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Original Article

δ-Cadinene inhibits the growth of ovarian cancer cells via caspase-dependent apoptosis and cell cycle arrest

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Abstract: Ovarian cancer is one of the most common causes of mortality among all cancers in females and is the primary cause of mortality from gynecological malignancies. The objective of the current research work was to evaluate a naturally occurring sesquiterpene-δ-Cadinene for its antiproliferative and apoptotic effects on human ovary cancer (OVCAR-3) cells. We also demonstrated the effect of δ-Cadinene on cell cycle phase distribution, intracellular damage and caspase activation. Sulforhodamine B (SRB) assay was used to evaluate the antiproliferative effect of δ-cadinene on OVCAR-3 cells. Cellular morphology after δ-cadinene treatment was demonstrated by inverted phase contrast microscopy, fluorescence microscopy and transmission electron microscopy. Flow cytometry was used to analyze the effect of δ-cadinene on cell cycle phase distribution and apoptosis using propidium iodide and Annexin V-fluorescein isothiocyanate (FITC)/PI kit. The results revealed that δ-cadinene induced dose-dependent as well as time-dependent growth inhibitory effects on OVACR-3 cell line. δ-cadinene also induced cell shrinkage, chromatin condensation and nuclear membrane rupture which are characteristic of apoptosis. Treatment with different doses of δ-cadinene also led to cell cycle arrest in sub-G1 phase which showed dose-dependence. Western blotting assay revealed that δ-cadinene led to activation of caspases in OVCAR-3 cancer cells. PARP cleavage was noticed at 50 μM dose of δ-cadinene with the advent of the cleaved 85-kDa fragment after exposure to δ-cadinene. At 100 μM, only the cleaved form of PARP was detectable. Pro-caspase-8 expression remained unaltered until 10 μM dose of δ-cadinene. However, at 50 and 100 μM dose, pro-caspase-8 expression was no longer detectable. There was a significant increase in the caspase-9 expression levels after 50 and 100 μM δ-cadinene treatments.

Keywords: Ovary cancer, apoptosis, δ-cadinene, Sulforhodamine B, caspases

Introduction

Ovarian cancer results from abnormal, uncontrolled cell division of ovary cells and has the ability to invade or spread to other parts of the body. Ovarian cancer is the sixth most common cancer in women. Despite having lower occurrence, this cancer has high mortality rates. The incidence rates of ovarian cancer vary greatly according to geographical location and are more common in United States of America than Asia, Africa, Latin America and Japan [1]. Interestingly the incidence rate of this cancer is more in developed countries as compared to the developing countries like Africa and India. Most of the ovarian cancer patients develop metastasis due to high difficulty in detecting this cancer at an early stage. In spite of advanced chemotherapy and surgical interventions, the prognosis of the disease yet remains poor and discouraging. The survival rate of patients with advanced ovarian cancer is only 10-20% [2]. A combination of cisplatin and paclitaxel chemotherapeutic drugs is given to the ovarian cancer patient nowadays [2]. However, the ovarian cancer in most of the cases after this first line chemotherapy recur and in these cases other more potent chemotherapeutic drugs are used like vinca alkaloids, anthracyclines or topoisomerase inhibitors like irinotecan which causes DNA strand breakage [3-5]. However, recently gemcitabine which is a pyrimidine analogue, has been embellished with lot of success in treating a variety of cancers with minimum serious side effects [6].

Cancer chemotherapy in ovarian cancer has two main problems, one is the serious side-effects of the drugs and the other is the development of resistance by the ovarian cancer

cells towards these anticancer agents which make this therapy ineffective. Thus it is imperative to gain a better understanding of the molecular pathways involved in ovarian cancer development and discover novel natural molecules which can target this cancer. The objective of the current research work was to demonstrate the molecular mechanism of action of a plant terpenoid δ-cadinene in inhibiting the growth of human ovarian cancer cells. We studied the effects of δ-cadinene on apoptosis, cellular morphology, cell cycle arrest and caspase activation in ovary cancer cells (OVCAR-3).

Materials and methods

Chemicals, antibodies, and other reagents

δ-Cadinene (purity greater than 98%) was obtained from Sigma (St. Louis, MO). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin/streptomycin solution, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and Hoechst 33342 were obtained from Mediatech, Inc. (Herndon, VA, USA). Dimethyl sulfoxide (DMSO) was purchased from Merck Co. (Darmstadt, Germany). Annexin V-FITC-Propidium Iodide Apoptosis Detection Kit was purchased from (Beyotime Institute of Biotechnology, Shanghai, China). All other chemicals and solvents used were of the highest purity grade. The cell-permeable pancaspase inhibitor, the caspase-3 specific inhibitor, the caspase-9 specific inhibitor, the caspase-8 specific inhibitor, and the caspase-2 specific inhibitor were purchased from (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). The rabbit polyclonal antibodies (Abs) against caspase-3, caspase-8, the mouse monoclonal Abs (mAbs) against poly (ADP-ribose) polymerase (PARP), and the rabbit mAb against the active form ofcaspase-3 were purchased from PharMingen (Cell Signaling Technology, Inc.).

Cell line and culture conditions

The human ovarian cancer cell lines OVCAR-3 was procured from Shanghai Institute of Cell Resource Center of Life Science (Shanghai, China). Cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 100 U/mL penicillin and 100 μg/mL streptomycin. Cells were cultured in CO_2 incubator (New Brunswick, Galaxy 170R, Eppendroff) with an internal atmosphere of 95% air and 5% CO_2 gas and the cell lines were maintained at 37°C. The media was stored at low temperature (2-8°C) and the medium for cryopreservation contained 20% PBS and 10% DMSO in growth medium.

Sulforhodamine B (SRB) cell viability assay

The antiproliferative effect of δ-cadinene on cell proliferation was evaluated using the sulforhodamine B (SRB) assay [7]. Briefly, human ovarian cancer cell lines OVCAR-3cells were seeded in 96-well plates (5000 cells/well) overnight followed by treatment with desired concentrations (0, 10, 20, 50, 75 and 100 μM) of δ-cadinene. After 48 h cells were fixed with 200 μl of 0.1% TCA, washed with tap water and stained with 100 μl of 0.4% (w/v) SRB dissolved in 1% acetic acid. Unbound dye was removed by washing three times with 0.5% acetic acid before air drying. Bound SRB was solubilized with 150 μl of 10 mM Tris base (pH 10.5) and the absorbance read at 570 nm. Each concentration was tested in repetitions of 5 and each experiment was repeated three times. Data denote mean ± SEM from two independent experiments.

Morphological study using inverted phase contrast microscopy

Human ovarian cancer cell lines OVCAR-3 cells were seeded in 6-well plates at 2×10^5 cells per well in 5 mL of complete growth medium, incubated for 24 hours and treated with δ-cadinene at various concentrations (0, 10, 50 and 100 μM). Control cells treated with 0.11% DMSO alone were also included. The morphological changes were observed and the images were captured under an inverted light microscope (Olympus, PA, USA) after 48 hours. The same spot of cells was marked and captured.

Morphological study of apoptosis using fluorescence microscopy

Human ovarian cancer cell lines OVCAR-3 cells were seeded in 6-cm dish and then treated with or without δ-cadinene at various doses (0, 10, 50 and 100 μM). After 48 h incubation, the harvested cells were washed with PBS and fixed with 1% glutaraldehyde for 30 min. After this the cells were stained with Hoechst 33258 for 15 min. The cells were yet again washed with phosphate-buffered saline (PBS) and then visualized by a UV fluorescence microscope (Olympus, Olympus Optical Co., LTD, Tokyo,

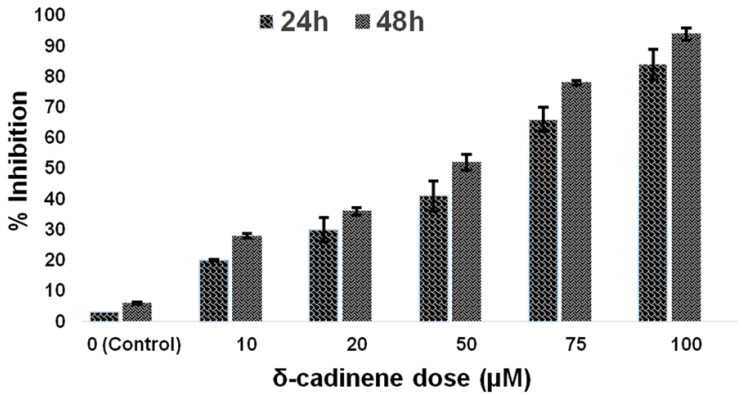

Figure 1. Antiproliferative activity of δ-cadinene against human ovary cancer cells (OVCAR-3) at different doses and time intervals.

Japan) using UV filter at 200 × magnification to detect morphological evidence of apoptosis.

Morphological study of apoptosis by fluorescence microscopy using acridine orange/ethidium bromide

OVCAR-3 cells 1 × 10^6/ml were taken in a Petri dish and treated with different concentrations of δ-cadinene (0, 10, 50 and 100 μM) for 48 h. Cells were washed with PBS and stained with a combination of acridine orange (50 μg/ml): ethidium bromide (50 μg/ml) 1:1 ratio for 15 min and 20 μl of the cell suspension was taken on a slide and images were scanned [8] using a fluorescence microscope (Olympus, Olympus Optical Co., LTD, Tokyo, Japan).

Annexin V-FITC/PI assay for cell apoptosis examination

Apoptotic cells were quantified using an Annexin V-fluorescein isothiocyanate (FITC)/PI kit (BD Biosciences, San Jose, CA, USA) and detected using flow cytometry using a FACS Calibur flow cytometer (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and analyzed using Modfit and CellQuest™ software. OVCAR-3 cells were plated at a density of 2 × 10^6 cells/well into 12-well plates and incubated overnight. Apoptosis was induced by treating cells with δ-cadinene at various concentrations (0, 10, 50 and 100 μM). Cells grown in media containing an equivalent amount of 0.11% DMSO without any drug served as control. Cells were then collected and resuspended in binding buffer. Cells were incubated with Annexin V-fluorescein isothiocyanate and PI for 20 min in the dark, before flow cytometric analysis. Annexin V-positive cells were considered to be in the early stage of apoptosis, whereas Annexin V and PI-positive cells were considered to be in the late stage of apoptosis.

Transmission electron microscopy (TEM) of ultrastructural changes in OVCAR-3 cells

TEM was involved to study ultrastructural alterations in the OVCAR-3 cells after 12 h δ-cadinene treatment. Briefly, the OVCAR-3 cells were treated with different doses of δ-cadinene (0, 10, 50 and 100 μM). After 12 h, the cells were harvested and fixed in 2% glutaraldehyde at 4°C overnight. After removal of theprimary fixative, cells were washed three times with3-(N-morpholino) propansulfonic acid (MOPS) buffer, post fixed in 1% osmium tetroxide (OsO4), dehydrated in graded alcohol and embedded in epoxy resin Epon 812 (Electron Microscopy Sciences, Fort Washington, PA, USA). Ultra-thin sections were double-stained with lead citrate/uranyl acetate before being examined by an electron microscope (H-600; Hitachi, Japan).

Determination of the effect of δ-cadinene on cell cycle phase distribution

Briefly, OVCAR-3 cells (1 × 10^5 cells/mL) were seeded into each well of 6-well plates and incubated for 24 h for cell attachment and recovery. The cells were treated with different concentrations (0, 10, 50 and 100 μM) of δ-cadinene. After incubation for 24 h, the cells were harvested and fixed with ice-cold 70% ethanol (2 mL) at -20°C for 1 h. Prior to analysis, the cells were washed with cold PBS and re-suspended in 450 μL of PBS, 25 μL PI and 25 μL RNase A. The DNA contents were recorded by a FACS Calibur flow cytometer (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) equipped with Cell Quest software.

Western blot analysis

Western blot assay was done as previously reported [9] with slight modifications. Bradford assay (Bio-Rad) was used to determine the protein content. After electrophoresing a total of 20-40 Ag of protein on 15% SDS-PAGE gels, it

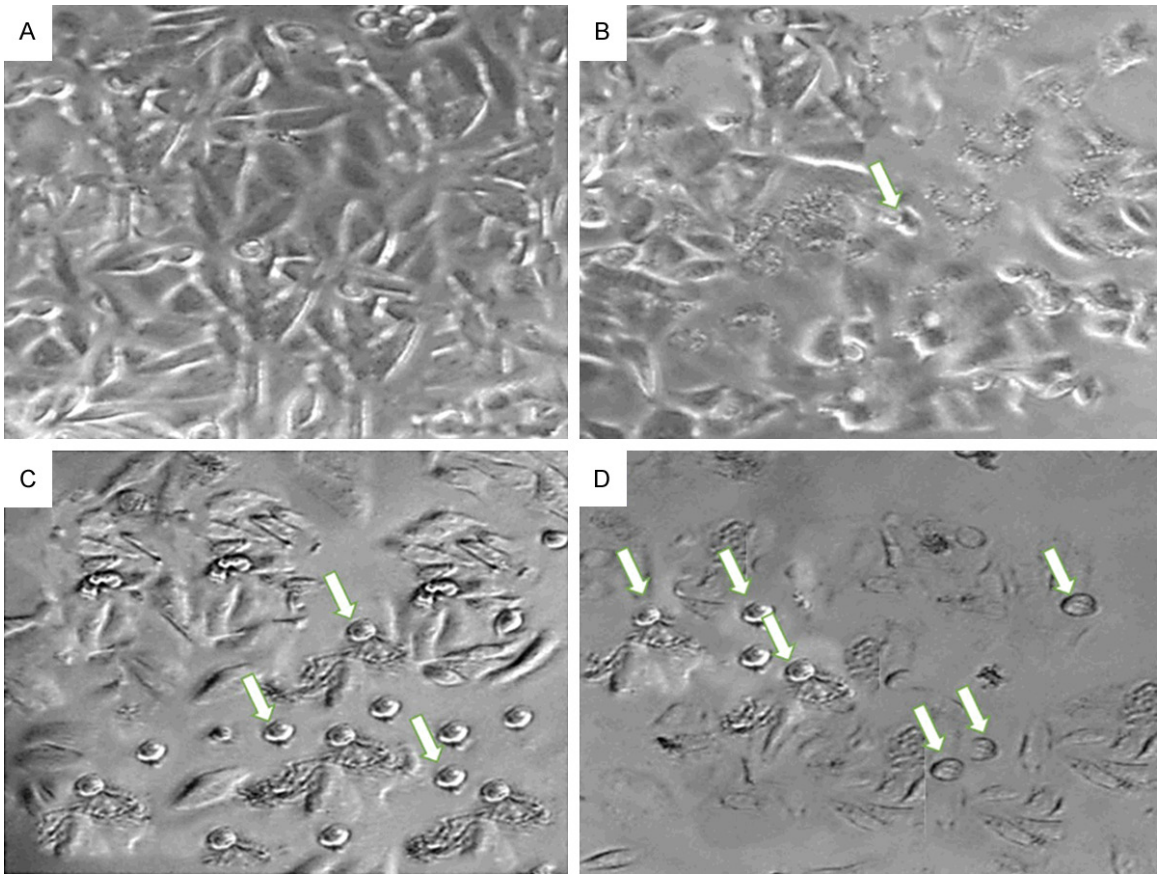

Figure 2. δ-cadinene induced morphological changes in human ovary cancer cells (OVCAR-3) as identified by phase contrast microscopy (magnification 400 ×). Cellular shrinkage and blebbing were observed in δ-cadinene-treated cells (arrows). A. Represents control (untreated cells), B-D. Represent effect of 10, 50 and 100 μM of δ-cadinene on cell morphology of OVCAR-3 cells.

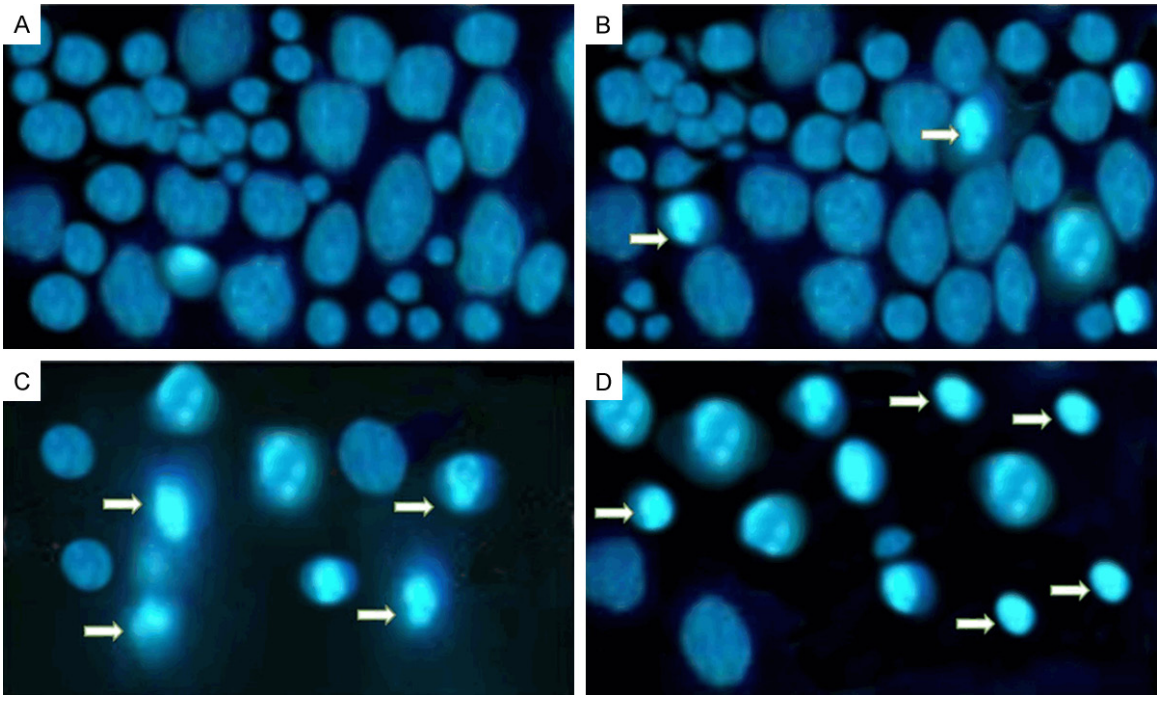

Figure 3. Morphological examination of δ-cadinene-induced apoptosis with Hoechst 33258 staining at actual mag nification 200 ×. OVCAR-3 cells were treated without (A) and with δ-cadinene10 μM (B), 50 μM (C), and 100 μM (D) for 48 hours. White arrows represent Apoptotic cells exhibiting chromatin condensation and nuclear fragmentation.

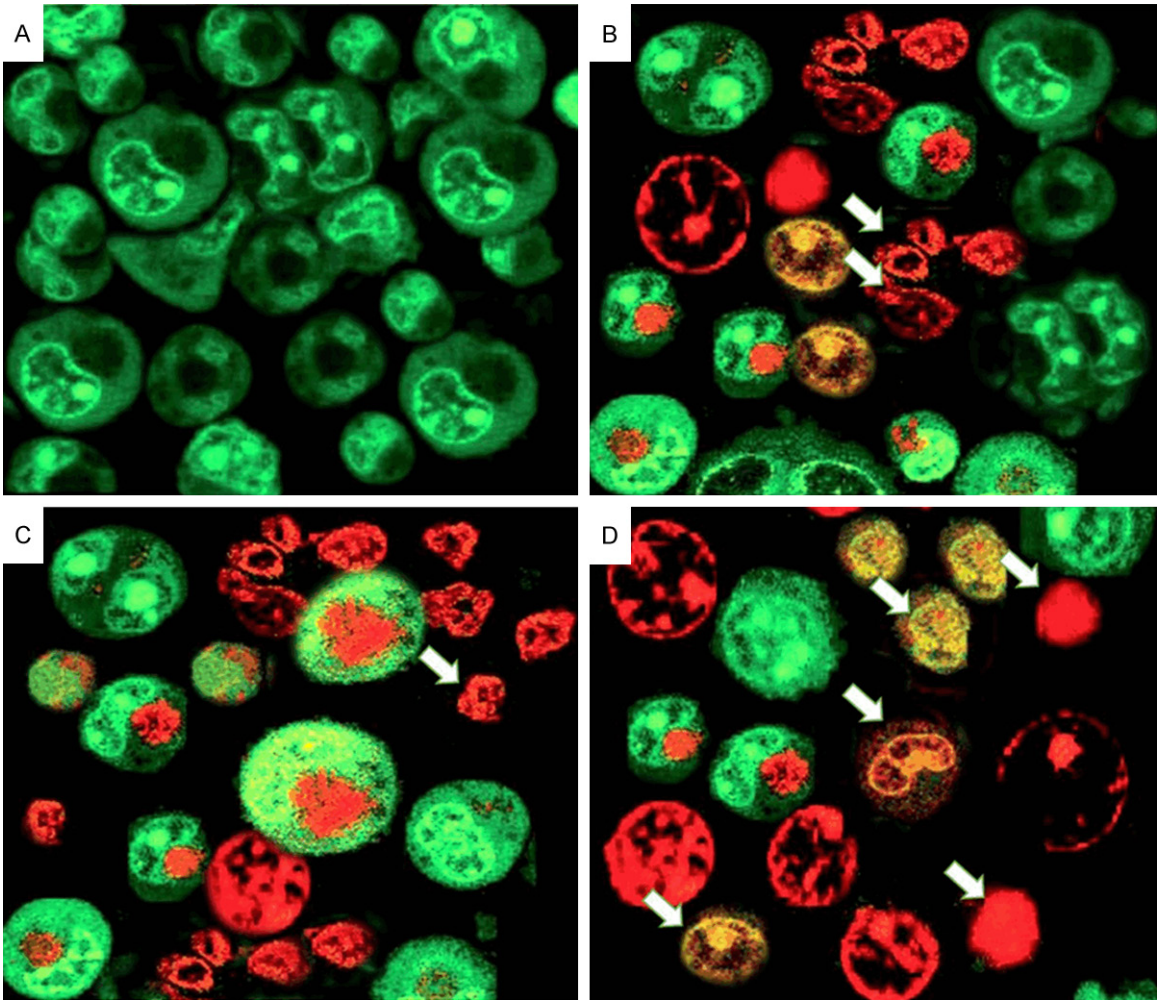

Figure 4. Fluorescence microscopic study of human ovary cancer cells stained with a combination of acridine orange: ethidium bromide (1:1 ratio). (A) shows untreated Control cells, (B) shows OVCAR-3 cells treated with 10 μM dose of δ-cadinene, (C) shows OVCAR-3 cells treated with 50 μM dose of δ-cadinene and (D) shows OVCAR-3 cells treated with 100 μM dose of δ-cadinene.

was transferred to nitrocellulose membranes. Membranes were blocked, incubated with primary Abs at the suitable dose, and consequently incubated with primary antibody, washed and incubated with horseradish peroxidase-conjugated secondary antibody (1:2500 dilution; Bio-Rad). Detection was performed using a chemiluminescent Western detection kit (Cell Signaling Technology, Inc., Danvers, MA, USA).

Statistical analysis

Unless otherwise indicated, each result is expressed as the mean ± SD of data obtained from triplicate experiments. Statistical analysis was performed using a paired Student t-test. Differences at $P < 0.05$ were considered statistically significant.

Results and discussion

Antiproliferative activity of δ-cadinene by Sulforhodamine B (SRB) cell viability assay

To examine the inhibitory effect of δ-cadinene on human ovary cancer cells (OVCAR-3), SRB assay was conducted. OVCAR-3 cells were treated with different doses (0, 10, 20, 50, 75 and 100 μM) of δ-cadinene dissolved in DMSO

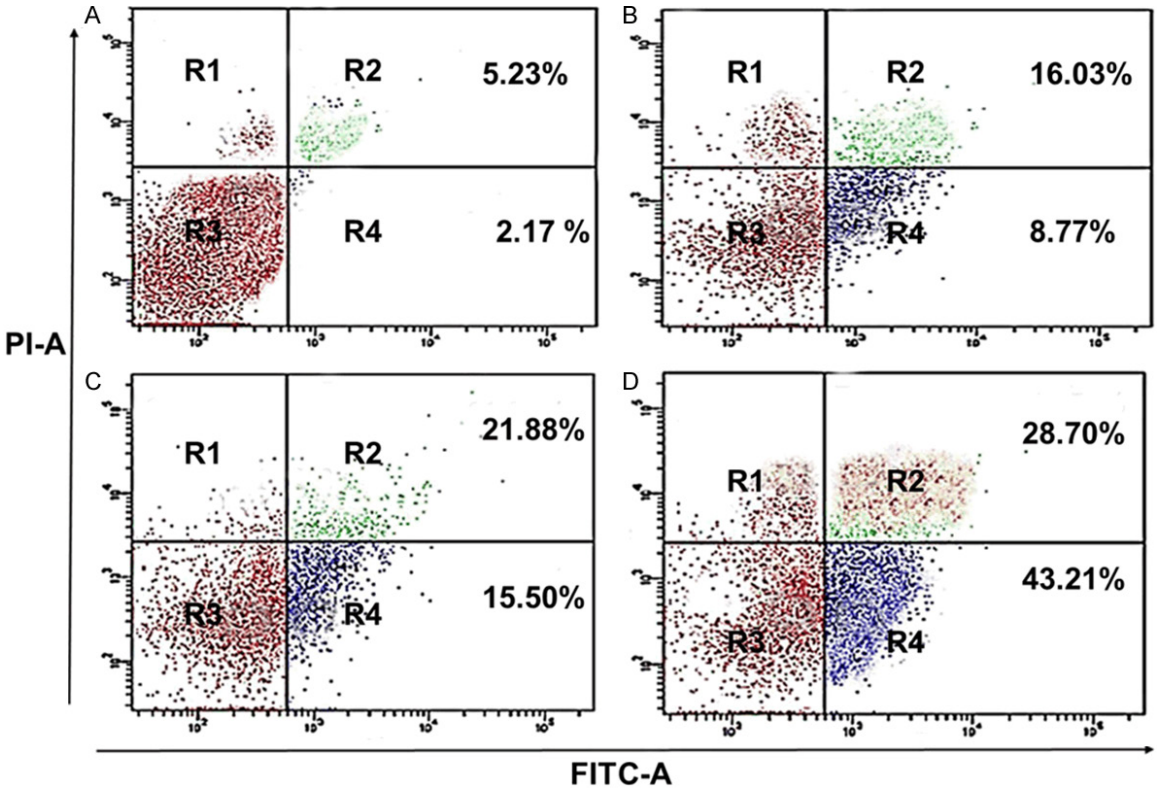

Figure 5. Quantification of δ-cadinene-induced apoptosis in human ovary cancer cells (OVCAR-3). The cells were subjected to different doses of δ-cadinene (0, 10, 50 and 100 μM) for 48 h and analyzed by flow cytometry with annexin V-FITC/PI staining. The different quadrants Q1, Q2, Q3 and Q4 represent necrotic cells, late apoptotic cells, viable cells and early apoptotic cell population respectively. Percentage of apoptotic cells increases from 7.4% in control cells (A), to 24.8%, 37.8% and 71.9% in 10 μM (B), 50 μm (C) and 100 μm (D) δ-cadinene-treated cells respectively.

and the same volume of solvent was used as a control. As shown in **Figure 1**, δ-cadinene inhibited the growth of OVCAR-3 cells in a concentration-dependent manner compared to the controls. The compound also showed time dependent growth inhibitory effects with 48 h time interval producing more potent effects as compared to the 24 h time.

Cellular morphological changes using inverted phase contrast and fluorescence microscopy

In this study, the morphological alterations of human ovary cancer cells (OVCAR-3) untreated and treated with δ-cadinene were observed under an inverted light microscope. The most conspicuous changes characteristic of apoptosis were observed in the treated cells that include the detachment of the cells from substratum, cell shrinkage, nuclear condensation, membrane blebbing as well as formation of apoptotic bodies. As revealed by inverted light microscopy, the untreated control cells were evenly distributed on the substratum. Decrease in the cell population was seen with the increase in the δ-cadinene concentration. As can be seen in **Figure 2A-D**, the cells with higher doses of δ-cadinene revealed that cellular shrinkage and blebbing occurred. This effect was shown to be related to δ-cadinene dose.

In case of fluorescence microscopy, OVCAR-3 cells were stained and evaluated for nuclear shape using a fluorescence microscope with Hoechst 33342 staining **Figure 3A-D**. The results revealed that δ-cadinene-treated cells revealed substantial chromatin condensation or dense staining fragmentation called apoptotic bodies, which implied an early apoptotic event. The appearance of such apoptotic bodies was related with δ-cadinene dose. OVCAR-3 cells underwent the morphologic changes typical of apoptosis after treatment with δ-cadinene.

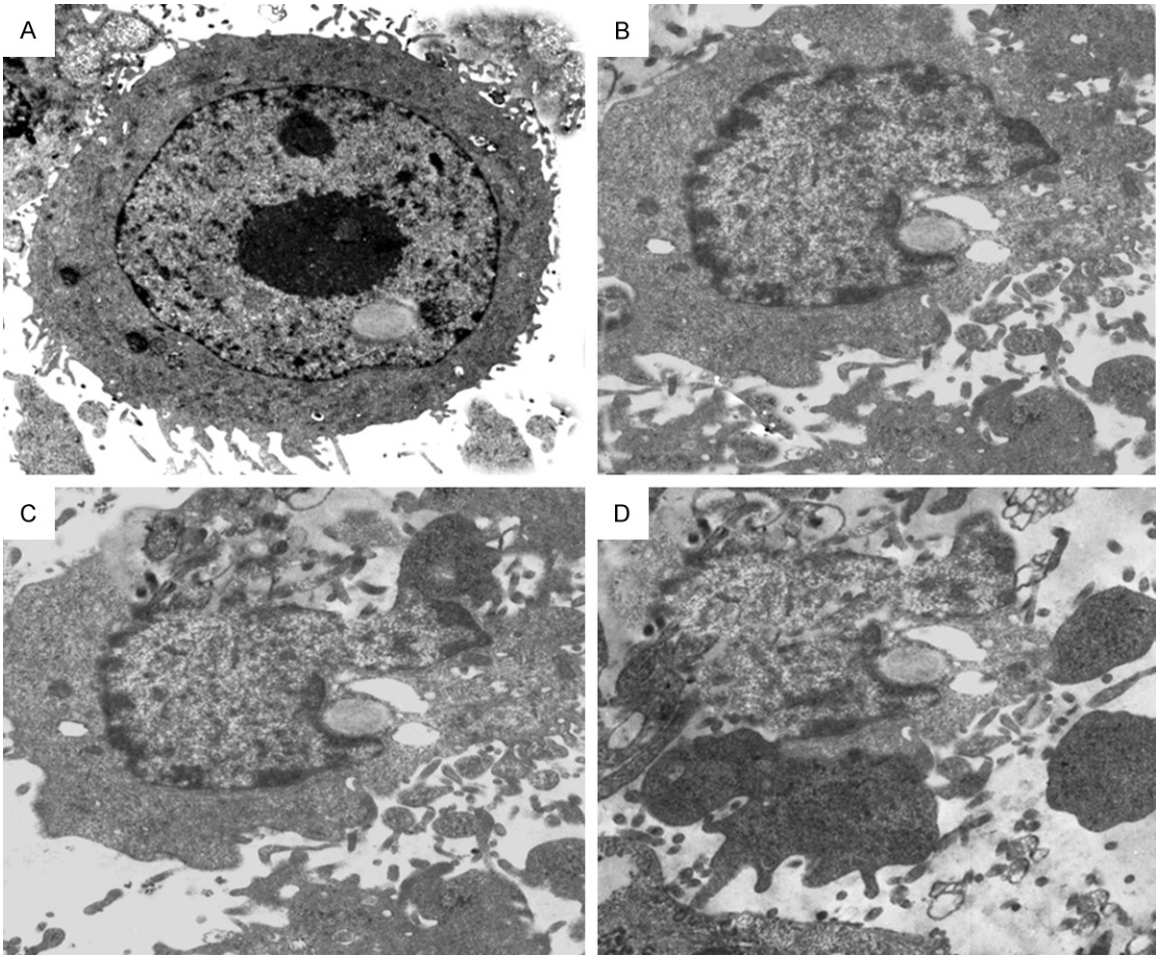

Figure 6. δ-Cadinene induced cellular ultrastructure alterations observed by transmission electron microscopy (TEM) in human ovary cancer cells (OVCAR-3). The cells were treated with 0, 10, 50 and 100 μM δ-Cadinene for 48 h. Clear nuclear fragmentation was observed in the treated OVCAR-3 cells. Magnification, × 2,000, Scale bar, 1.5 μm.

Further, the effects of δ-cadinene on OVCAR-3 cellular morphology were demonstrated by acridine orange/ethidium bromide staining using fluorescence microscopy. The results of this experiment **Figure 4A-D** were similar to other two experiments (**Figures 2** and **3**) indicating that δ-cadinene induces cell death through the mediation of apoptosis.

Apoptosis quantification by Annexin V-FITC/PI assay

Annexin V/PI double staining was used to detect apoptosis in the human ovary cancer cells (OVCAR-3) **Figure 5A-D**. OVCAR-3 cells were treated with different concentration (0, 10, 50 and 100 μM) of δ-cadinene for 48 h. δ-cadinene induced both early and late apoptosis in a concentration-dependent manner (**Figure 5B-D**) as compared to the untreated control cells (**Figure 5A**). The Percentage of apoptotic cells increased from 7.4% in control cells (**Figure 5A**), to 24.8%, 37.8% and 71.9% in 10 μM (**Figure 5B**), 50 μm (**Figure 5C**) and 100 μm (**Figure 5D**) δ-cadinene-treated cells respectively.

Transmission electron microscopy (TEM)

Transmission electron microscopy was used to observe the ultrastructure of the δ-cadinene -treated OVCAR-3 cells and to assess the intracellular damage caused by δ-cadinene for 48 h. δ-cadinene-treated cells revealed morphological changes characteristic of apoptosis. As shown in **Figure 6A-D**, clear nuclear fragmentation was found in the treated OVCAR-3cells, while the untreated OVCAR-3 cells exhibited a fairly intact morphology.

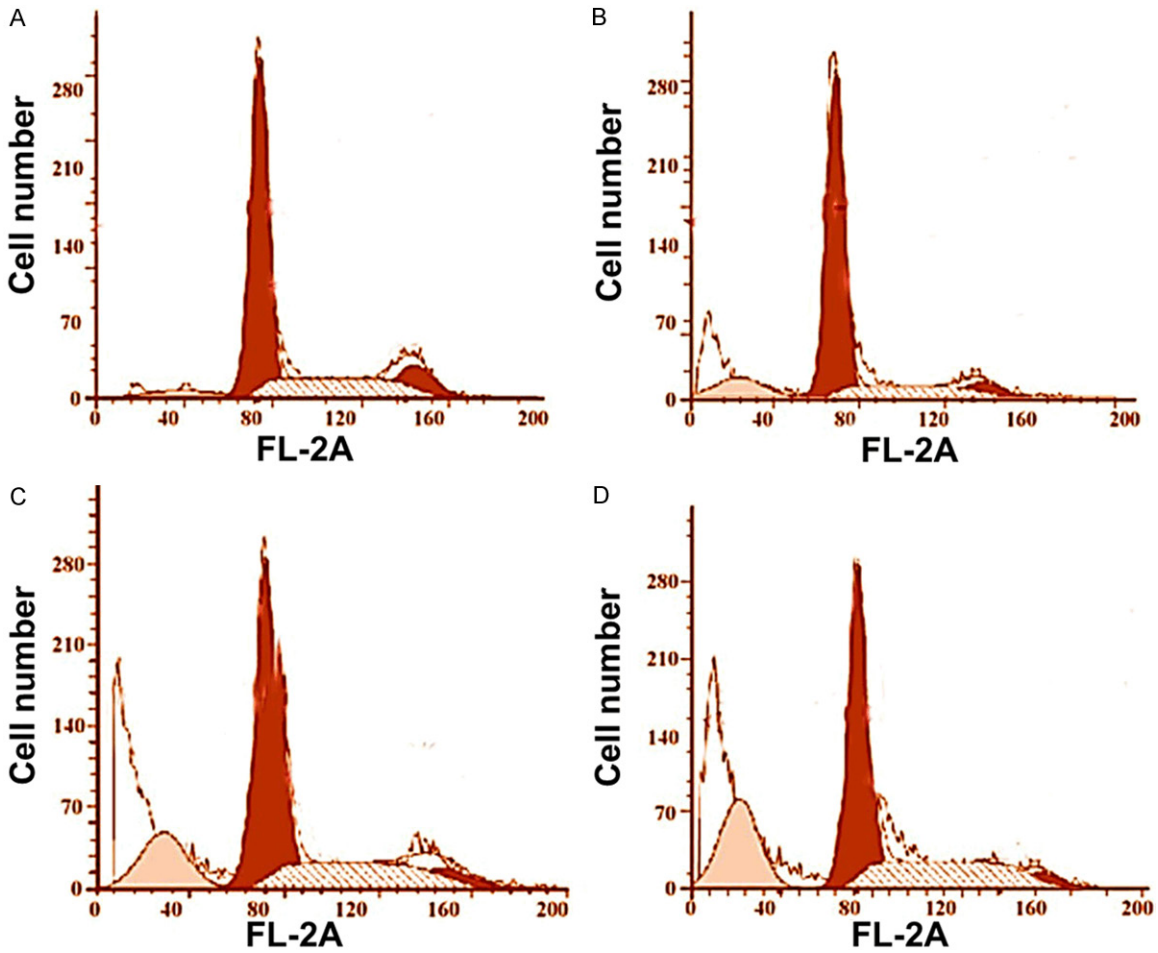

Figure 7. Effect of different doses of δ-cadinene on the cell cycle phase distribution of human ovary cancer cells (OVCAR-3) using flow cytometry analysis of the DNA content after staining with propidium iodide. C. Data expressed as mean ± SD from three independent experiments. A-D. Show effect of 0, 10, 50 and 100 μM dose of the compound on cell cycle respectively.

Effect of the δ-cadinene on cell cycle phase distribution in OVCAR-3 cells

Apoptosis and cell cycle dysfunction are intimately related biochemical processes, and any disturbance in cell cycle progression may finally lead to apoptotic cell death. In order to have a mechanistic overview of the growth inhibitory effect exerted by δ-cadinene in OVCAR-3 cancer cells, flow cytometry analysis was carried out to detect whether the compound induces cell cycle arrest in this cell line. The results showed that treatment with different concentrations of δ-cadinene for 48 h induced sub-G1-phase growth arrest in OVCAR-3 cells. As is apparent in **Figure 7**, following different doses (0, 10, 50 and 100 μM) of the compound treatment to the ovarian cancer cells, considerable G0/G1 cell cycle growth arrest was seen. Apoptotic cells were labeled as shrunken cells with degraded chromatin, high side scatter (SSC) and low forward scatter (FSC) properties. The rise in the sub-G1 cell population (hypodiploid DNA content) may be due to DNA fragmentation which eventually results in apoptotic cell death.

δ-cadinene induces caspase activation and apoptosis in ovary cancer (OVCAR-3) cells

In order to demonstrate whether caspase activation was connected in δ-cadinene -induced apoptosis, we examined caspase-3 activation in OVCAR-3 cells by using Western blot analysis as shown in **Figure 8**. The functional activity of the activated caspase-3 was examined against PARP caspase-3 substrate. As shown in **Figure 8**, PARP cleavage was noticed at 50 μM dose of

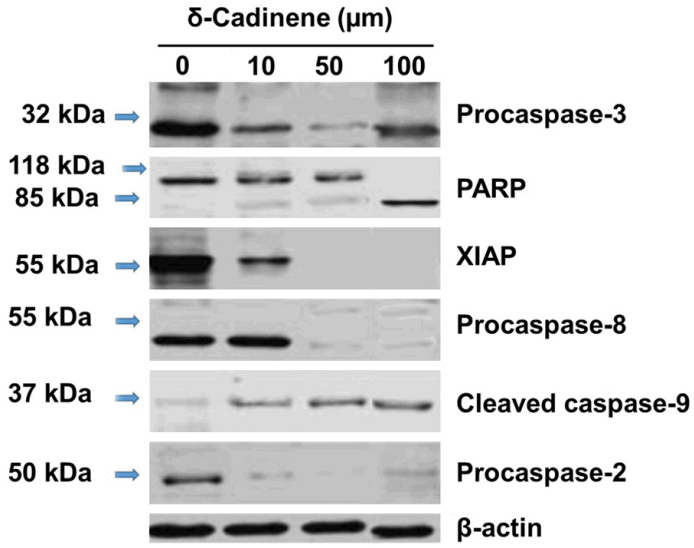

Figure 8. Activation of caspases by δ-cadinene treatment in OVCAR-3 cells. δ-cadinene induced cleavage of pro-caspase-3, -8, -9, and -2 and PARP, and XIAP. OVCAR-3 cells were treated with δ-cadinene at varying doses (0, 10, 50 and 100 μM). Whole cell lysates were subjected to Western blot analysis. Western blot analysis of β-Actin levels was included to show that equivalent amounts of protein were loaded in each lane.

δ-cadinene with the advent of the cleaved 85-kDa fragment after exposure to δ-cadinene. At 100 μM, only the cleaved form of PARP was detectable. **Figure 8** also shows the effect of δ-cadinene treatment on X-linked IAP (XIAP) expression. XIAP expression was decreased at 10 μM dose of δ-cadinene and was no longer detectable at 50 and 100 μM concentration of δ-cadinene. Further, we demonstrated that pro-caspase-8 expression remained unaltered until 10 μM dose of δ-cadinene. However, at 50 and 100 μM dose, pro-caspase-8 expression was no longer detectable. In case of caspase-9, we observed that treatment with 10 μM of δ-cadinene resulted in expression of the cleaved caspase-9 and there was a significant increase in the caspase-9 expression levels after 50 and 100 μM δ-cadinene treatments **Figure 8**. Pro-caspase-2 was almost fully cleaved at 10 μM dose of δ-cadinene.

Discussion

Apoptosis is a cellular suicide program that eradicates undesirable, faulty and potentially dangerous cells during the development and maintenance of cell homeostasis [10]. Inducing apoptosis is a key tactic to eliminate cancer cells without stimulating an inflammatory reaction. Regulation of apoptotic signaling pathways encompasses a complicated system consisting of several elements. Several conventional drugs are currently used in anticancer chemotherapy, which are believed to induce cell apoptosis via activation of these elements [10, 11]. Therefore, the ability of cancer cells to induce the apoptotic program has been recognized as one of the major mechanisms which might serve for the development of novel approaches to treat cancer.

Dysregulation in the cell division and apoptosis are connected to the development of most cancers. Amongst the two apoptotic pathways, the intrinsic pathway is primarily controlled by the members of the Bcl-2 family proteins [12, 13]. Many anticancer drugs function primarily to induce apoptosis in cancer cells and prevent tumor development [14, 15]. The morphological changes of apoptosis observed in most cell types initially start with a reduction in cell volume and condensation of the nucleus [16]. In many cases extensive DNA damage leads to activation of cell cycle check points and results in cell cycle arrest and apoptosis [17].

The mechanisms involved in induction of apoptosis by natural chemotherapeutic agents are believed to be largely mediated by the mitochondrial apoptotic pathway [18]. This involves release of mitochondrial apoptotic proteins such as cytochrome c [19], apoptosis inducing factor (AIF) [20], second mitochondrial-derived activator of caspase/direct inhibitor of apoptosis (IAP) protein binding protein with low pH [21].

In the present study, we demonstrated antiproliferative effects of a naturally occurring sesquiterpene, δ-cadinene against human ovary cancer (OVCAR-3) cells along with demonstrating the underlying mechanism of action. Sul forhodamine B (SRB) assay was used to evaluate the antiproliferative effect of δ-cadinene which showed that this compound induced potent, dose-dependent as well as time-dependent growth inhibitory effects on OVCAR-3

cells. Further, using phase contrast microscopy along with fluorescence microscopy revealed that δ-cadinene was capable of inducing cellular shrinkage, chromatin condensation and apoptotic body formation. Transmission electron microscopy showed that δ-cadinene could induce powerful and dose-dependent nuclear membrane rupture which is hallmark of apoptosis. The quantification of apoptotic cells was convincingly done by using Annexin V-fluorescein isothiocyanate (FITC)/PI kit which showed that δ-cadinene induced both early and late apoptosis in a concentration-dependent manner (**Figure 5B-D**) as compared to the untreated control cells (**Figure 5A**). The percentage of apoptotic cells increased from 7.4% in control cells (**Figure 5A**), to 24.8%, 37.8% and 71.9% in 10 μM (**Figure 5B**), 50 μm (**Figure 5C**) and 100 μm (**Figure 5D**) δ-cadinene-treated cells respectively. Effects of δ-cadinene on cell cycle analysis were carried out by flow cytometry using propidium iodide as a probe. The results showed that treatment with different concentrations of δ-cadinene for 48 h induced sub-G1-phase growth arrest in OVCAR-3 cells. As is apparent in (**Figure 7**), following different doses (0, 10, 50 and 100 μM) of the compound treatment to the ovarian cancer cells, considerable G0/G1 cell cycle growth arrest was seen.

Effect of δ-cadinene on caspase activation in OVCAR-3 cells was done by western blotting assay (**Figure 8**) in order to reveal whether there occurs any link between apoptosis and caspase activation. PARP cleavage was noticed at 50 μM dose of δ-cadinene with the advent of the cleaved 85-kDa fragment after exposure to δ-cadinene. At 100 μM, only the cleaved form of PARP was detectable. XIAP expression was decreased at 10 μM dose of δ-cadinene and was no longer detectable at 50 and 100 μM concentration of δ-cadinene. Pro-caspase-8 expression remained unaltered until 10 μM dose of δ-cadinene. However, at 50 and 100 μM dose, pro-caspase-8 expression was no longer detectable. In case of caspase-9, we observed that treatment with 10 μM of δ-cadinene resulted in expression of the cleaved caspase-9 and there was a significant increase in the caspase-9 expression levels after 50 and 100 μM δ-cadinene treatments.

In conclusion, we summarize that δ-cadinene shows potent anticancer effects against human ovary cancer cells (OVCAR-3) through the mediation of apoptosis, nuclear membrane rupture, cell cycle arrest and caspase activation.

Disclosure of conflict of interest

None.

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