnoticed until advanced stages due to its vague symptoms and lack of effective detection tests. A major obstacle in its treatment is chemoresistance, particularly to conventional therapies like cisplatin, leading to recurrent tumors and adverse side effects[80]. This presents a significant challenge to explore novel approaches that enhance the efficacy of existing treatments while minimizing the side effects of conventional therapeutics targeting OC. The drug combination has evolved into an innovative method to investigate the anticancer potential of existing compounds when combined with novel therapeutic alternatives.

Numerous studies in cancer research have demonstrated the promising effects of plant-derived agents, either used alone or in combination with other drugs. These natural agents possess important features such as cost affordability, bioavailability, and biosafety. One such phytochemical is thymoquinone, derived from black cumin, has shown promise in the treatment of various cancers. While there have been promising findings regarding the effects of TQ on OC cells, further research is needed to fully understand its impact[81]. This study aims to investigate the effects of TQ alone and in combination with CDDP on the proliferation, viability, migration, and stemness of OC cells.

To evaluate the efficacy of TQ alone and in combination with CDDP we used two OC cell lines OVCAR-420 and SKOV-3, chosen for their relevance in OC research

and representing distinct pathological and behavioral profiles. While OVCAR-420's origin is debated, its genetic profile aligns with HGSC. Whereas, SKOV-3 is consistently reported to be derived from malignant ascites of a patient with non-serous adenocarcinoma [82].

Treatment with TQ alone and in combination with CDDP significantly reduced the proliferation and viability of OVCAR-420 and SKOV-3 cells. TQ alone demonstrated in several studies a substantial suppression of survival and proliferation in the OVCAR-420 and SKOV-3 cell lines. This hypothesis aligns with prior research across various cancer types, consistently highlighting TQ's efficacy as an inhibitor of cell proliferation[83]. Interestingly in osteosarcoma cell lines, treatment with 25μM of TQ induced both apoptosis and cell-cycle arrest, a concentration same as that used in our MTT, trypan blue, and migration assays on OVCAR-420, which demonstrated a reduction in cell proliferation, viability and migration 24 hours post-treatment, mirroring our findings of significant inhibitory effects.

Despite HGSC typically being aggressive, the non-serous SKOV-3 displayed higher migratory abilities as compared with OVCAR-420. It was observed during the scratch assay that the migration rate is greater by approximately 20% in SKOV-3 post-treatment due to its advanced disease stage and genetic difference compared to OVCAR-420. Previous studies have linked this reduced migratory ability to the inhibition of NF-kB activity, a transcription factor involved in cellular proliferation and survival[84]. Inhibition of migration and invasion was also observed in human pancreatic carcinoma cell lines treated with TQ[85]. Our results align with these findings, where a more pronounced 80% and 60% suppression were detected to hinder the migratory ability of cells when 25μM and 10μM of TQ were combined with

different concentrations of CDDP for OVCAR-420 and SKOV-3, respectively, proving a synergistic effect when combining TQ with CDDP. This is consistent with the literature, where SKOV-3 has shown a higher invasive potential, with an invasion rate 23 times greater than OVCAR-420[82]. Genomic mutations, particularly in TP53, alter signaling pathways that enhance cell survival. Additionally, interactions with the extracellular matrix activate integrin and Akt signaling[86], further contributing to their aggressive behavior.

Given that the recurrence of OC is primarily due to the presence of slowly dividing dormant CSCs. This research is significant as it represents the first comprehensive analysis of TQ's potential solely and in combination with CDDP to target OC stem cells, which are known to contribute to treatment resistance and tumor recurrence in OC, and obtained promising results. TQ alone or in combination with CDDP remarkably reduced the size of the sphere and inhibited its sphere-forming ability. The concentrations of TQ required to inhibit spheroid formation were notably lower than those needed to impede cellular viability, proliferation, and migration.

In contrast to proliferation and viability assays, where SKOV-3 showed a better response to both drugs, in the 3D cell culture, it exhibited a lower response compared to OVCAR-420. This difference could be attributed to the higher cancer stem cell-like properties of SKOV-3 compared to OVCAR-420. To substantiate this hypothesis, further studies should be conducted to evaluate the expression of stem cell-like markers in these cell lines. Notably, previous studies have evaluated the inhibitory effects of TQ on various cancers, including prostate, pancreatic, colon, breast, and lung cancers. However, this study is the first to demonstrate the combined effect of TQ and CDDP on

OC cell lines SKOV-3 and OVCAR-420, with TQ enhancing the efficacy of CDDP, overcoming its resistance, and targeting cancer stem cells.

The concentrations of TQ combined with CDDP in these studies are consistent and fall within the range utilized in our MTT, trypan, and scratch assays, reinforcing the relevance of our findings in enhancing the therapeutic efficacy against OC. Furthermore, TQ enhanced cisplatin-mediated in OC cells in vitro and a mouse syngeneic model, with effects associated with increased DNA damage. However, some results caution that TQ treatment alone may have a deleterious effect on immunocompetent hosts through stimulation of ascites[77]. Since TQ is a potential candidate for future clinical trials in OC patients, this finding has considerable relevance to the clinic.

Future perspective, TQ, like other flavonoids, faces challenges of poor solubility, stability, and thermal/light sensitivity, reducing its therapeutic efficacy. To overcome these issues, TQ has been incorporated into nanoparticles such as nanoliposomes, nano micelles, and silica nanoparticles. These nano-delivery systems enhance TQ's solubility, stability, and efficacy against tumor cells by protecting it from degradation and improving its bioavailability. Nanocarriers offer improved drug-targeted delivery, reduced toxicity, and enhanced pharmacokinetic properties. Intravenous administration of TQ-loaded nanoparticles further enhances its carrying efficiency, suggesting that TQ can be effectively delivered to target cancer cells[87, 88].

In conclusion, our study contributes to the growing body of literature on the anti-cancer properties of TQ, providing evidence for its potential as an adjunctive therapy for OC. Our findings demonstrate that TQ, both alone and in combination with CDDP, exhibits anti-proliferative and anti-migratory effects on OC cells. Additionally,

TQ effectively inhibits the sphere-forming ability of the cells and reduces the size of the formed spheres, indicating its promise in targeting the stem cell population of ovarian tumors. This research, alongside previous studies, underscores the potential of this natural, and affordable plant extract as an anti-cancer agent. Further preclinical and clinical studies are warranted to validate the efficacy and safety of TQ-based combination therapies in OC patients. Ultimately, the combination of TQ with CDDP highlights the importance of exploring alternative treatment strategies to improve therapeutic outcomes for OC patients.

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