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RESEARCH PAPER

Investigating the effect of cadmium telluride quantum dots coated with *Nerium oleander* hydroalcoholic extract on apoptosis induction in MCF7 cancer cells

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ABSTRACT

Objective(s): Breast cancer, a prevalent malignancy, poses significant challenges despite current clinical strategies. Herbal medicine, such as *Nerium oleander* (*N. oleander*), and nanocarriers like Cadmium Telluride Quantum Dots (CdTe QDs), offer potential solutions. This study aims to evaluate the apoptosis-inducing effects of *N. oleander* extract using CdTe QDs as nanocarriers on the MCF-7 cell line, highlighting their potential in breast cancer treatment.

Materials and Methods: *N. oleander* specimens were collected and processed to obtain a hydroalcoholic extract. CdTe QDs were biosynthesized using the extract. MCF-7 cancer cells were cultured and treated in different groups, including the control group, extract group, QDs group, and extract + QDs group. Cell viability, Phosphatidylserine externalization, indicators of apoptosis, Caspases 3, 8, and 9 activity, DNA fragmentation, Nitric oxide (NO) levels, alkaline phosphatase activity and Clonogenic assays were determined.

Results: In our study, all tested substances, including *N. oleander* extract, CdTe QDs, and *N. oleander*-coated CdTe QDs, showed concentration and time-dependent cytotoxic effects on MCF-7 cells. *N. oleander*-coated CdTe QDs exhibited the highest cytotoxicity. These QDs also induced a significantly higher rate of apoptosis in treated MCF-7 cells after 24 hours. Moreover, the activity of caspases 3, 8, and 9 was notably increased in cells treated with *N. oleander* extract and *N. oleander*-coated CdTe QDs. Electrophoresis confirmed DNA fragmentation in these treated cells. *N. oleander* extract reduced nitric oxide production, with even lower levels observed in the extract + QDs group. Treatment with *N. oleander* extract and *N. oleander*-coated CdTe QDs also elevated alkaline phosphatase activity. Furthermore, *N. oleander*-coated CdTe QDs effectively reduced cell colony formation in a dose-dependent manner.

Conclusion: *N. oleander*-coated CdTe QDs demonstrated superior cytotoxicity and apoptosis-inducing effects on MCF-7 cells compared to *N. oleander* extract and CdTe QDs. This suggests their potential as a promising candidate for cancer therapy.

Keywords: *Breast cancer, Apoptosis, N. oleander, Cadmium Telluride Quantum dots, MCF-7 cell line*

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INTRODUCTION

Breast cancer ranks as one of the most prevalent malignancies affecting women [1]. Given the increasing prevalence of breast cancer, its evaluation holds special importance within the global community [2]. According to the latest report from the International Agency for Cancer Research (IACR), this form of cancer stands as the fifth leading cause of mortality among common female cancers, underscoring the urgency of implementing preventive and control measures for breast cancer like never before[3, 4]. Despite the implementation of multiple clinical strategies, including surgical resection, radiotherapy, endocrine therapy, targeted therapy, and

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chemotherapy, the battle against breast cancer encounters several challenges. These challenges encompass drug resistance, a dearth of effective drugs against metastatic breast cancer, and the absence of a more refined treatment for the triple negative subtype [5]. Consequently, ongoing efforts to discover novel treatment options that overcome these limitations persist, with herbal medicine emerging as one potential solution [6].

The plant kingdom comprises a vast array of 250,000 species, of which only 10% have undergone screening for their potential effects in cancer treatment [7]. Among these, *Nerium oleander (N. oleander)*, an evergreen ornamental shrub belonging to the Apocynaceae family, stands out. Despite its toxic nature, *N. oleander* boasts numerous properties, including antibacterial, antifungal, antidiabetic, antioxidant, antitumor, and hepatoprotective effects [8, 9]. These effects are attributed to the presence of bioactive compounds found in various parts of the plant, such as alkaloids, flavonoids, carbohydrates, tannins, phenolics, saponins, cardenolides, cardiac glycosides, pregnanes, triterpenoids, triterpenes, and steroids [10]. For instance, Oleandrin, a glycoside compound found in *N. oleander*, has been shown to possess antitumor properties [11].

Quantum dots (QDs), nanoparticles with diameters ranging from 2 to 10 nm, serve as nanocarriers and fluorescent biomarkers[12]. Their remarkable luminescence, narrow emission spectra, low toxicity, and biocompatibility render them an excellent choice for applications in bioimaging, diagnostics, and biosensing [13]. Cadmium telluride quantum dots (CdTe QDs), approximately 2.5–4.0 nm in diameter and emitting light within the 520–650 nm range, represent a prominent subset of QDs widely utilized in cosmetics, the pharmaceutical industry, drug delivery, and medicine [12, 14]. Additionally, the cytotoxic effects of CdTe QDs on cancer cells have been substantiated through the induction of Reactive Oxygen Species (ROS) generation [15].

In this study, CdTe QDs were employed as nanocarriers to assess the apoptosis-inducing effects of hydroalcoholic extracts from *N. oleander* on the MCF7 cell line.

MATERIALS AND METHODS

Plant material and extract preparation

N. oleander specimens were gathered from Noorabad County in the spring (Fars province, Iran). The collected samples underwent a process involving drying in a dark and dry environment, followed by grinding. A total of 50 gr of the dried and powdered samples (the aerial parts of the plant) were soaked in 200 ml of 70% methanol (Merck, Germany), for 48 hours at room temperature. The resulting mixture underwent filtration using filter paper. The residual material was soaked once again in 70% methanol, and the subsequent filtrate was combined with the filtrate from the previous step. Using a Rotary Evaporator System (Heidolph, Germany), the filtrate was evaporated at a temperature of 40 °C, and then further drying was conducted using a Freeze Dryer (Zirbus Technology, Germany). The resulting extract was stored at a temperature of 4 °C to be utilized in subsequent experiments [16].

Biosynthesis of CdTe QDs

Twenty-five milliliter of resulting extract (pH=9) was placed in a balloon and 50 ml of an aqueous solution of $Cd(NO3)_2 \cdot 7H_2O$ (7.0 mM, Sigma, USA) was added drop-wise to the extract under argon gas flow (with continuous stirring at room temperature). Then, 50 mL of tellurium solution (5.0 mM, Sigma, USA) was added drop-wise to the balloon and the resulting solution was incubated at 70 °C for 30 min. Finally, the obtained solution was centrifuged (1000 rcf for 10 min at room temperature) and the supernatant was collected for further analysis. The morphology and the diameter of QDs were evaluated with Scanning Electron Microscopy (Sigma, Germany)(Figure 1) [17, 18].

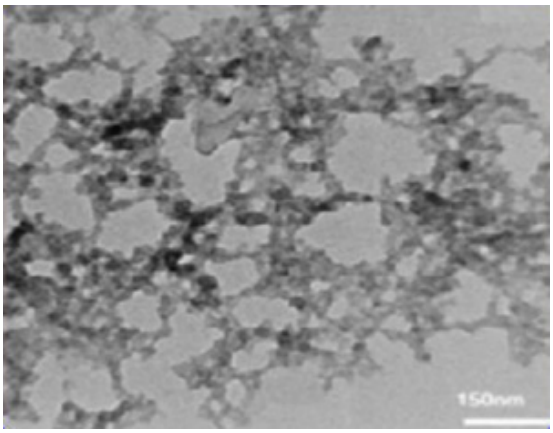


Fig. 1. Scanning electron microscopy image of QDs with microwave method at 270 watts. QDs; Quantum Dots. QDs: quantum dots.

Cell culture

The MCF-7 cell line was obtained from the Pasteur Institute in Tehran, Iran. The cells were thawed and cultured in Roswell Park Memorial Institute medium (RPMI medium) (Gibco, USA), supplemented with 10% Fetal Bovine Serum (FBS) and 1% Penicillin/Streptomycin (Gibco, USA). The cell culture flask (SPL, South Korea) was used, held the cells and was placed in an incubator at 37°C with a 5% CO2 level and appropriate humidity.

The cell treatment groups consisted of the following:

- MCF-7 (Control) Group: MCF7 cells cultured without any treatment.
- Extract Group: MCF7 cells treated with the prepared extract from *N. oleander*.
- QDs Group: MCF7 cells treated with nanoparticles synthesized from CdTe.
- Extract+ QDs Group: MCF7 cells treated with CdTe QDs coated with the extract from *N. oleander*.

Tetrazolium (MTT) assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay was performed in triplicate for each concentration to evaluate cell viability in the different concentrations of *N. oleander* extract, CdTe QDs and *N. oleander*-coated CdTe QDs. Briefly, MCF7 cells were seeded into a 96-well plate (9000 cells/well) and incubated at 37 °C, PCO2 5% and appropriate humidity for 24 h. After that, each well was treated with a certain concentration of the *N. oleander* Extract, CdTe QDs and *N. oleander*-coated CdTe (10, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220 and 240 µg/mL). After 24- and 48-hour incubation, 10 µL MTT reagent 5 mg/mL (Sigma, USA) was added to each well and the plate was incubated at 37 °C, PCO2 5% for 3h. Then, 100 µL of dimethyl sulfoxide was added and the plate were placed in the dark for 30 min. The intensity of the produced purple color indicates cell viability. The absorbance of the wells was measured at 540 nm with a microplate reader (Bio-Rad , USA) [19].

Measuring of the phosphatidylserine externalization from the inner layer to the outer layer of the plasma membrane

Phosphatidylserine exposure on the outer layer of the plasma membrane is characterized as a unique feature of apoptotic cells [20]. The externalization rate of phosphatidylserine was measured using the flow cytometry method. The MCF7 cells which had been treated with the effective dose of the extract, were trypsinized and separated from the bottom of the plate. After inhibiting trypsin using a culture medium containing FBS, about 1.5 million cells were suspended in binding buffer 1X. Then, the cells were incubated with 5 µl of Annexin V-FITC and 5 µl of Propidium iodide (Sigma USA), at room temperature and in the dark for 5 min. The green fluorescence from Annexin V-FITC was measured using a FL1 detector that has a filter with a specific band at 530 nm, and the red fluorescence from PI was measured using a FL2 detector that has a filter with a specific band at 585 nm. Annexin V binds to phosphatidylserine. Therefore, only the detection of Annexin V-FITC signal is related to early stage of apoptosis, while both Annexin V-FITC and PI signals indicate its final stages [21].

Activity measurement of caspases 3, 8 and 9

After twenty-four hours treatments with an effective dosage of extract, MCF7 cells of the control and treatment groups were separated from the bottom of the plate wells using trypsin and centrifuged. The supernatant of each microtube was removed and 50 µL of lysis buffer was added to the microtube. The microtubes were incubated on ice for 10 min. Then, the tubes were centrifuged (10000 ×g, 4 °C, 20 min) and the supernatant was aliquoted to measure caspases 3, 8 and 9 activity, and protein content. Caspases 3, 8 and 9 activity were measured using available commercial kits (Biogen, USA) according to the manufacturer's instructions. The protein content of the samples also was assayed by the Bradford method and caspase activity was reported as IU/mg protein.

Evaluation of DNA fragmentation

DNA was extracted using with DNA extraction kit for cell culture (Cinnagen, Iran) based on manufacturer's instructions. After separating of the cells from the bottom of the flask, about 2 × 10^6 cells of each groups were transferred into microtube and washed twice with PBS.100 µL lysis buffer was added to each tube and the tubes were centrifuged. The samples were washed by washing buffer two times. Then, the supernatant was removed and 10µL of lysing buffer was added to each tube. The samples were washed using with washing buffer and obtained DNA was dissolved in

10µL solvent buffer. Finally, extract DNA of each samples was electrophoresed on agarose gel 1% (70 V, 90 min) to evaluate DNA fragmentation.

Measurement of released Nitric oxide (NO)

The Griess method was performed to measure NO. MCF7 cells were seeded at an initial density of 1×10^4 in each well of a 96-well plate and incubated Dulbecco's Modified Eagle Medium (DMEM) for 24 h. 100 µL effective dosage of *N. oleander*-coated CdTe QDs were added to each well. After 24 h, 100 µL of cell supernatant was mixed with 100 µL of Griess reagent (1% sulfanilamide, 0.1% naphthlethylenediamine dihydrochloride in 2.5% phosphoric acid) and placed in the dark for 15 min. The formation of pink color indicates the presence of NO in the environment. The absorbance of samples was read at 540 nm with a microplate reader (Bio-Rad , USA) to determine the NO level.

Alkaline phosphatase activity assay

Alkaline phosphatase activity was measured using with available commercial kit according to the manufacturer's instructions. The basis of the assay in the kit was based on the conversion of para nitrophenyl phosphate to nitrophenol by the enzyme activity present in the sample.

Clonogenic assay

In this procedure, a total of 1,000 cells were initially placed into each well of a 6-well plate. After a 24-hour incubation period, distinct concentrations of *N. oleander*-coated CdTe QDs (10, 20, 40, and 60 µg/ml) were individually administered to the wells. Following nine days post-treatment, the growth medium was aspirated from the wells, and the cells underwent a thorough wash with phosphate-buffered saline (PBS). To immobilize the cells, a fixative solution comprising methanol and acetic acid (in a ratio of methanol to acetic acid as 3:1) was applied for 5 minutes. After fixation, the fixative solution was meticulously discarded, and a 0.5% solution of crystal violet dye was employed to stain the cells for 15 minutes. Following the staining process, excess dye was gently removed via rinsing with distilled water. The evaluation entailed the enumeration of colonies wherein the cell count exceeded 50 cells. This enumeration was conducted using an Olympus SZX16 stereo microscope for each well.

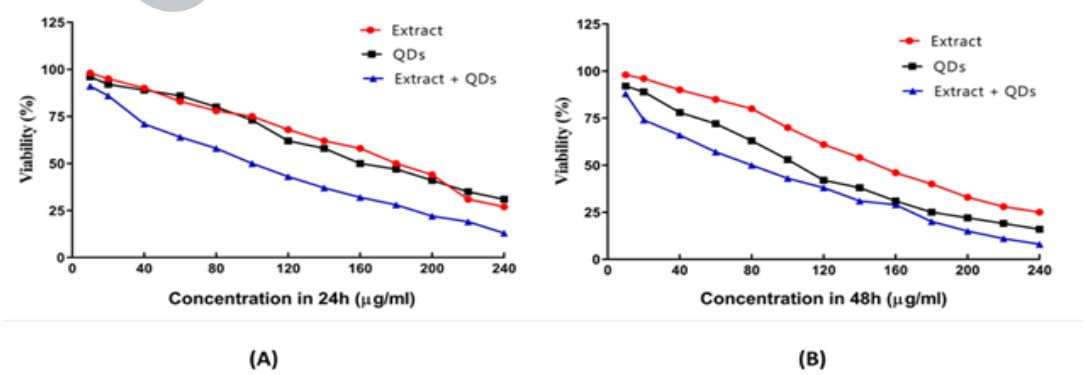
Statistical analysis

All the measurements were performed triplicate manner and data were statistically analyzed by GraphPad Prism 6 software. Shapiro-Wilk statistical test was used to check if the data has a normal distribution pattern or not. The data was analyzed by One-way ANOVA or Kruskal-Wallis statistical testes according to Shapiro-Wilk results. The significance level was also considered $p<0.05$ in the present study.

RESULTS

Cytotoxicity evaluation of N. oleander extract, CdTe QDs and N. oleander-coated CdTe QDs on MCF-7 cells

The MTT assay was used to determine the cytotoxicity of the extract and QDs (IC50) at varying concentrations over 24 and 48 hours (Figure 2 and Table 1). The results revealed that *N. oleander*



(A) (B)

Fig. 2 Cytotoxic effect of different concentrations of N. oleander extract, CdTe QDs and N. oleander extract coated-CdTe QDs on the proliferation of MCF-7 in comparison with untreated cells (control) by MTT method After 24 (A) 48 (B) hours. Extract: Group treated with N. oleander, QDs: Group treated with CdTe QDs, Extract + QDs: Group treated with N. oleander-coated CdTe QDs. CdTe: cadmium telluride; N. oleander: Nerium oleander; QD: quantum dots.

Table 1. IC50 values obtained for the cell treatment groups in different concentration (24 and 48 hours).

Groups	IC50 value (µg/mL)	
	Concentrations in 24 h	Concentrations in 48 h
QDs	158.6	106.1
Extract	177.2	151.5
Extract + QDs	97.6	76.8

*** Extract Group: MCF7 cells treated with the prepared extract from *N. oleander*.**
*** QDs Group: MCF7 cells treated with nanoparticles synthesized from CdTe.**
*** Extract+ QDs Group: MCF7 cells treated with CdTe QDs coated with the extract from *N. oleander.***

CdTe: cadmium telluride; *N. oleander*: *Nerium oleander*; QDs: quantum dots.

extract, CdTe QDs, and *N. oleander*-coated CdTe QDs exhibited cytotoxic effects against the MCF7 cell line, impeding cell growth and proliferation in a concentration and time-dependent manner. After 24 hours, the IC50 values of *N. oleander* extract, CdTe QDs, and *N. oleander*-coated CdTe QDs on MCF7 were 168.7, 162.3, and 89.6 µg/ml, respectively. *N. oleander*-coated CdTe QDs had significantly lower IC50 values than both the extract and CdTe QDs ($p<0.05$), while there was no significant difference between the IC50 values of CdTe QDs and the extract.

After 48 hours, the IC50 values were 146.6, 99.8, and 68.5 µg/ml for *N. oleander* extract, CdTe QDs, and *N. oleander*-coated CdTe QDs, respectively. Furthermore, the IC50 values of *N. oleander*-coated CdTe QDs were significantly lower than those of the extract ($p<0.05$), with no significant difference between the IC50 values of CdTe QDs and *N. oleander*-coated CdTe QDs. Interestingly, the toxicity pattern remained consistent over both 24 and 48-hour treatments, with *N. oleander*-coated CdTe QDs exhibiting the highest toxicity and *N. oleander* extract showing the lowest, in descending order.

The phosphatidylserine externalization from the inner layer to the outer layer of the plasma membrane

As aforementioned above, phosphatidylserine externalization is considered a unique property of apoptotic cells [20]. Analysis of flow cytometry data by software (Flow JO) showed that the percentage of apoptotic cells in cells treated with *N. oleander*-coated CdTe QDs was significantly higher than the amount of apoptosis in untreated cells after 24 h (Table 2 and Figure 3) ($p<0.001$).

Table 2. Apoptosis percentage in MCF7 cells after 24 h of treatment with N. oleander-coated CdTe QDs.

Groups	Apoptosis percentage
Untreated	1.79±0.11
Extract+QDs	27.76±1.3^{a***}

Extract+QDs: Group treated with *N. oleander*-coated CdTe QDs
a: significant with untreated group

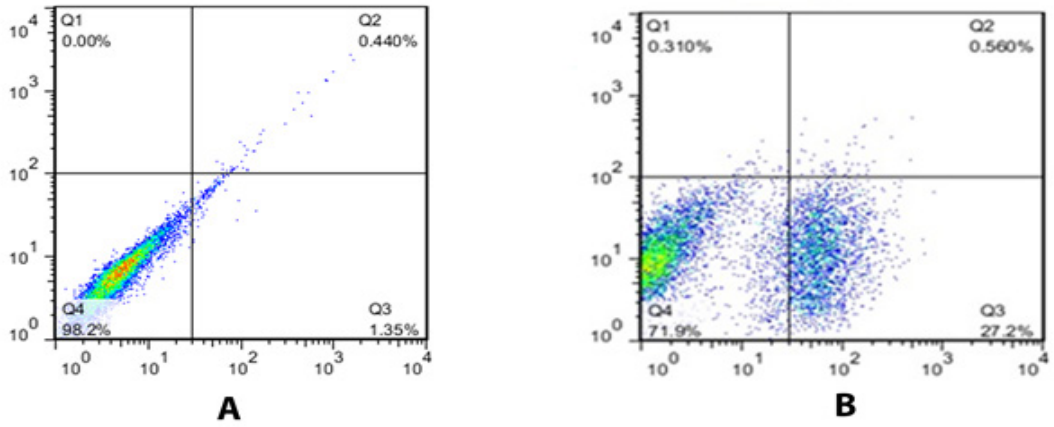


Fig 3. Apoptosis percentage in MCF7 cells after 24 h of treatment with *N. oleander*-coated CdTe QDs. A. Untreated group. B. Extract+QDs: Group treated with *N. oleander*-coated CdTe QDs. CdTe: cadmium telluride; *N. oleander: Nerium oleander*; QDs: quantum dots.

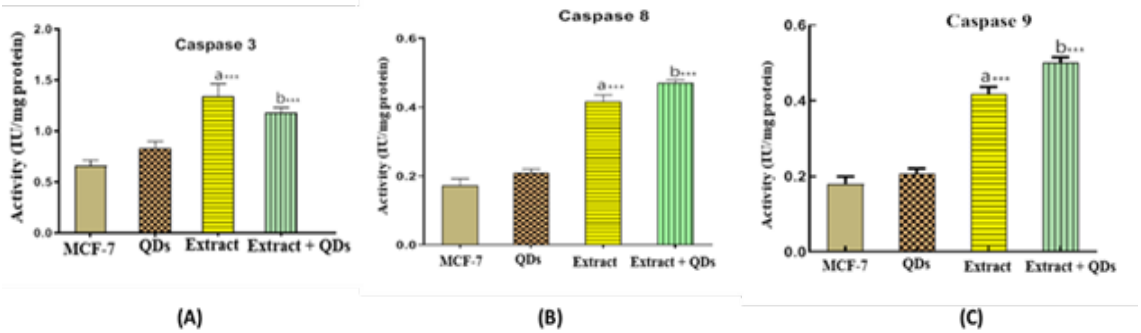


Fig. 4. The activity of caspases 3 (A),8 (B)and 9 (C) in the studied groups.
Extract: treated with *N. oleander* extract, QDs: treated with CdTe QDs, Extract+ QDs: treated with *N. oleander* extract coated-CdTe QDs. a: significant difference with MCF7 and QDs groups, b: significant difference with QDs group, *** :p < 0.001. CdTe: cadmium telluride; *N. oleander: Nerium oleander*; QD: quantum dots.

The effects of treatment with N. oleander extract, CdTe QDs and N. oleander-coated CdTe QDs on the activity of caspases 3, 8, 9 of the MCF-7 cells

The findings of the present study on measuring the activity of caspases 3, 8, and 9 are shown in Figure 4. The activity of all three enzymes in the cells that were treated with the effective dose of the *N. oleander* extract and *N. oleander*-coated CdTe QDs for 24 hours was significantly higher than the untreated MCF cells (p<0.001), in contrast to no significant difference was observed between CdTe QDs group and the untreated MCF cells. In addition, the activity of Caspase 8 and 9 in the Extract+Nano group was significantly higher than the Extract group (p<0.001).

The effects of treatment with N. oleander extract, CdTe QDs and N. oleander-coated CdTe QDs on the DNA fragmentation of the MCF-7 cells

Figure 5 shows the results of electrophoresis of extracted DNA samples from Extract and Extract+QDs groups. The presence of a smear in the sample which was extracted from the Extract+QDs group revealed the occurrence of the apoptosis process in this group.

The effect of N. oleander extract, CdTe QDs and N. oleander-coated CdTe QDs on the release rate of nitric oxide as a free radical

The findings of the present study about the amount of produced NO are summarized in Figure 6A. Treatment with *N. oleander* extract significantly prevented increasing NO production compared with the untreated cells (p<0.01). This result showed that produced NO in the Extract+QDs group significantly was lower than in untreated cells and QDs groups (p<0.01).

The effect of N. oleander extract, CdTe QDs and N. oleander-coated CdTe QDs on the alkaline phosphatase activity

According to the findings of the present study which are presented in the Figure 6B, treatment with an effective dosage of *N. oleander* extract and *N. oleander*-coated CdTe QDs led to increased alkaline phosphatase activity in the Extract and Extract+QDs groups in comparison with untreated cells and QDs groups (p<0.001).

The effect of N. oleander-coated CdTe QDs on the MCF7 colony formation

Figure 6C shows the percentage of colonies that formed after 9 days-treatment with *N. oleander*-coated CdTe QDs in different doses (in

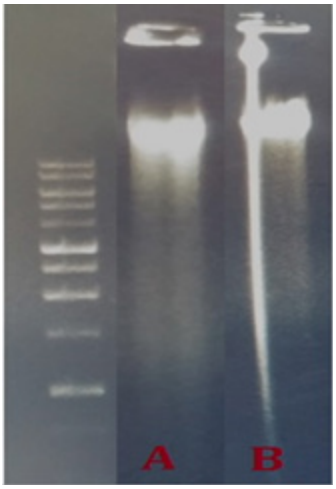


Fig. 5. Evaluation of DNA fragmentation in treated MCF-7 cells. The cell treated with an effective *N. oleander* extract(A), The cells treated with *N. oleander* -coated CdTe QDs (B). CdTe: cadmium telluride; *N. oleander: Nerium oleander*; QD: quantum dots.

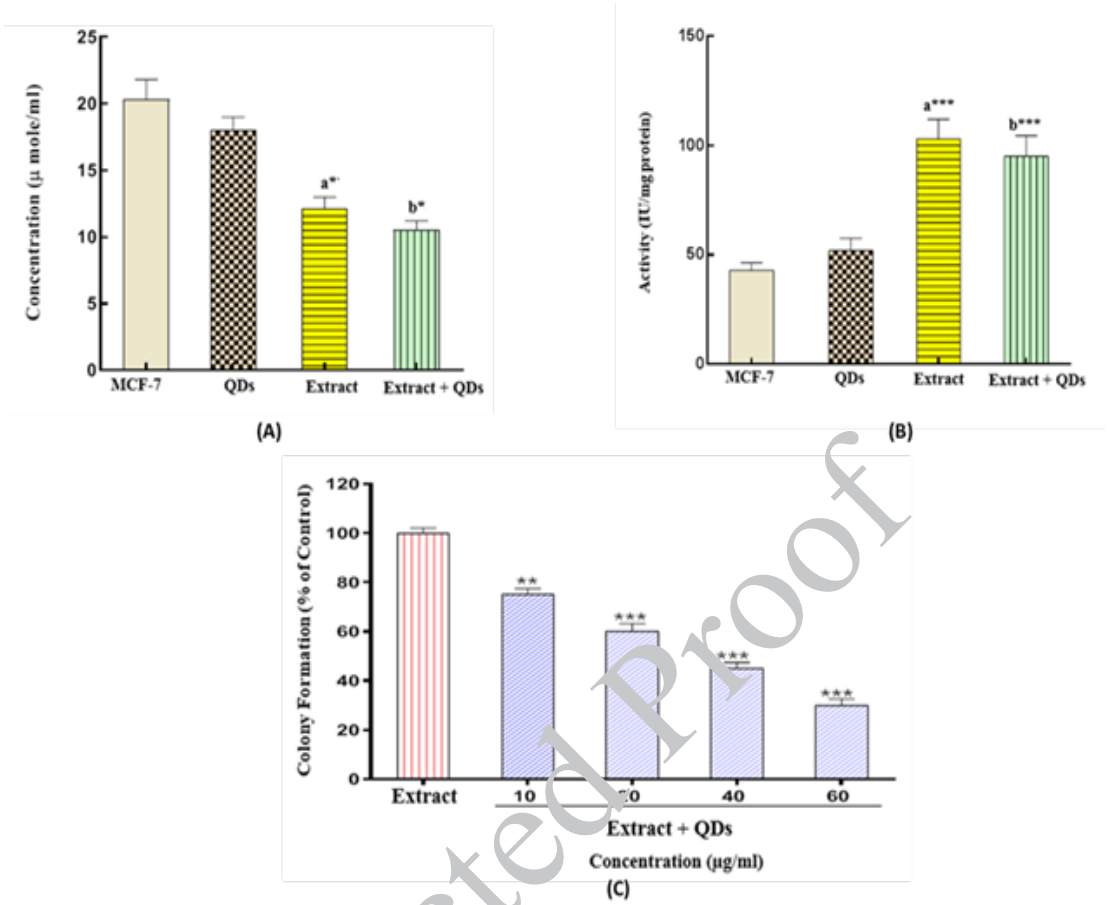


Fig. 6. Findings of measuring the amount of produced ROS (A), alkaline phosphatase activity (B) and percentage of colony formation in the studied groups (C).Extract: Group treated with *Nerium oleander,* QDs: Group treated with CdTe QDs, Extract + QDs: Group treated with *N. oleander*-coated CdTe QDs. a: significant difference with MCF7 and QDs groups, b: significant difference with QDs group, ** :p < 0.01,*** :p < 0.001. CdTe: cadmium telluride; *N. oleander: Nerium oleander*; QD: quantum dots.

comparison with the Extract group). Treatment with *N. oleander*-coated CdTe QDs significantly reduced the number of cell colonies formed in a dose-dependent manner in compared with the *N. oleander* extract alone (p<0.001).

DISCUSSION

In the present study, MCF-7 cells were exposed to different concentrations of *N. oleander* extract, Cadmium Telluride Quantum Dots (CdTe QDs) and *N. oleander*-coated CdTe QDs from 10 to 240 µg/ml for 24 and 48 h to evaluate their cytotoxic effects. The results revealed that all three substances exhibited cytotoxic effects on the MCF7 cell line, impeding cell growth and proliferation in a concentration and time-dependent manner. The IC50 values of three substances were found to be different after 24 and 48-hour treatments and these values were lower after 48-hour treatments compared to 24-hour treatments, indicating that longer exposure to these substances may increase the cytotoxic effects. The study also found that *N. oleander*-coated CdTe QDs had significantly lower IC50 values than the extract, indicating that the QDs were more cytotoxic than the extract. Furthermore, the pattern of toxicity was consistent across both 24 and 48-hour treatments. The findings of this study are consistent with previous studies that have reported the cytotoxic effects of *N. oleander* extract [22-24] and CdTe QDs [25-29] on various cancer cell lines. The cytotoxic effects observed in this study may be attributed to the substances' ability to induce oxidative stress and apoptosis in cancer cells. CdTe QDs have been reported to generate ROS, leading to oxidative stress and apoptosis in cancer cells [15]. *N. oleander* extract contains various bioactive compounds, such as oleandrin, that have been reported to

induce apoptosis and inhibit tumor growth [10]. *N. oleander* is a highly toxic plant that contains a variety of cardiac glycosides, which are known to have cytotoxic effects on cancer cells [11]. The mechanism behind this increased toxicity is not yet fully understood, but it is believed to involve the inhibition of the Na+/K+-ATPase pump, which plays a crucial role in maintaining intracellular ion homeostasis [30]. By disrupting this process, *N. oleander* may induce apoptosis in cancer cells, thereby reducing the growth and proliferation of tumors. The combination of these two substances (*N. oleander* extract and CdTe QDs) may enhance their cytotoxic effects and reduce their toxicity.

Phosphatidylserine externalization, a unique characteristic of apoptotic cells, was examined in the study. Flow cytometry analysis revealed that the percentage of apoptotic cells treated with *N. oleander*-coated CdTe QDs was significantly higher compared to untreated cells after 24 hours. This finding suggests that the combination of *N. oleander* extract and CdTe QDs induced apoptosis in the MCF7 cell line. This finding aligns with previous studies that have demonstrated

observed that the *N. oleander* extract triggered apoptosis in the cells and displayed cytotoxic properties [31-33].

The results of this study provide valuable insights into the apoptotic mechanisms induced by *N. oleander* extract and nanoparticles in MCF cells. The increased activity of caspases 3, 8, and 9 indicates that both the extract and nanoparticles can trigger apoptosis through various pathways [34]. The lack of significant caspase activation in the CdTe QDs group compared to untreated cells suggests that these nanoparticles alone might not be potent inducers of apoptosis.

The observed synergistic effect of the combination treatment (Extract+QDs) on caspase 8 and 9 activity implies that the nanoparticles might enhance the apoptotic potential of the extract. This could be due to a variety of factors, such as increased cellular uptake of the extract when delivered with nanoparticles or potential interactions between the extract's bioactive compounds and the nanoparticles. Caspase 3 is an executioner caspase, playing a pivotal role in the cleavage of cellular substrates during apoptosis [35]. The elevated activity of caspase 3 observed in response to the extract and nanoparticles indicates the activation of the caspase cascade, ultimately leading to the dismantling of critical cellular components and programmed cell death. Caspase 8, often referred to as an initiator caspase, plays a central role in the extrinsic apoptotic pathway [36]. Its increased activity in the presence of both the extract and nanoparticles implies the activation of death receptor-mediated apoptosis. This suggests that external signals may have contributed to the initiation of apoptosis, potentially through the engagement of death receptors and subsequent activation of caspase 8 [36, 37]. Caspase 9, on the other hand, is a key initiator caspase in the intrinsic apoptotic pathway, primarily activated by mitochondrial-mediated signals[37]. The heightened caspase 9 activity indicates that mitochondria may have played a crucial role in apoptosis induction by both the extract and nanoparticles. This suggests that these agents might have disrupted mitochondrial integrity, leading to the release of pro-apoptotic factors and the subsequent activation of caspase 9 [37].

DNA damage repair mechanisms play a vital role in maintaining genomic stability [38]. When these repair pathways are compromised, cells become more susceptible to accumulating mutations that can contribute to cancer development [38, 39]. Some anti-cancer compounds exploit these vulnerabilities by causing extensive DNA damage, overwhelming the repair systems and ultimately triggering cell death pathways [39]. The presence of a smear in the DNA sample extracted from the group treated with *N. oleander*-coated CdTe QDs suggests that apoptosis has occurred in this particular group. The mechanism might involve the interaction of the *N. oleander* compounds with the QDs, resulting in their delivery to the cells. Once inside the cells, these compounds could initiate a signaling cascade that triggers apoptosis pathways. This could include disrupting DNA replication and repair processes, leading to DNA fragmentation, a hallmark of apoptosis [39].

The results obtained in this study and other research showed that the increase in serum alkaline phosphatase level is one of the symptoms of many cancers [40-43]. Because cancer cells treated with *N. oleander* extract showed increased production of intracellular alkaline phosphatase. Therefore, it can be said that these treatments have probably led to partial recovery of cancer cells. Intracellular alkaline phosphatase increases greatly. This increase can either be due to the increase in the expression of different intracellular

genes related to different proteins, of which the alkaline phosphatase gene can be one of them, or due to changes in the physical structure or composition of plasma membrane components [44].

One of the potential mechanisms studied for the cytotoxic function is the effect of their extract and nanoparticles on Nitric oxide (NO) inhibition. The results showed that when cells were treated with *N. oleander* extract, there was a notable prevention of the increase in NO production compared to untreated cells. The results of this study align perfectly with numerous similar studies that have assessed the effect of plant extracts and their derivatives on inhibiting nitric oxide production. These extracts inhibited the activation of the transcription factor NF-κB and, consequently, nitric oxide synthase in astrocytoma (T67) cells [45]. NO is a crucial free radical with diverse physiological functions. Excessive NO production, mainly by nitric oxide synthase, is associated with diseases like cancer. Inhibiting NO synthesis holds promise as a preventive and therapeutic approach[46]. In a 2012 study by Abrahim et al., a specific plant extract reduced nitric oxide production in MCF-7 cells in a dose- and time-dependent manner [47]. Plant extracts, alkaloid compounds, and their derivatives have also been shown to lower nitric oxide levels, suggesting their potential cytotoxic role by inhibiting NO production [48]. Also, this study showed that *N. oleander*-coated CdTe QDs significantly reduced the production of NO in cells compared to untreated cells and the group treated with QDs alone. This suggests that using *N. oleander* extract together with nanoparticles enhances its ability to reduce NO production in cells.

The provided passage delves into a study concerning the impact of *N. oleander*-coated CdTe QDs on cellular colonies. Over nine days, it was administered various doses of these QDs and compared the outcomes with a control group treated solely with *N. oleander* extract. The results indicated that the treatment involving these coated QDs led to a substantial reduction in the formation of cell colonies, and this reduction occurred in a manner that was directly related to the dosage applied. This dose-dependent relationship became even more pronounced when contrasted with the effects of the *N. oleander* extract on its own. Perhaps the most significant finding lies in the fact that the impact of *N. oleander*-coated CdTe QDs on reducing cell colonies is directly linked to the dosage administered. This dose-dependent response reflects the intricate and calibrated manner in which the QDs engage with cellular processes. The passage underscores that as the dosage of QDs increases, the reduction in cell colony formation becomes more pronounced. This pattern signifies the potential for these QDs to be tailored for specific therapeutic interventions by adjusting their dosages.

CONCLUSION

This study demonstrated the cytotoxic effects of *N. oleander* extract, CdTe QDs, and *N. oleander*-coated CdTe QDs on MCF-7 cells. All three substances inhibited cell growth and proliferation in a concentration and time-dependent manner, with *N. oleander*-coated CdTe QDs displaying higher cytotoxicity than the extract alone. These effects suggest that oxidative stress and apoptosis induction as potential mechanisms. Combining *N. oleander* extract with CdTe QDs appeared to enhance apoptosis, as evidenced by increased caspase activity and DNA damage. The study also noted alterations in intracellular alkaline phosphatase levels and nitric oxide production, potentially contributing to cytotoxicity. This research highlights the need for future studies to explore the safety and therapeutic potential of *N. oleander* extract and nanoparticles in breast cancer treatment.

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CONFLICT OF INTEREST

The authors have no relevant financial or non-financial interests to disclose.

CONSENT TO PARTICIPATE

The nature of the study was explained to patients and written informed consent was obtained from all applicants.

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