Original Article

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Anti-Cancer Effect of Taxol Extracted from Schizophyllum Radiatum Against SKOV-3 Cell Line

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Abstract

The extraction process involved obtaining Taxol from the fruiting bodies of Schizophyllum Radiatum, a fungal species known for its taxane-producing capabilities. The study employed various analytical techniques, including chromatography and spectroscopy, to confirm the purity and identify the chemical profile of the extracted Taxol. In vitro experiments were conducted to evaluate the cytotoxicity of Taxol against SKOV-3 cells, employing standard assays to assess cell viability, apoptosis induction, and cell cycle alterations. The results revealed a dose-dependent reduction in cell viability, indicating the potential of Taxol from Schizophyllum Radiatum as an anti-cancer agent against ovarian cancer cells. Furthermore, mechanistic studies were undertaken to elucidate the underlying pathways involved in the anti-cancer activity. The study investigated the impact of Taxol on key molecular targets associated with cell survival and apoptosis, providing insights into its potential as a targeted therapeutic agent.

Keywords:

Human health care, Death rates, Pneumonia, tuberculosis, influenza

Introduction

The twentieth century has been a significant turning point in the history of human health care. The recent human health care has shown improvement in the human longevity through a decrease in death rates from contagious diseases, such as Pneumonia, tuberculosis and influenza. However, this heath progress has its limitations in which there was a high increase of cancer diseases [1, 2].

Cancer is a dangerous disease which is formed by changes in normal cells which leads to the cells growth in abnormal shape. As a result, countless cells are produced with no control or order, which leads to forming tissues mass named Tumor. This process is conducted in various and gradual stages and in a series of vital changes that lead to increase in the accumulation of abnormal traits, which eventually the cell gains the carcinogenic features. These features are found in the malignant tumors, and the cells become more abnormal than the sound and healthy cells. They also acquire the ability to be divided in an unusual speed leading to forming the cancerous cell [3]. People annually die due to cancer, which is considered the second most lethal disease in the world. It is expected that cancer would become the first most lethal disease in the upcoming decades with a high rate of deaths reaching to 1000 human death per hour [4].

Over the years, there have been discoveries and experiments of many biological and industrial tools so as not spread the cancer or treat it. However, in the current time, the focus is shifted to the natural compounds, such as the Paclitaxel, an effective anti-cancer drug. This drug is commercially known as (Taxol), which is a tricyclic diterpenoid compound with on secondary metabolite. This drug is naturally produced from the barks of Yew trees, its molecular formula is (C47H51NO14) [5].

Due to its unique anti- cancer mechanism, Taxol is considered one of the most natural anti- cancer drugs which is the most successful and the most commonly used. On the contrary from the other anti- cancer drugs that is associated with Tubulin.

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These drugs prevent the accumulation of tubulin in precision tubes. The Paclitaxel reinforces the accumulation of tubulin in precision tubes, prevents disassembling of precision tubes, prevents the progress of cell cycle, prevents the division, and inhibit the cancerous cell [6, 7]. The structure of PTX was published for the first time in 1971, and the first clinical experiments started in 1984, and the second in 1985 [8]. After a series of clinical experiments, the food and drug administration (FDA) proved that it uses Paclitaxel for the treatment of the advanced ovarian cancer in 1992. From then, the use of Paclitaxel has become widely spread around the world in treating breast cancer, colorectal cancer, squamous cell carcinoma of the bladder. Moreover, Paclitaxel is used in treating other cancer and diseases, such as lung cancer and HIV disease [9, 10]. This study amid to testing the activity of taxol extracted from the local isolate Schizophyllum radiatum in inhibiting the growth of ovarian cancer cells.

Material and Methods

Maintenance of cell cultures

RPMI-1640 with 10% Fetal bovine serum, 100 units/mL penicillin, and 100 g/mL streptomycin was used to cultivate the SKOV-3 and MCF-10 cell lines. Trypsin-EDTA reseeding was used at 80% confluence twice weekly, and cells were kept in a 37 °C incubator [11].

Cytotoxicity Assays

Using 96-well plates, the cytotoxic impact of Taxol was measured using the MTT test [12]. One multiplicity of 104cells per well was used to seed the cell lines. After a period of 24 hours. After a monolayer of SKOV-3 and MCF-10 cells had formed, Taxol was added. After 24 and 48 hours of treatment, cell viability was determined by washing the cells with PBS, replacing the medium with 28 L of a 2 mg/mL solution of MTT, and incubating the mixture at 37 °C for 2.5 hours. After the MTT solution was discarded, the crystals in the wells were solubilized by adding 130 L of DMSO (Dimethyl Sulphoxide) and incubating the plates at 37 °C for 15 minutes while shaking [13]. The absorbance was measured using a microplate reader set to 492 nm, and the experiment was run three times for accuracy. The following formula was used to determine the percentage of cytotoxicity (rate of cell growth inhibition). [14, 15]:-

Inhibition rate = A- B/A*100

where A is the optical density of control, and B is the optical density of the samples [16]. Cells were seeded onto 24-well micro-titration plates at a density of 1105 cells mL1 and cultured for 24 h at 37 °C before being seen under an inverted microscope to determine their shape. After then, cells were treated with Taxol for a whole day. Crystal violet stain was used after the exposure period, and the plates were incubated at 37 °C for 10-15 minutes [17]. Tap water was used to gently scrub off the stain until all traces of the color had been erased. Images of the cells were taken using a digital camera fitted to an inverted microscope at a magnification of 100x [18].

Cytotoxicity = A-B/A *100

Where A and B are the optical density of control and the optical density of test.

Acridine Orange–Ethidium Bromide Staining

AO/EtBr (Sigma-Aldrich, USA) staining was used to determine how many SKOV-3 cells were killed by a given drug. SKOV-3 cells were seeded in 24-well plates, and then treated with Taxol at an IC50 concentration of 10, followed by 20 further hours of incubation. Phosphate-buffered saline was used to wash the cells twice. After allowing the cells to adhere for 2 minutes, we added 10 L of each fluorescent dye to the wells. At last, a fluorescent microscope was used to study the cells [19].

Identification of DNA Damage

Genotoxicity Assay

The alkaline comet assay was used to examine the possible genotoxicity of taxol towards DNA. At 37 degrees Celsius, the IC50-treated SKOV-3 cells were harvested and suspended in a 1:10 (v/v) solution of molten LMAgarose (Trevigen, USA). After preparation, the comet slide was loaded with the samples.. A lysis solution (4 degrees Celsius, 1 hour) was applied to the slides, and then an alkaline unwinding solution (pH >13.0, room temperature, 20 minutes) was added. The slides were prepared as usual and then put in a NaOH-EDTA solution-filled electrophoresis slide tray. Electrophoresis was carried out in an appropriate setting (21 V, 30 min). Cells in the dried agarose circle were stained using SYBR Green solution (room temperature, 30 min). After the samples were stained, they were seen using a confocal laser scanning microscope. Comet Assay was used to assess the results data.

ROS determination

Flow cytometry (FACSCalibur flow cytometer) wFlow cytometry (using a FACSCalibur flow cytometer) was utilized to assess cellular reactive oxygen species (ROS) production. SKOV-3 cells were seeded in six-well plates and incubated foras used to measure the generation of ROS in cells. SKOV-3 cells were plated on six-well plates at density 1*106. Cells then incubated overnight, and treated with Taxol at IC50 concentration for 8 hr. Then, ROS probe (DCFH-DA) (15 μM) added to new media, and the mixture was incubated at 37 degrees for 30 minutes. Fluorescence intensity was measured in collected cells using a flow cytometer (BD Biosciences).

Mitochondrial Membrane Potential Assay

Rhodamine (Rh123) fluorescence dye was utilized to examine Taxol's impact on mitochondrial activity in SKOV-3 cells. The mitochondrial membrane potential

was analyzed using this dye both before and after Taxol therapy. To sum up, cells were treated with Taxol and stained with 5 M Rh123 dye for 2 hours at 37°C 24 hours after they were seeded in 96-well plates. Next, 0.2 mL of 5% trypsin-EDTA was used to dissociate the cells, and then they were centrifuged at 300 rpm for 5 minutes to remove debris. Flow cytometry analysis was performed after the cells were resuspended in FACS buffer and histograms were produced.

Measurement of Caspase-8 and p53 Levels

Flow Cytometry Assay

We used a fluorescein caspase-3 and p53 staining kit to check for their activation levels (Thermo Fisher Scientific, USA). The SKOV-3 cancer cells were grown in 5 mL of media at 37°C for 24 hours (at a concentration of 4 104 cells.mL1). After 24 hours incubation, cells were treated with Taxol at the IC50 concentration.

The treated cells were collected at the end of the incubation time and washed twice with 1 ice-cold PBS. Collecting the pellets, we next used fresh growth media to bring the cell density back up to the original setting of 1 106 cells.mL1. After the cells were ready, 1 mL of FITC-IETD-FMK was added to the dish and incubated at 37°C for 60 minutes to detect caspase-8 and FITC-anti-p53. Wash buffer was used twice after the incubation time to remove any remaining cells (0.5 mL). After the cells were labeled, they were put into flow cytometry tubes. The BD Accuri C6 program was used for the calculations and analysis.

Statistical analysis

An unpaired t-test in GraphPad Prism 6 was used for statistical analysis of the collected data (Bahjat et al., 2021; Al-Omar et al., 2021). Measurements were done in triplicate, and the data were reported as the mean SD.

Results

The cytotoxic effect of Taxol against SKOV-3 cells was studied. The anti proliferative activity of the Taxol was tested by studying their ability to inhibit the cells proliferation. The results demonstrated that the Taxol have highly cytotoxic effect against SKOV-3 cell line as shown in Figures (1-3). The results demonstrated that the Taxol made clear morphological changes in SKOV-3 cells lines after treated as in. While, the results shows have no cytotoxic effect against normal breast cell line MCF-10 as shown in figures(4-6).

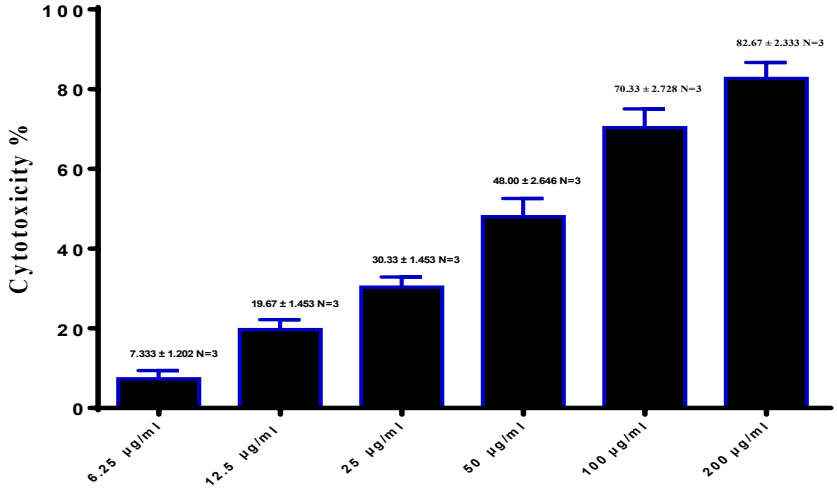

Figure 1. Cytotoxicity effect of Taxol in SKOV-3 cells. IC50=24.73 µg/ml.

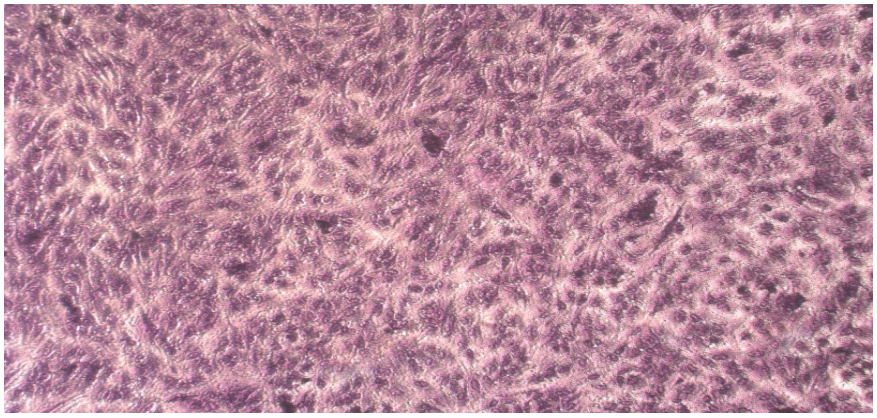

Figure 2. Control untreated SKOV-3 cells. Magnification power 10x.

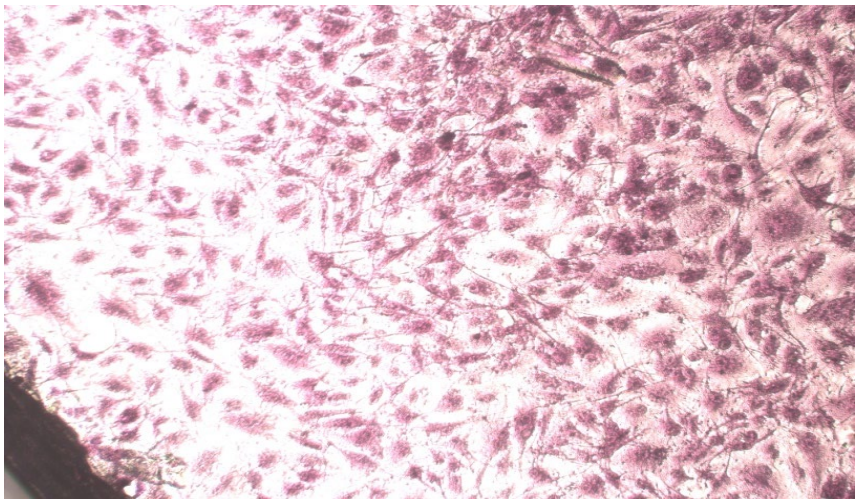

Figure 3. Morphological changes in SKOV-3 cells after treated with Taxol. Magnification power 10x.

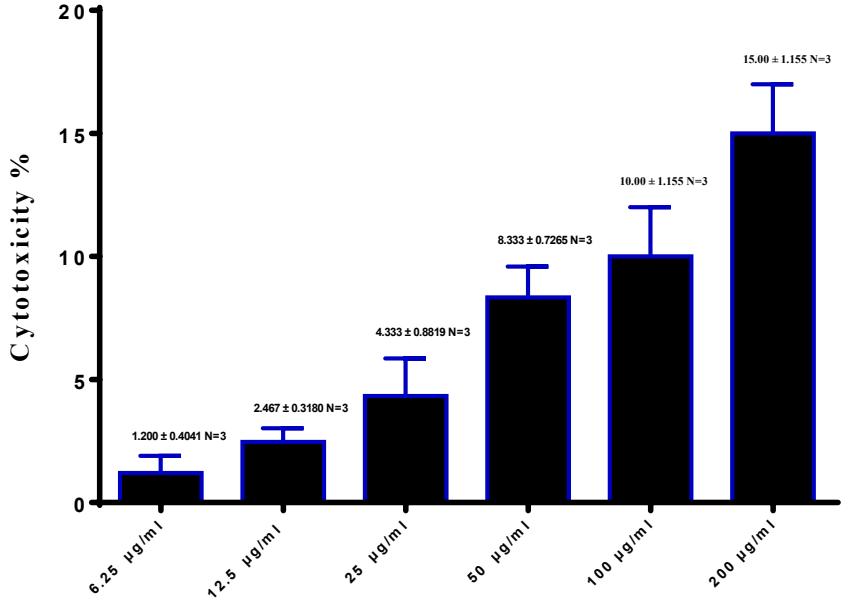

Figure 4. Cytotoxicity effect of Taxol in MCF-10 cells. IC50=377.06 µg/ml.

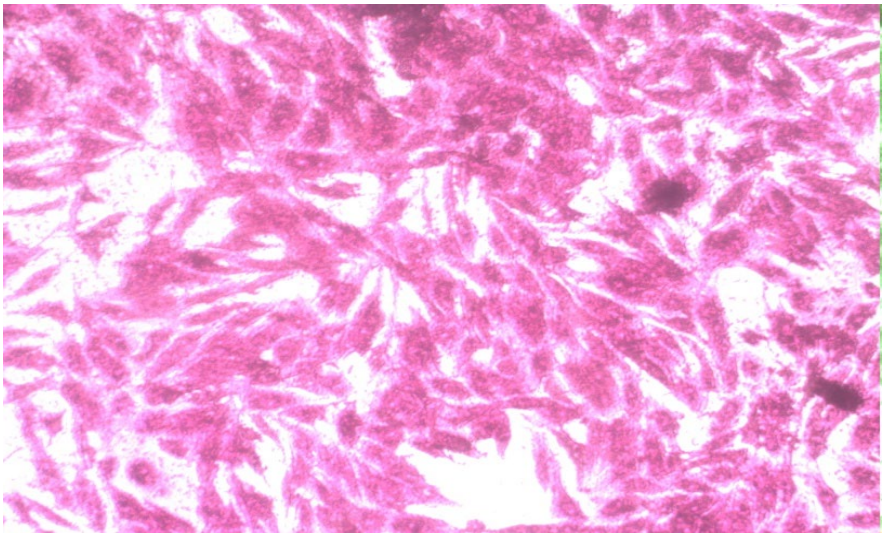

Figure 5. Control untreated MCF-10 cells. Magnification power 10x.

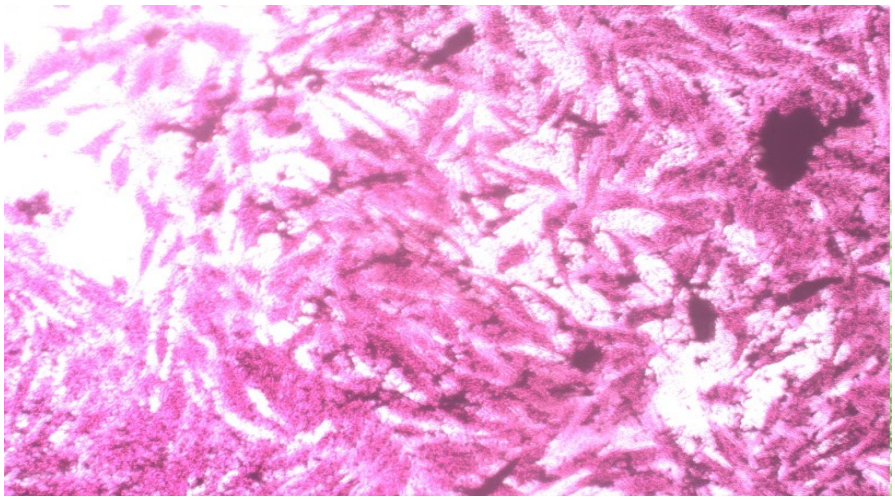

Figure 6. Morphological changes in MCF-10 cells after treated with Taxol. Magnification power 10x.

Taxol induce SKOV-3 cell death

This work adds to the growing body of evidence that Taxol may trigger programmed cell death (apoptosis) in ovarian cancer cells. Gene expression levels are altered due to the triggering of a programmed cell death mechanism [20], which is responsible for the suppression of cell proliferation. Additionally, nuclear morphology was assessed by dual labeling with acridine orange and ethidium bromide in treated cells.

DNA damage was used as a metric for assessing apoptotic cells. The effectiveness of Taxol was also studied here. The various apoptotic characteristics of the nuclear changes were analyzed using AO-EB staining. After being stained with AO-EtBr, non-apoptotic cells showed green while apoptotic cells appeared orange or red. As may be seen in Figure 7. New studies show that Taxol may induce cell death (apoptosis) in ovarian cancer cells. The inhibition of cell growth results from changes in gene expression levels caused by the activation of a programmed cell death mechanism. The nucleus morphology of treated cells was also examined using acridine orange-ethidium bromide dual labeling. To quantify apoptotic cells, DNA damage was analyzed [21]. In this study, we looked at how well Taxol works. AO-EB labeling was used to examine the various nuclear alterations associated with apoptosis. Cells that were not apoptotic showed up green after AO-EtBr staining, whereas apoptotic cells showed up orange or red. This is seen in Figure 7.

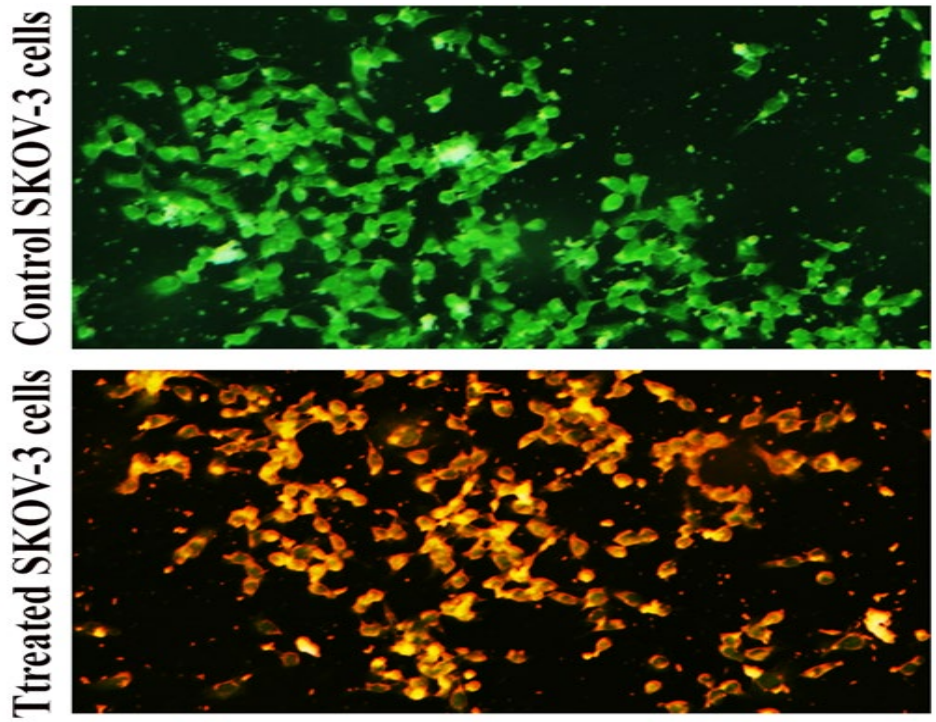

Figure 7: Apoptosis markers in SKOV-3 cells following treatment with Taxol. SKOV-3 cells treated as indicated. Untreated cells are shown normal structure of cell without significant apoptosis or necrosis, after treated, red color indicated apoptosis.

Identification of DNA damage

The DNA damage Figure 8 displays the DNA damage in SKOV-3 cells as a function of the degree of destruction and the length of their tails. The comet test findings in the present research showed that cells treated with Taxol had a lengthy tail length. These findings suggest that nucleic acid damage was induced.

The findings revealed that even untreated cells had a halo-like structure specifically surrounding their nuclei. The duration of nuclear emigration was also prolonged in the treated SKOV-3 cells ased on the degree of damage and the length of the SKOV-3 cells' tails are shown in Figure 8.

The comet test findings in the present research showed that cells treated with Taxol had a lengthy tail length. These findings suggest that nucleic acid damage was induced. A halo was found to be confined around the cell nucleus of untreated cells. Additionally, the length of nucleic acid migration was enhanced in the treated SKOV-3 cells [22].

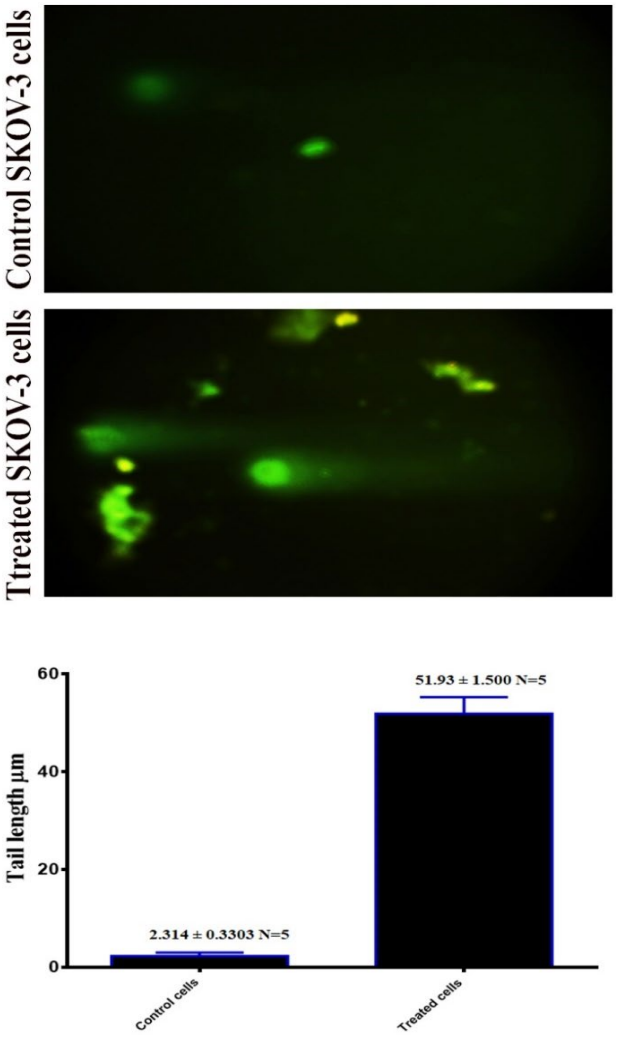

Figure 8. DNA damage caused by Taxol in SKOV-3 cells, in Comet assay was performed to quantify DNA damage (long tail) in SKOV-3 cells after treatment with the specified agents, as shown in the top panel. The size of DNA's tail was shown in the lower panel. Mean Standard Deviation is used to display the data

Taxol induce ROS generation in SKOV-3 cells

In aerobic organisms, the production of ROS is essential for maintaining cellular redox equilibrium and for the transmission of signals. In addition to aiding tumor cells, reactive oxygen species (ROS) are also useful therapeutic weapons in the fight against cancer (47). In this work, intracellular ROS generation in the SKOV-3 cell line was measured using a DCFH-DA probe. After 24 hours of therapy [23], Taxol dramatically enhanced ROS production as measured by flow cytometry.as indicated in Figure 9.

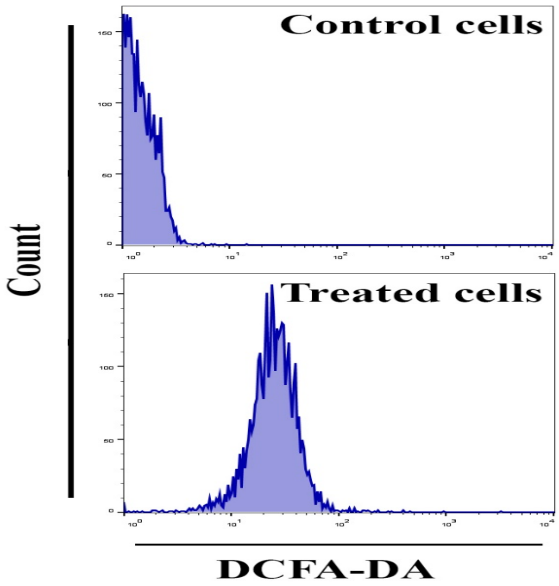

Figure 9. Taxol induces ROS generation in SKOV-3 cells.

Taxol induces mitochondrial dysfunction of SKOV-3 cells

Since mitochondrial failure is a prominent feature of apoptotic cells, matrix metalloproteinase (MMPs) are valuable indicators and possible therapeutic targets of malignancies. Apoptotic events may be triggered by a variety of triggers that target mitochondria. Caspase-3 is activated through the caspase-9 pathway when the mitochondrial membrane potential (m) is reduced and the cytochrome c protein is released into the cytoplasm.

In the current investigation [24], we used a commercially available flow cytometry test to identify MMPs. Dropping the mitochondrial membrane potential is a major indicator of apoptotic cell death. After labeling SKOV-3 cells with the Rh123 probe, mitochondrial membrane potential was determined by flow cytometry. We looked at how much apoptosis occurred in SKOV-3 cells when they were exposed to Taxol.

As can be seen in Figure 10, the use of these drugs led to a significant uptick in apoptosis. Compared to control untreated SKOV-3 cells, SKOV-3 cells that were treated with Taxol for 24 hours showed a significant drop in Rh123 staining, indicating the lowering of the mitochondrial membrane potential.

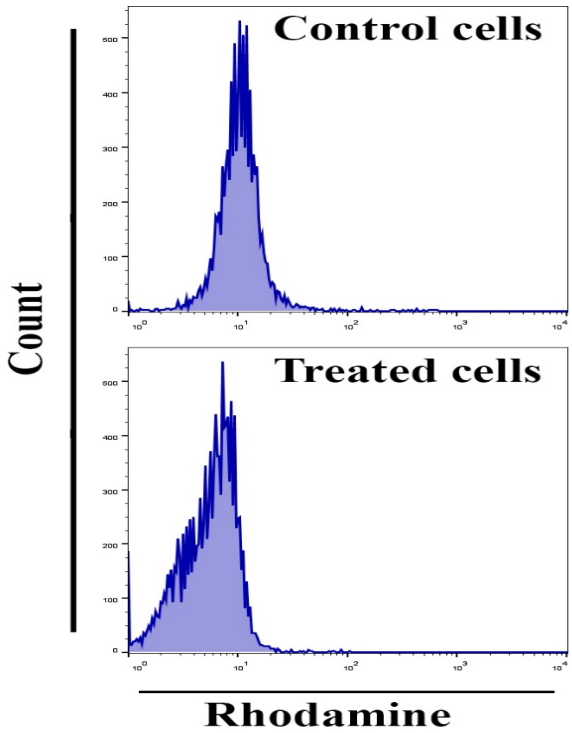

Figure 10. Dysfunction of MMP in SKOV-3 cells after treated with Taxol

Taxol induces secretion of Caspase-3 and p53

Taxol's capacity to trigger an apoptotic response based on mitochondrial dysfunction was shown in a prior test. The extrinsic route (death receptor-mediated) and the intrinsic pathway are two of the most important pathways required for apoptosis induction (regulated at the level of mitochondria) [25].

The surface molecule signals (Fas/FasL) trigger the recruitment of the Fas-associated death domain through the extrinsic route (FADD). Inducing procaspase-8 is a function of the FADD-Fas complex. The death-inducing signaling complex (DISC) is a highly dynamic and essential complex that may activate and trigger caspase-3, ultimately leading to the induction of apoptosis. Flow cytometry was used to examine caspase-3 and p53 activity.

Figures 10 and 11 show the findings of a flow cytometry analysis showing that cells treated with Taxol displayed a dramatic shift to the right in the levels of caspase-3 and p53, markers of the apoptosome. During apoptotic processes, apoptosomes are present and play a role in the orderly breakdown of the cell. We evaluated p53 and caspase-3 expression in the current investigation. Both P53 and caspase-3 were found to be significantly elevated after Taxol treatment of SKOV-3 cells compared to the control.

As a result, caspase-3 was activated in apoptotic processes through both the extrinsic and intrinsic apoptotic routes. The fragmentation of nucleic acids is an essential step in the apoptotic process, and here is where caspase-3 shines.

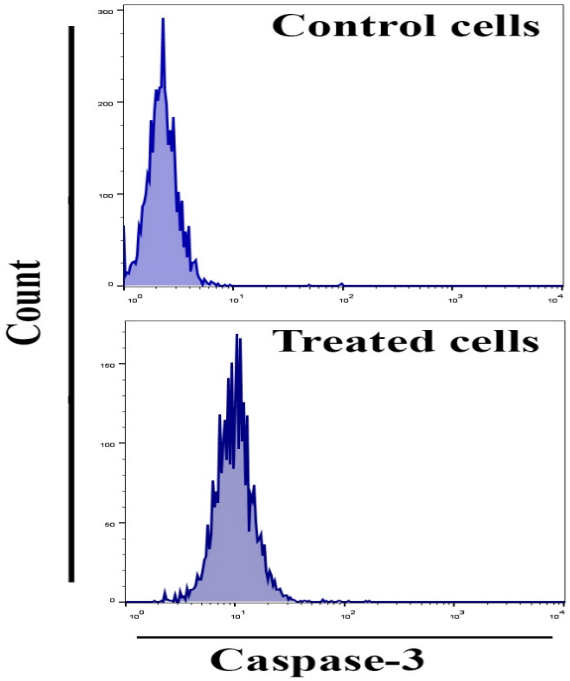

Figure 11. Taxol induces of caspase-8 secretion in SKOV-3 cells.

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