RESEARCH ARTICLE

Doxorubicin Loaded in Niosomal Nanoparticles Improved Inhibitory Effect of Free Doxorubicin on Cancer Stem Cell Markers in Human Breast cancer Cells

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ABSTRACT

Doxorubicin (DOX) is an effective chemotherapy drug for breast cancer treatment. However, its side effect and breast cancer resistance limit its clinical application. Therefore, drug delivery systems were designed to decrease its side effects and improve therapeutic efficacy. Here, we prepared and characterized DOX-loaded niosomes (Nio-DOX) and evaluated cytotoxicity and inhibitory effect of Nio-DOX on cancer stem cell markers in MCF-7 cells. Noisome were prepared with mixture of nonionic surfactant (Tween 40, span 40) and cholesterol at 50:50 wt/wt % ratio using film hydration method. Characteristics of noisome, including size, zeta potential, morphology, drug entrapment efficiency (EE) and in vitro release behavior were studied. Cytotoxicity against MCF-7 and inhibitory effect on expression of CSC markers were evaluated by MTT assay and Real time PCR. DOX-Nio showed spherical structure with size, zeta potential, and EE values of 67.81 ± 3.25 nm, -3.48 ± 0.16 mV, and 92.03±2.95 %, respectively. The release profiles of DOX-Nio revealed the extended release over of 5 h. The IC50 values of DOX and Nio-DOX were 5.6 and 1.6 μg/ml after 48h, respectively which show higher cytotoxicity of Nio-DOX against MCF-7. DOX-Nio significantly decreased CD44, CD1133, ALDH1, SOX2 and ABCB1, while free DOX had no significant effect on these markers. The resulting data showed that Nio-DOX has stronger inhibitory effects on MCF-7 viability and expression of CSC markers compared to free drug. Therefore, noisome could be a good carrier system for targeted delivery of the drugs in the treatment of breast cancer.

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INTRODUCTION

Breast cancer remains the most commonly diagnosed cancer among women worldwide and represents the second leading cause of cancer-related death in this population [1]. Common therapeutic drugs include anthracyclines (e.g., doxorubicin/DOX), taxanes (paclitaxel, docetaxel), fluorouracil, and cyclophosphamide. While DOX is a potent chemotherapeutic option, its clinical efficacy is limited by poor blood-brain barrier permeability, low biological stability, and a pronounced first-pass metabolism. Because of these limitations, large administered dose concentrations is required which result in severe side effects for healthy tissues [2]. Chemoresistance is also a problem that limits the effectiveness of chemotherapy and leads to metastasis and relapse of cancer [3]. The possible mechanism of multi-drug resistance in breast cancer cells includes increased resistance to apoptosis, increased DNA repair capacity, overexpression of drug efflux transporters, enzyme systems inactivating antitumor drugs by affecting their metabolism, etc. [4]. All the chemoresistance mechanisms mentioned above were identified in a small subpopulation of cells within tumors

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with self-renewal and differentiation capabilities named cancer stem cells (CSCs) [4, 5]. In conclusion, undesired side effects, drug resistance and recurrence following longer exposure to the drugs, are still the main drawbacks of current chemotherapy drugs.

In order to solve the weaknesses of cancer therapy by chemotherapy drugs, various studies have been done to design drug delivery systems including nano micelles, microemulsions liposomes, polymer-based vesicles, hydrogel, nanoparticulate and niosomes systems for anti-cancer agents such ad DOX [6-10]. These nanocarriers improve absorption, permeability, water solubility, stability, bioavailability distribution, and metabolism of drug and enhance the efficiency of tumor targeting, simultaneously decrease the DOX adverse effects and solve the problem of multidrug resistance [11-13]. In recent years, advancements in nanotechnology within the pharmaceutical field have led to increased research on niosomes as drug delivery systems for cancer therapy. Niosomes are vesicular nanocarriers capable of encapsulating hydrophilic and hydrophobic drugs. These nanocarriers are composed of non-ionic surfactant and lipid such as cholesterol and have biodegradation and biocompatibility properties, adequate encapsulation ability, cost effective preparation and suitable stability [14, 15].

In this study, we loaded DOX into noisome by thin-film hydration (THF) method. To the best of our knowledge, this study is the first one to compare the inhibitory effect of free DOX and Nio-DOX on down regulation of some cancer stem cell markers in MCF-7 cell line.

MATERIAL AND METHODS

Nio-DOX preparation

Niosomes were prepared by film hydration method. A mixture of 300 µM Tween 40 and Span 40/cholesterol (at a 50:50 weight ratio) was dissolved in chloroform for 30 min. The organic solvent was then removed using a rotary evaporator (Heidolf, Germany) at 60 °C, resulting in the formation of a thin film on the bottom of the round-bottom flask. This thin film was subsequently hydrated with phosphate-buffered saline (PBS, pH 7.4) containing DOX (304 mg/ml) and stirred (120 rpm, 40 min). Then, to obtain a uniform niosomes, the hydrated niosomes was sonicated using a sonicator (sonopuls DK 255 P, Bandelin, Germany) with 10 kHz frequency for 3 min (30s on and 10s off). The niosomal suspensions were kept at room temperature for 24 hours to ensure thorough annealing and drug distribution within the lipid bilayer, after which they were refrigerated for further analysis [16, 17]

Niosomes characterization

Size, morphology, and zeta potential assessment

The morphology of niosomes was executed by SEM (KYKY SEM China; EM-3200). The mean size of the niosomes was conducted using a dynamic light scattering (DLS) technique. The evaluation of the zeta potential of the niosomes was undertaken utilizing the Zetasizer Nano ZS (Malvern Instruments). The stability of drug-loaded niosomes was evaluated under different conditions. Samples were stored for three months at 4–8 °C and 25 °C. Particle size and drug entrapment efficiency were measured to assess stability. Each procedure was repeated three times.

Drug entrapment efficiency

The entrapment efficiency of the niosomes was assessed through centrifugation. Specifically, 1 mL of the niosomal suspension was centrifuged at 13,000 rpm for 120 minutes using a Hettich centrifuge (Germany). After centrifugation, the supernatant was separated, and the pellet containing the niosomal lipid bilayers was disrupted with isopropyl alcohol. The concentration of DOX in both the supernatant and the pellet was measured using a UV-spectrophotometer at 491 nm (UV 1800, Shimadzu Co., Japan). The drug entrapment percentage was calculated using the formula:

$$EE\% = (Cp \times 100) / (Cp + Cs),$$

where Cp is the concentration of DOX in the pellet and Cs is the concentration in the supernatant.

In vitro drug release study

The in vitro release study used Franz cells with a diameter of 1. 5 cm and a 20 mL receptor phase volume (Ashke-shisheh CO., Iran). A dialysis membrane (Sigma, MWCO 12400) was soaked in 50% ethanol for 24 h then placed on top of the Franz cells. Nio-DOX and a blank noisome (1 cc) were added to a dialysis bag, and the donor compartment contained 50% ethanol, stirred at 37 ± 0. 2 ˚C. Samples were taken at intervals for 5 hours and analyzed with a UV spectrophotometer at 491 nm [16].

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Stability studies of Nio-DOX

Stability studies involved storing samples at 4° C and 25° C for three months. Measurements of particle size, Zeta potential, and encapsulation efficiency occurred right after preparation and after 30 days.

Cell culture

The human breast MCF-7 cell line was obtained from Pasteur Institute Cell Bank, Tehran, Iran. All cells were markedly cultured in high glucose DMEM medium supplemented with 10% FBS, and 1% penicillin/streptomycin. MCF-7 cells were cultured in incubator with 5% CO2 and at 37 °C. Upon reaching 90% confluency, the cells underwent a washing process using sterile phosphate-buffered saline, followed by detachment from the culture surfaces through trypsinization employing 0.25% trypsin–EDTA. Finally, cells were re-suspended in a complete DMEM medium and used for MTT.

Cell viability assay

The effect of different concentrations of free DOX and Nio-DOX on MCF7 cell viability was assessed by using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide). Briefly, around 12,000 MCF-7 cells were seeded into each well of 96-well plate with 100 mL cell media (DMEM, 10% FBS). For cell adherence, 96-well plate incubated overnight at 37°C and 5% CO2. Different concentrations of free DOX and the Nio-DOX (0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 mM) were added in different wells to see their cytotoxic effects. Control wells for free DOX were treated with medium and for Nio-DOX were treated with blank noisome. After 48 h of exposure to free DOX or Nio-DOX, 10 mL of MTT solution (5 mg/mL PBS) was added to each well. After incubation for 4h, cell media was removed and 100 mL of DMSO was added to dissolve the formazan crystals and then read the absorbance at 570 nm. All experiments were performed in triplicates. The half maximal inhibitory concentration (IC_{50}) values of agents were calculated using Prism 6.0 (GraphPad Software, Inc., San Diego, California, USA).

Drug treatment

Cultured MCF-7 cells were seeded into three groups: a control group (untreated group), a group treated with IC_{50} concentration of DOX, a group treated with IC_{50} concentration of Nio-DOX.

Gene expression analysis by real-time PCR

RNA extraction and cDNA synthesis

Total RNA isolation kit (DENAzist Asia, Mashhad, Iran) was used to extract total RNA from untreated and treated cells and then quantity and quality of RNA were determined using nanodrop and agarose gel electrophoresis. For reverse transcription–polymerase chain reaction, mix 1 µg of the template RNA, 0.5 µg oligo (dt)18 (Cat. No. YT4551; Yekta Tajhiz Azma), 1 mM dNTPs (Cat. No. YT5553; Yekta Tajhiz Azma), and 200 U of M-MuLV reverse transcriptase (Cat. No. EP0441; Thermo Scientific) and DEPS-treated water up to final reaction volume of 20 µl. Mix the above mixture by quick vortex then incubate at 42°C for 60 min and then terminate the reaction by incubation at 70°C for 15 min.

Quantitative real-time PCR

The specific primers for OCT4, NANOG, ALDH1A1, ABCB1, CD133, CD44, SOX2 and B2m (as internal control) were designed using Oligo 7 software (Table 1). Experimental reaction was performed by adding the components in this order, 2x SYBR Green master mix (Cat. No. C101021; Parstous, Iran) (5 ml), primer F and R mix 10 mM (1 ml), cDNA (1 ml), PCR-grade H_2O (3 ml). The reaction microtubes were placed in Rotor-Gene real-time thermal cyclers, and the appropriate program according to the manufacturer's instructions was run, (1 Cycles, 15 min Duration of a cycle, 95 °C Temperature), and (40 Cycles, 20 s duration of cycle in 95 °C Temperature, and 20 s duration of cycle in 62 °C Temperature and 10 s duration of cycle in 72 °C). After real-time RT-PCR, the temperature increased from 60 °C to 95°C at a rate of 1°C/5s to construct a melting curve. The specificity of amplified target genes were verified by agarose gel electrophoresis and melting curve analysis. The $2^{-\Delta\Delta CT}$ *method* was used to analyze the relative changes in gene expression between control and treat group from real-time quantitative PCR experiments. β2M was used as housekeeping genes in real-time PCR data analysis.

Statistical Analysis

GraphPad prism 5 software was used for data analysis. Data are expressed as the mean ± standard deviation of triplicate measurements. One-way ANOVA analysis was also used to determine significant differences between treatment groups and untreated control. Data were presented as the

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Table 1. List of different PCR primers used in the study

Gene name	Sequence (5´ to 3´)	Product size (bp)
Octamer-binding transcription factor 4 (OCT4)	F:CCGAAAGAGAAAGCGAACCAGTAT R: CCACACTCGGACCACATCCTTC	145
Nanog homeobox (NANOG)	F: AATACCTCAGCCTCCAGCAGATG R: CTGCGTCACACCATTGCTATTCT	149
SRY-box (SOX2)	F: GGGAAATGGGAGGGGTGCAAAAGAGG R: TTGCGTGAGTGTGGATGGGATTGGTG	151
Aldehyde dehydrogenase 1 family, member A1 (*ALDH1A1*)	F: TCAGCAGGAGTGTTTACCAA R: CTTACCACGCCATAGCAA	98
ATP Binding Cassette Subfamily B Member 1 (ABCB1)	F: CACCACTGGAGCATTGACTR R: CAGTGTTAGTTGCCAACCAT	151
Prominin 1 (CD133)	F: ACCGACTGAGACCCAACATC R: GGTGCTGTTCATGTTCTCCA	101
CD44	F: AAGGTGGAGCAAACACAACC R: AACTGCAATGCAAACTGCAAG	116
Beta-2-Microglobulin (*β2M*)	F: CTCCGTGGCCTTAGCTGTG R: TTTGGAGTACGCTGGATAGCCT	69

mean ± standard deviation and the significance level was considered at * $p < 0.05$ and ** $p < 0.01$.

RESULTS

Characterizations of the niosomes

The size and morphology of niosomes are shown in figure 1a, b, c. The niosomes showed a spherical vesicular structure with an average size of 67.81 ± 3.25 nm. The zeta potential of the optimized niosomes was -3.48 ± 0.16 mV, which suggests that the charged niosomes are stable against aggregation. Zeta potential was an key factor in drugs penetration since typically negatively charged particles exhibit strong interactions with cell membranes and drug [18].

Drug entrapment efficiency

In this investigation, the optimal DOX niosomes entrapment efficiencies were around 92.03 % ± 2.95. A perfect drug delivery system would have a compact structure, high encapsulation efficiency, and be able to carry sufficient drugs to enter the cell.

Drug release

Figure 1d shows the *in vitro* drug release profile of DOX from the loaded noisome. The formulation showed a sustained release for it. *In vitro* release after 5 h from niosomal dispersion was 51.0 % while about 88.81 % of DOX were released from free solutions. The current experiments obviously showed that the drug amount released from niosomal formulation is effectively delayed compared to the free drug solution selected as control.

Stability studies of Nio-DOX

The stability of Nio-DOX in terms of vesicle particle size, zeta potential, and entrapment efficacy after 3 months storage at both 4-8 and 25 °C have been reported in Table 2.

In vitro cytotoxicity assay

Cytotoxic effects of free DOX and Nio-DOX on MCF-7 cells were assessed by MTT assay. MCF-7 were exposed to various concentrations of free DOX and Nio-DOX (0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 mM) and cell proliferation was assessed after 48 h. As shown in figure 2, free DOX and Nio-DOX significantly inhibited the proliferation of MCF-7 cells in a dose-dependent manner. When comparing the same concentration in both exposures, it clearly appears that the Nio-DOX has enhanced growth inhibitory effects in MCF7 cells

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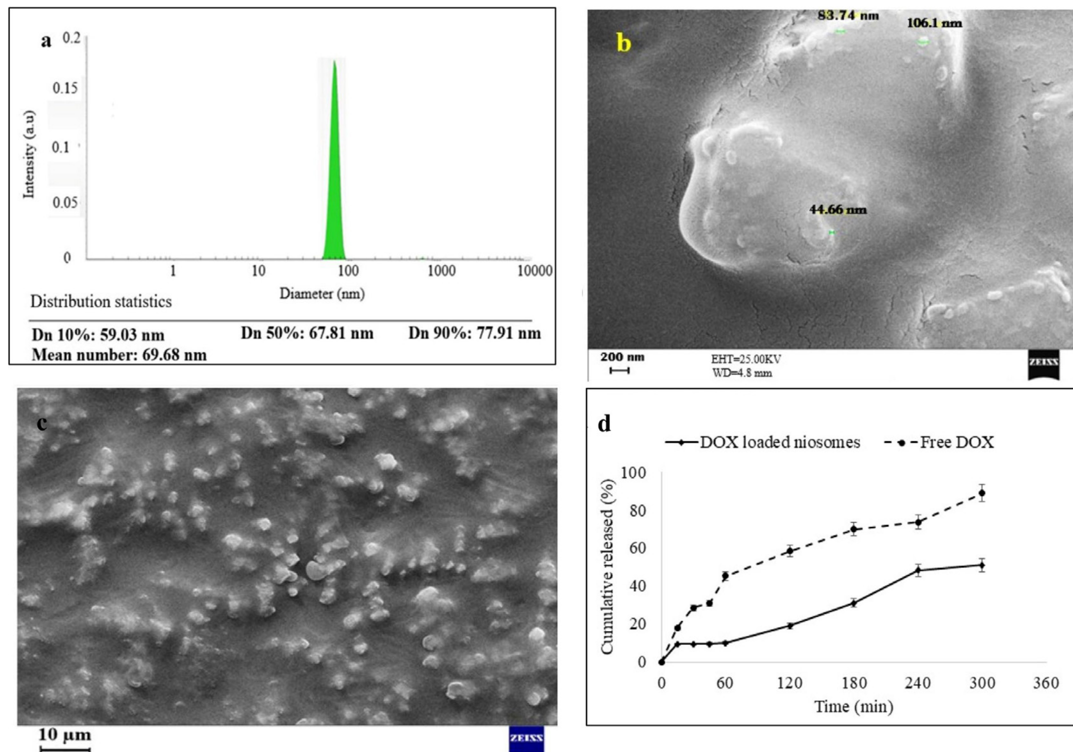

Fig. 1. Characteristics of optimal noisome. Droplet size distribution of optimized niosome using dynamic light scattering technique (a). FE-SEM image (200 nm nanoscale) (b), FE-SEM image (10 µm nanoscale) (c). *In vitro* drug release profile of doxorubicin -loaded noisome (d) (Data represent mean ± SD, n = 3).

Table 2. Stability of doxorubicin noisome: Size, zeta-potential and Entrapment efficiency.

	At 25±2°C			At 4-8°C		
	48 h	1 month	3 months	48 h	1 month	3 months
Size (nm± SD)	67.81±3.25	113.34±3.42	176.14±3.55	67.81±3.25	78.34±2.42	83.14±1.52
Zeta potential (mV± SD)	-3.48 ± 0.16	-7.11±0.95	-13.22±1.2	-3.48 ± 0.16	-4.28±0.71	-7.32±0.71
Entrapment (%± SD)	92.03±2.95	73.15±2.12	58.21±3.13	92.03±2.95	88.26±2.05	85.64±2.98

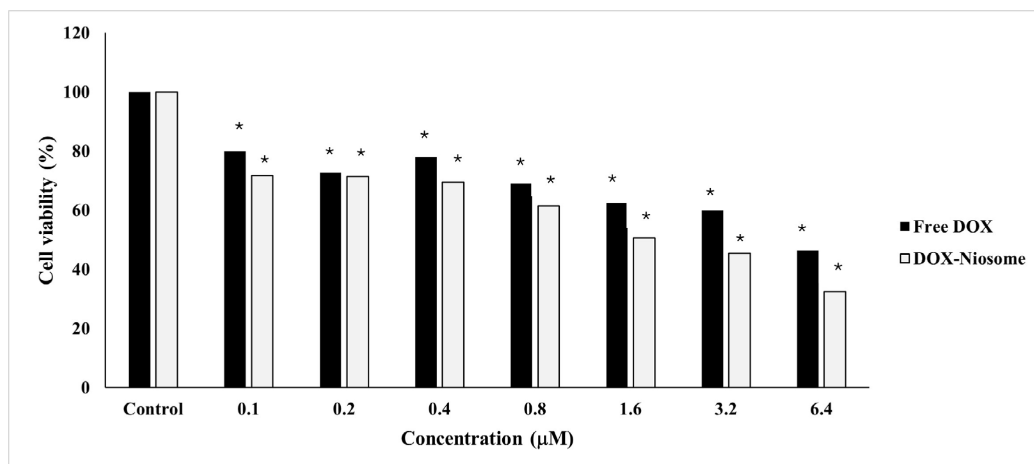

Fig. 2. Cytotoxic effects of DOX and Nio-DOX on human breast cancer MCF-7 cells. The cytotoxicity of different concentrations of free DOX and Nio-DOX on MCF-7 viability after 48 h was assessed by MTT assay. Data are presented in percent (%) in comparison to control. Cell viability in the absence of drug is expressed as 100%. Data are presented as mean ± SD. * $p < 0.05$ indicates significant differences compared to the control group.

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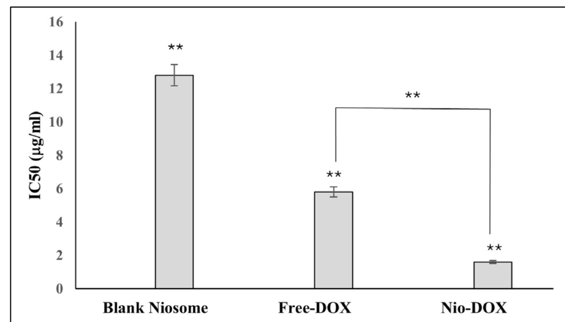

Fig. 3. The bar graph indicates IC_{50} value of empty noisome, free DOX and Nio-DOX after 48 h treatment on MCF-7 cancer cell. To determine the IC_{50} of drugs, percentage of live MCF-7 was measured after 48 h treatment with different concentrations of drugs. Results are represented by mean ± SD (n = 3). ** $p < 0.01$

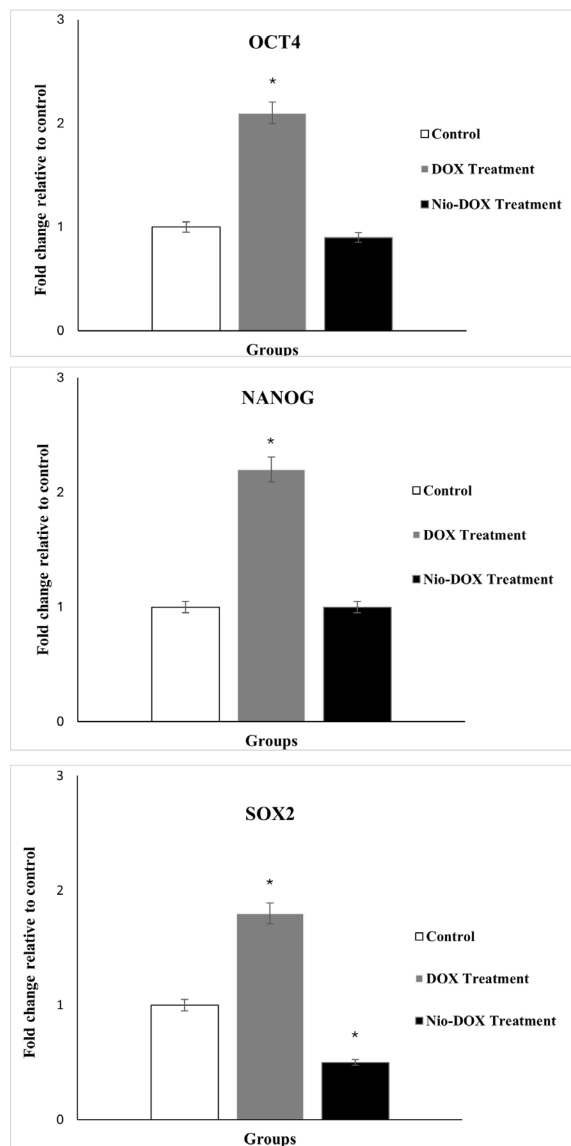

Fig. 4. Expression of pluripotency- associated genes (OCT4, NANOG and SOX2) after treatment of MCF-7 with DOX and Nio-DOX was measured by quantitative real-time PCR and compared to untreated cells as control (designated as 1.0). Results were expressed as mean ± standard error of the mean (SEM) at least three independent experiments. The asterisk indicates a significant (*$p < 0.05$) difference in mRNA expression in comparison with untreated cells.

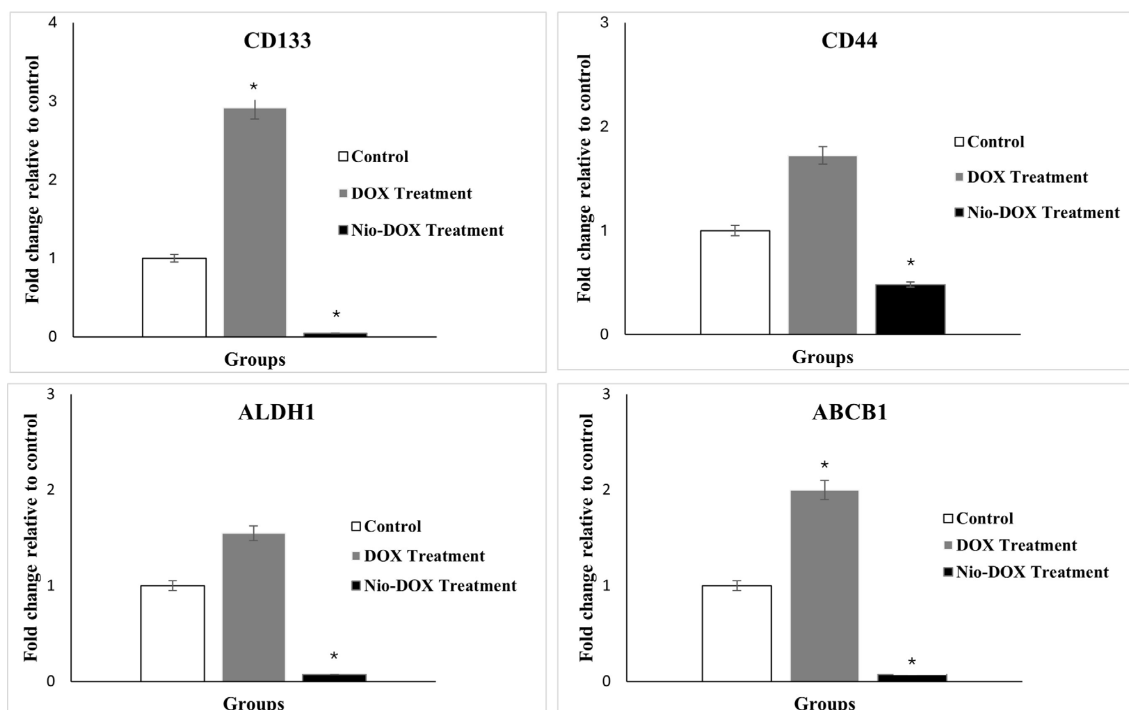

Fig. 5. qRT-PCR analysis of CD44, CD133, ABCB1 and ALDH1 gene expression after treatment of MCF-7 with DOX and Nio-DOX. Results were expressed as mean ± standard error of the mean (SEM) at least three independent experiments, and significant differences were compared to the untreated MCF-7 control cells (designated as 1.0). The asterisk indicates a significant (*p < 0.05) difference in mRNA expression in comparison with untreated cells.

in comparison to free DOX. IC_{50} doses of free DOX and Nio-DOX were determined and Nio-DOX (IC_{50} value 1.6 mM) exhibited a 3.5-fold reduction in IC_{50} compared to free DOX (IC_{50}: 5.6 mM). Although formulation of Nisome was optimized to have the least toxicity, it showed little toxicity due to its constituent detergent compounds (IC_{50} value of empty noisome was more than 12.8 mM) (Figure 3).

Expression analysis of CSC markers

QRT-PCR was performed to investigate expression values of some important CSC markers at mRNA level after MCF-7 treatment with free DOX and Nio-DOX. As shown in Figure 4, expressions of OCT4, NANOG and SOX2 increased about 2-fold after treatment with free DOX. However, treatment with Nio-DOX didn't change expression of OCT4 and NANOG significantly and reduced expression of SOX2 about 2-fold. Furthermore, as shown in Figure 5, expressions of CD44, CD133, ALDH1 and ABCB1 genes increased 1.5-2.9-fold after treatment with free DOX. However, our results demonstrated that the expression of CD44: 52%, CD133: 95%, ALDH1: 92% and ABCB1: 97% significantly decreased in MCF-7 cells after Nio-DOX treatment compared with control group. Therefore, our results showed that noisome nanoparticles increased the inhibitory effects of DOX.

DISCUSSION

In this study we prepared noisome with spherical structure as a promising delivery system with size, zeta potential, and entrapment efficiency values of 67.81 ± 3.25 nm, -3.48 ± 0.16 mV, and 92.03±2.95 %, respectively which indicates an efficient encapsulation of DOX. The prepared noisomes remained stable for three months at -4 ˚C. The release profiles of DOX loaded in niosomes revealed the extended release over of 5 h. Considering the physical and chemical properties of Nio-DOX, they demonstrated a stable, permeable, and cost-effective nanocarrier for delivering DOX to cancer cells. Based on cytotoxicity studies, blank noisome showed no significant toxicity effect on cells confirming cytocompatibility of the nanocarrier. Moreover, Nio-DOX showed a 3.5-fold reduction in IC_{50} values compared with free DOX which show better cytotoxic effects of Nio-DOX against breast

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cancer cells compared with free administered DOX, probably due to better internalizations of niosomes and a sustained release of DOX. Present data also indicates a greater effect of Nio-DOX on down regulation of CSCs markers including CD44, CD133, ALDH1 and ABCB1 in comparison with free DOX. Among pluripotent transcription factors, although, SOX-2 down regulate two-fold after Nio-DOX treatment, expression of OCT4 and NANOG didn't change.

DOX is a potent chemotherapy drug approved by the US Food and Drug Administration for the treatment of breast cancer [13, 19-23]. Despite the positive effects of it, severe side toxic effects including fatigue, alopecia, nausea, hair loss, vomiting, and oral sores and long treatment durations are vexing drawbacks in the use of this drug [24]. DOX has also adverse effects on the immune system, making the patient susceptible to infections [13]. Physical barrier to diffusion of drugs and insufficient drug delivery within the tumor is another limiting factor. Moreover, resistance to DOX has frequently appeared in reports on breast cancer [25, 26]. Breast CSCs are a subpopulation of breast cancers that express stem cell- associated markers and have high capacity for tumor formation [27-29]. Drug resistance of Breast CSCs to traditional chemotherapy (e.g. DOX) lead to anti-cancer treatment failure and cancer recurrence. [30, 31].

According to our results, expression of most CSCs markers that were investigated increased following treatment with free DOX which show drug resistance of cancer stem-like cells to DOX. Similarly, another study showed an increase in expression of stem-cell marker in spheroids formed from doxorubicin-treated cells when compared to non-treated cells [32]. Moreover, in another research, a DOX-resistant MCF-7 cell line was established through a series of long-term DOX *in vitro* treatment and data revealed that DOX-resistant MCF-7 cells showed increased cancer stemness, multi-drug resistance compared and invasiveness compared with parental cells [29].

Role of nanocarriers in enhancing chemotherapy efficacy of drugs and overcoming the limitations of conventional chemotherapy for cancer treatment is demonstrated in a lot of research [33]. Noisome is considered as a new drug delivery systems that are preferable to other nanocarriers, due to simple preparation, low cost and stability, safety, biodegradability, biocompatibility and prominent quality for solubilizing hydrophobic agents [34]. Noisome can preserve drugs from degradation, metabolism and clearance, enzyme inhibition by encapsulating them and thus improving the bioavailability of drugs [35-39]. Therefore, controlled-release Nuisomes lead to increased drug persistence and stability in the target tissue while simultaneously reducing side effects, including toxicity and hemolysis [39, 40].

In this study, we encapsulated DOX into noisome as a safe nanocarrier with little cytotoxicity and indicated that noisome can intensify the cytotoxicity of DOX against MCF-7 cells. Encapsulation of DOX inside noisome were also carried out in previous studies. Nio-DOX demonstrated a slight decrease in the IC50 value when assessed against the doxorubicin-resistant ovarian cell line, while simultaneously exhibiting an enhanced anticancer efficacy against the MCF-7, MDA MB 468 cell lines, as well as KG-1 acute myeloblastic leukemia cells; notably, the findings of this investigation align with those previously reported in the literature [2, 41, 42]. Niosomal DOX showed lower IC_{50} value compared with free administered DOX against MCF-7 cells which was similar to our result [43]. In another study, niosomal formulations of DOX overcame the limitation of DOX to cross the blood-brain barrier and ultimately improved the delivery of DOX into the brain for the treatment of brain tumors [44]. *In vitro* cytotoxicity studies showed improved cytotoxicity of Tamoxifen- and DOX-loaded niosomes on MCF-7 cells as well as better synergistic effect compared to the free drug combination [45]. Furthermore cationic PEGylated niosomes co-loaded with DOX, quercetins, and short interfering RNAs have also yielded promising results for treatment of gastric cancer [46]. Melittin-loaded niosomes has been shown to down-regulate the expression of Bcl2, MMP2, and MMP9 genes while up-regulate the expression of Bax, Caspase3 and Caspase9 genes in breast cell lines. They have also enhanced the apoptosis rate and inhibited cell migration, invasion compared to the free melittin. Therefore, melittin-loaded niosomes showed more anti-cancer effects than free melittin and niosomes are proper vesicle carriers for melittin [17]. Furthuremore, a number of researches have reported the use of niosomes as drug carriers for the delivery of many anti-cancer drugs such as artemisone, estradiol, gemcitabine, cisplatin, 5-FU, paclitaxel, gamma oryzanol, balanocarpol and tamoxifen for the treatment of cancers. In all

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studies, niosomal formulation of drugs can resolve their inadequate aqueous solvability and restricted permeability and enhance the solubility of drugs and help to drug retention which is important for long-term delivery [34, 47-58].

To our knowledge, no studies have been conducted to investigate the effect of Nio-DOX on expression of CSC markers. According to our results, while Nio-DOX treatment showed significant effect on down regulation of some CSC markers including CD44, CD133, ALDH1, ABCB1 and SOX-2, treatment with free DOX increased them. CSCs in solid tumors like breast tumors are distinguished by expression of certain cell surface biomarkers, such as CD133, CD44 [59]. CD133 and CD44 are membrane glycoproteins involved in migration, cell adhesion, and drug resistance and originally classified as a CSC marker in many solid tumors [60-64]. High ALDH1 activity is defined as a marker for CSC populations in many cancer types [65, 66]. Increased expression of ATP binding cassette (ABC) transporter superfamily which actively pump various chemotherapeutic drugs out of cancer stem cells, leads to multidrug resistance (MDR) in these cells by reducing intracellular drug accumulation and thus impairing chemotherapy effectiveness [67]. The expression of NANOG, OCT4 and SOX2 is crucial for stemness maintenance in normal and cancer stem cells and considered as integral regulators of pluripotency [68, 69].

Encapsulating DOX in niosomes increases its effectiveness in reducing CSC markers in MCF-7 cells primarily due to enhanced drug delivery and sustained release. The niosome's surface characteristics, including its size and composition, can influence its uptake by cells, including CSCs. Therefore, concentration of drugs within the cell can be increased, potentially overcoming the effect of ABC transporters that are trying to remove the drug. This contributed to better drug efficacy and potentially overcome drug resistance. Furthermore, Niosomes act as a nano-carrier, improving stability and protecting DOX from degradation and allowing it to be selectively transported to the target cells. This results in higher DOX concentrations at the site of action, thus improving its ability to inhibit CSC markers [70, 71]. Finally, CSCs are notorious for pumping drugs out via efflux pumps like ABC transporters. Encapsulation can sometime bypass these mechanisms because the noisome get inside by endocytosis, delivering the drugs inside the cell where it is harder to pump out. Furthuremore, niosomes can indirectly affect the activity of ABC transporters, which are also involved in drug efflux and drug resistance in CSCs. Inhibition of P-glycoprotein (P-gp) by noisome lead to enhanced bioavailability of anticancer drugs and higher efficiency in drug delivery to the CSCs in comparison with free drugs [12, 13, 34, 54].

Various nanodrug delivery systems such as solid lipid nanoparticles, magnetic nanoparticles, polymeric nanoparticles, dendrimers, micelles, liposomes, quantum dots, etc are utilized for chemotherapeutic drug delivery. These nanocarriers help to overcome drug resistance by bypassing the drug efflux mechanisms mediated by ABC transporters, which typically pump drugs out of cells and reduce their effectiveness [72]. For example, some research revealed the potential of DOX solid lipid nanoparticles (SLNs) in overcoming P-gp-mediated multi drug resistance in resistant leukemia cells and Adriamycin-resistant breast cancer cell line. Greater accumulation of DOX SLNs in resistant cells with enhanced apoptotic cell death and decreased cell viability compared to plain DOX revealed the potential of DOX SLNs to overcome chemoresistance [73-75]. According to another study, DOX liposomes increased cytotoxicity on vincristine resistant HL60 cells due to directly interaction with P-gp and inhibition of P-gp through endocytosis [76]

CONCLUSION

In conclusion, noisome are promising nanocarriers for encapsulating anti-cancer drugs to improve their release and, thus therapeutic affect and biological activity. Noisome encapsulated drugs significantly enhanced antitumor activity and cellular uptake of drugs as compared to free solution of the drug [34, 47-55]. According to our results, Nio-DOX have greater cytotoxicity against MCF-7 cells and inhibitory effect on CSC markers thus rendering noisome a promising nanocarrier for the potential treatment of metastatic breast cancer. However, niosomal applications as a delivery systems for drugs have some difficulties due to some disadvantages such as aggregation, leakage of the entrapped drug, time-consuming preparation procedure, high cost of preparation, difficulties for large-scale production, large amount of drugs that remain unencapsulated during the synthesis and other challenges that still have to be solved to make niosomes clinically applicable [43, 48, 71].

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-49. https://doi.org/10.3322/caac.21660
2. Di Francesco M, Celia C, Cristiano MC, d'Avanzo N, Ruozi B, Mircioiu C, et al. Doxorubicin hydrochloride-loaded nonionic surfactant vesicles to treat metastatic and non-metastatic breast cancer. ACS Omega. 2021;6(4):2973-89. https://doi.org/10.1021/acsomega.0c05350
3. Mansoori B, Mohammadi A, Davudian S, Shirjang S, Baradaran B. The different mechanisms of cancer drug resistance: a brief review. Adv Pharm Bull. 2017;7(3):339-48. https://doi.org/10.15171/apb.2017.041
4. Ji X, Lu Y, Tian H, Meng X, Wei M, Cho WC. Chemoresistance mechanisms of breast cancer and their countermeasures. Biomed Pharmacother. 2019;114:108800. https://doi.org/10.1016/j.biopha.2019.108800
5. Jordan CT, Guzman ML, Noble M. Cancer stem cells. N Engl J Med. 2006;355(12):1253-61. https://doi.org/10.1056/NEJMra061808
6. Khutale GV, Casey A. Synthesis and characterization of a multifunctional gold-doxorubicin nanoparticle system for pH triggered intracellular anticancer drug release. Eur J Pharm Biopharm. 2017;119:372-80. https://doi.org/10.1016/j.ejpb.2017.07.009
7. Rahdar A, Sargazi S, Barani M, Shahraki S, Sabir F, Aboudzadeh MA. Lignin-stabilized doxorubicin microemulsions: Synthesis, physical characterization, and in vitro assessments. Polymers (Basel). 2021;13(4):641. https://doi.org/10.3390/polym13040641
8. Rahdar A, Hajinezhad MR, Barani M, Sargazi S, Zaboli M, Ghazy E, et al. Pluronic F127/Doxorubicin microemulsions: Preparation, characterization, and toxicity evaluations. J Mol Liq. 2022;345:117028. https://doi.org/10.1016/j.molliq.2021.117028
9. Sriraman SK, Salzano G, Sarisozen C, Torchilin V. Anti-cancer activity of doxorubicin-loaded liposomes co-modified with transferrin and folic acid. Eur J Pharm Biopharm. 2016;105:40-9. https://doi.org/10.1016/j.ejpb.2016.05.023
10. Kim K, Oh KS, Park DY, Lee JY, Lee BS, Kim IS, et al. Doxorubicin/gold-loaded core/shell nanoparticles for combination therapy to treat cancer through the enhanced tumor targeting. J Control Release. 2016;228:141-9. https://doi.org/10.1016/j.jconrel.2016.03.009
11. Hosseinian S, Rad AK, Roshan NM, Havakhah S, Shafiee S. The protective effect of Nigella sativa against cisplatin-induced nephrotoxicity in rats. Avicenna J Phytomed. 2016;6(1):44-54.
12. Basati G, Khaksarian M, Abbaszadeh S, Lashgarian HE, Marzban A. Cancer stem cells and nanotechnological approaches for eradication. Stem Cell Investig. 2019;6:6. https://doi.org/10.21037/sci.2019.10.07
13. Tacar O, Sriamornsak P, Dass CR. Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. J Pharm Pharmacol. 2013;65(2):157-70. https://doi.org/10.1111/j.2042-7158.2012.01567.x
14. Akbarzadeh I, Shayan M, Bourbour M, Moghtaderi M, Noorbazargan H, Eshrati Yeganeh F, et al. Preparation, optimization and in-vitro evaluation of curcumin-loaded niosome@calcium alginate nanocarrier as a new approach for breast cancer treatment. Biology (Basel). 2021;10(3):173. https://doi.org/10.3390/biology10030173
15. Nematollahi MH, Pardakhty A, Torkzadeh-Mahanai M, Mehrabani M, Asadikaram G. Changes in physical and chemical properties of niosome membrane induced by cholesterol: a promising approach for niosome bilayer intervention. RSC Adv. 2017;7(78):49463-72. https://doi.org/10.1039/C7RA07834J
16. Mohamadi N, Soltanian S, Raeiszadeh M, Moeinzadeh M, Ohadi M, Sharifi F, et al. Characteristics and in vitro anti skin aging activity and UV radiation protection of morin loaded in niosomes. J Cosmet Dermatol. 2022;21(11):6326-35. https://doi.org/10.1111/jocd.15273
17. Dabbagh Moghaddam F, Akbarzadeh I, Marzbankia E, Farid M, Khaledi L, Reihani AH, et al. Delivery of melittin-loaded niosomes for breast cancer treatment: an in vitro and in vivo evaluation of anti-cancer effect. Cancer Nanotechnol. 2021;12(1):14. https://doi.org/10.1186/s12645-021-00085-9
18. Mohamad Saimi NI, Salim N, Ahmad N, Abdulmalek E, Abdul Rahman MB. Aerosolized niosome formulation containing gemcitabine and cisplatin for lung cancer treatment: Optimization, characterization and in vitro evaluation. Pharmaceutics. 2021;13(1):59. https://doi.org/10.3390/pharmaceutics13010059
19. Carvalho C, Santos RX, Cardoso S, Correia S, Oliveira PJ, Santos MS, et al. Doxorubicin: the good, the bad and the ugly effect. Curr Med Chem. 2009;16(25):3267-85. https://doi.org/10.2174/092986709788803312
20. Khasraw M, Bell R, Dang C. Epirubicin: is it like doxorubicin in breast cancer? A clinical review. Breast. 2012;21(2):142-9. https://doi.org/10.1016/j.breast.2011.12.012
21. AbuHammad S, Zihlif M. Gene expression alterations in doxorubicin resistant MCF7 breast cancer cell line. Genomics. 2013;101(4):213-20. https://doi.org/10.1016/j.ygeno.2012.11.009
22. Sritharan S, Sivalingam N. A comprehensive review on time-tested anticancer drug doxorubicin. Life Sci. 2021;278:119527. https://doi.org/10.1016/j.lfs.2021.119527
23. Minotti G, Recalcati S, Mordente A, Liberi G, Calafiore AM, Mancuso C, et al. The secondary alcohol metabolite of doxorubicin irreversibly inactivates aconitase/iron regulatory protein-1 in cytosolic fractions from human myocardium. FASEB J. 1998;12(7):541-52. https://doi.org/10.1096/fasebj.12.7.541
24. Tavano L, Vivacqua M, Carito V, Muzzalupo R, Caroleo MC, Nicoletta F. Doxorubicin loaded magneto-niosomes for targeted drug delivery. Colloids Surf B Biointerfaces. 2013;102:803-7. https://doi.org/10.1016/j.colsurfb.2012.09.019

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25. Greife A, Tukova J, Steinhoff C, Scott SD, Schulz WA, Hatina J. Establishment and characterization of a bladder cancer cell line with enhanced doxorubicin resistance by mevalonate pathway activation. Tumour Biol. 2015;36(5):3293-300. https://doi.org/10.1007/s13277-014-2959-9
26. Arora HC, Jensen MP, Yuan Y, Wu A, Vogt S, Paunesku T, et al. Nanocarriers enhance doxorubicin uptake in drug-resistant ovarian cancer cells. Cancer Res. 2012;72(3):769-78. https://doi.org/10.1158/0008-5472.CAN-11-2890
27. Anand U, Dey A, Chandel AKS, Sanyal R, Mishra A, Pandey DK, et al. Cancer chemotherapy and beyond: Current status, drug candidates, associated risks and progress in targeted therapeutics. Genes Dis. 2023;10(4):1367-401. https://doi.org/10.1016/j.gendis.2022.02.007
28. Paramanantham A, Jung EJ, Kim HJ, Jeong BK, Jung JM, Kim GS, et al. Doxorubicin-resistant TNBC cells exhibit rapid growth with cancer stem cell-like properties and EMT phenotype, which can be transferred to parental cells through autocrine signaling. Int J Mol Sci. 2021;22(22):12438. https://doi.org/10.3390/ijms222212438
29. Chong KH, Chang YJ, Hsu WH, Tu YT, Chen YR, Lee MC, et al. Breast cancer with increased drug resistance, invasion ability, and cancer stem cell properties through metabolism reprogramming. Int J Mol Sci. 2022;23(21):12875. https://doi.org/10.3390/ijms232112875
30. Alfarouk KO, Stock CM, Taylor S, Walsh M, Muddathir AK, Verduzco D, et al. Resistance to cancer chemotherapy: failure in drug response from ADME to P-gp. Cancer Cell Int. 2015;15:71. https://doi.org/10.1186/s12935-015-0221-1
31. Xiang D, Shigdar S, Bean AG, Bruce M, Yang W, Mathesh M, et al. Transforming doxorubicin into a cancer stem cell killer via EpCAM aptamer-mediated delivery. Theranostics. 2017;7(17):4071-86. https://doi.org/10.7150/thno.20168
32. Balahmar RM, Deepak V, Sivasubramaniam S. Doxorubicin resistant choriocarcinoma cell line derived spheroidal cells exhibit stem cell markers but reduced invasion. 3 Biotech. 2022;12(9):184. https://doi.org/10.1007/s13205-022-03243-x
33. Ghazal H, Waqar A, Yaseen F, Shahid M, Sultana M, Tariq M, et al. Role of nanoparticles in enhancing chemotherapy efficacy for cancer treatment. Next Mater. 2024:100128. https://doi.org/10.1016/j.nxmate.2024.100128
34. Kanaani L, Ebrahimifar M, Khiyavi AA, Mehrdiba T. Effects of cisplatin-loaded niosomal nanoparticles on BT-20 human breast carcinoma cells. Asian Pac J Cancer Prev. 2017;18(2):365-72. https://doi.org/10.31557/apjcb.2017.2.2.27-29
35. Bagheri A, Chu BS, Yaakob H. Niosomal drug delivery systems: Formulation, preparation and applications. World Appl Sci J. 2014;32(8):1671-85.
36. Sharma V, Anandhakumar S, Sasidharan M. Self-degrading niosomes for encapsulation of hydrophilic and hydrophobic drugs: an efficient carrier for cancer multi-drug delivery. Mater Sci Eng C Mater Biol Appl. 2015;56:393-400. https://doi.org/10.1016/j.msec.2015.06.049
37. Kumar GP, Rajeshwarrao P. Nonionic surfactant vesicular systems for effective drug delivery-an overview. Acta Pharm Sin B. 2011;1(4):208-19. https://doi.org/10.1016/j.apsb.2011.09.002
38. Reddy BS, Padman JSC, Santosh V. Niosomes as nanocarrier systems: a review. Int J Pharm Sci Res. 2012;3(6):1560-7.
39. Xu YQ, Chen WR, Tsosie JK, Xie X, Li P, Wan JB, et al. Niosome encapsulation of curcumin: characterization and cytotoxic effect on ovarian cancer cells. J Nanomater. 2016;2016:9248531. https://doi.org/10.1155/2016/6365295
40. Moghassemi S, Hadjizadeh A. Nano-niosomes as nanoscale drug delivery systems: an illustrated review. J Control Release. 2014;185:22-36. https://doi.org/10.1016/j.jconrel.2014.04.015
41. Uchegbu IF, Double JA, Kelland LR, Turton JA, Florence AT. The activity of doxorubicin niosomes against an ovarian cancer cell line and three in vivo mouse tumour models. J Drug Target. 1996;3(5):399-409. https://doi.org/10.3109/10611869608996831
42. Bahrami-Banan F, Sheikhha MH, Ghasemi N, Majdizadeh M, Haghiralsadat BF. Preparation and study of nano-niosomes containing doxorubicin and evaluation of its toxicity on acute myeloblastic leukemia cell line KG-1. Payavard Salamat. 2018;12(4):309-23.
43. Sargazi S, Barani M, Zargari F, Arshad R, Sharma RK. Preparation of pH-Responsive Vesicular Doxorubicin: Evidence from In-Vitro and In-Silico Evaluations. Curr Appl Sci. 2022:31-48.
44. Bragagni M, Mennini N, Ghelardini C, Mura P. Development and characterization of niosomal formulations of doxorubicin aimed at brain targeting. J Pharm Pharm Sci. 2012;15(1):184-96. https://doi.org/10.18433/J3230M
45. Kulkarni P, Rawtani D. Application of box-behnken design in the preparation, optimization, and in vitro evaluation of self-assembly-based tamoxifen-and doxorubicin-loaded and dual drug-loaded niosomes for combinatorial breast cancer treatment. J Pharm Sci. 2019;108(8):2643-53. https://doi.org/10.1016/j.xphs.2019.03.020
46. Hemati M, Haghiralsadat F, Jafary F, Moosavizadeh S, Moradi A. Targeting cell cycle protein in gastric cancer with CDC20siRNA and anticancer drugs (doxorubicin and quercetin) co-loaded cationic PEGylated nanoniosomes. Int J Nanomedicine. 2019;14:6575-85. https://doi.org/10.2147/IJN.S211844
47. Hajizadeh MR, Parvaz N, Barani M, Khoshdel A, Fahmidehkar MA, Mahmoodi M, et al. Diosgenin-loaded niosome as an effective phytochemical nanocarrier: Physicochemical characterization, loading efficiency, and cytotoxicity assay. Daru. 2019;27(1):329-39. https://doi.org/10.1007/s40199-019-00277-0
48. Ge X, Wei M, He S, Yuan WE. Advances of non-ionic surfactant vesicles (niosomes) and their application in drug delivery. Pharmaceutics. 2019;11(2):55. https://doi.org/10.3390/pharmaceutics11020055
49. Wang J, Sui M, Fan W. Nanoparticles for tumor targeted therapies and their pharmacokinetics. Curr Drug Metab. 2010;11(2):129-41. https://doi.org/10.2174/138920010791110827
50. Dwivedi A, Mazumder A, Du Plessis L, Du Preez JL, Haynes RK, Du Plessis J. In vitro anti-cancer effects of artemisone nano-vesicular formulations on melanoma cells. Nanomedicine. 2015;11(8):2041-50. https://doi.org/10.1016/j.nano.2015.07.010
51. Gude R, Jadhav M, Rao S, Jagtap A. Effects of niosomal cisplatin and combination of the same with theophylline and with activated macrophages in murine B16F10 melanoma model. Cancer Biother Radiopharm. 2002;17(2):183-92. https://doi.org/10.1089/108497802753773801
52. Cosco D, Paolino D, Muzzalupo R, Celia C, Citraro R,

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Caponio D, et al. Novel PEG-coated niosomes based on bola-surfactant as drug carriers for 5-fluorouracil. Biomed Microdevices. 2009;11(5):1115-25. https://doi.org/10.1007/s10544-009-9328-2
53. Shaker DS, Shaker MA, Hanafy MS. Cellular uptake, cytotoxicity and in-vivo evaluation of Tamoxifen citrate loaded niosomes. Int J Pharm. 2015;493(1-2):285-94. https://doi.org/10.1016/j.ijpharm.2015.07.041
54. Mahale N, Thakkar P, Mali R, Walunj D, Chaudhari S. Niosomes: novel sustained release nonionic stable vesicular systems-an overview. Adv Colloid Interface Sci. 2012;183-184:46-54. https://doi.org/10.1016/j.cis.2012.08.002
55. Bayindir ZS, Yuksel N. Characterization of niosomes prepared with various nonionic surfactants for paclitaxel oral delivery. J Pharm Sci. 2010;99(4):2049-60. https://doi.org/10.1002/jps.21944
56. Shah HS, Gotecha A, Jetha D, Rajput A, Bariya A, Panchal S, et al. Gamma oryzanol niosomal gel for skin cancer: formulation and optimization using quality by design (QbD) approach. AAPS Open. 2021;7:1-15. https://doi.org/10.1186/s41120-021-00041-2
57. Mohamad Saimi NI, Salim N, Ahmad N, Abdulmalek E, Abdul Rahman MB. Aerosolized niosome formulation containing gemcitabine and cisplatin for lung cancer treatment: Optimization, characterization and in vitro evaluation. Pharmaceutics. 2021;13(1):59. https://doi.org/10.3390/pharmaceutics13010059
58. Akbarzadeh I, Fatemizadeh M, Heidari F, Niri NM. Niosomal formulation for co-administration of hydrophobic anticancer drugs into MCF-7 cancer cells. Arch Adv Biosci. 2020;11(2):1-9.
59. Wright MH, Calcagno AM, Salcido CD, Carlson MD, Ambudkar SV, Varticovski L. Brca1 breast tumors contain distinct CD44+/CD24- and CD133+ cells with cancer stem cell characteristics. Breast Cancer Res. 2008;10(1):R10. https://doi.org/10.1186/bcr1855
60. Baba T, Convery P, Matsumura N, Whitaker R, Kondoh E, Perry T, et al. Epigenetic regulation of CD133 and tumorigenicity of CD133+ ovarian cancer cells. Oncogene. 2009;28(2):209-18. https://doi.org/10.1038/onc.2008.374
61. Artells R, Moreno I, Diaz T, Martinez F, Gel B, Navarro A, et al. Tumour CD133 mRNA expression and clinical outcome in surgically resected colorectal cancer patients. Eur J Cancer. 2010;46(3):642-9. https://doi.org/10.1016/j.ejca.2009.11.003
62. Wiranowska M, Ladd S, Smith SR, Gottschall PE. CD44 adhesion molecule and neuro-glial proteoglycan NG2 as invasive markers of glioma. Brain Cell Biol. 2006;35:159-72. https://doi.org/10.1007/s11068-007-9009-0
63. Bendardaf R, Algars A, Elzagheid A, Korkeila E, Ristamäki R, Lamlum H, et al. Comparison of CD44 expression in primary tumours and metastases of colorectal cancer. Oncol Rep. 2006;16(4):741-6. https://doi.org/10.3892/or.16.4.741
64. Brugnoli F, Grassilli S, Al-Qassab Y, Capitani S, Bertagnolo V. CD133 in breast cancer cells: more than a stem cell marker. J Oncol. 2019;2019:7512632. https://doi.org/10.1155/2019/7512632
65. Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, et al. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell. 2007;1(5):555-67. https://doi.org/10.1016/j.stem.2007.08.014
66. Balicki D. Moving forward in human mammary stem cell biology and breast cancer prognostication using ALDH1. Cell Stem Cell. 2007;1(5):485-7. https://doi.org/10.1016/j.stem.2007.10.015
67. Begicevic RR, Falasca M. ABC Transporters in Cancer Stem Cells: Beyond Chemoresistance. Int J Mol Sci. 2017;18(11):2362. https://doi.org/10.3390/ijms18112362
68. Leis O, Eguiara A, Lopez-Arribillaga E, Alberdi M, Hernandez-Garcia S, Elorriaga K, et al. Sox2 expression in breast tumours and activation in breast cancer stem cells. Oncogene. 2012;31(11):1354-65. https://doi.org/10.1038/onc.2011.338
69. Hepburn A, Steele R, Veeratterapillay R, Wilson L, Kounatidou E, Barnard A, et al. The induction of core pluripotency master regulators in cancers defines poor clinical outcomes and treatment resistance. Oncogene. 2019;38(22):4412-24. https://doi.org/10.1038/s41388-019-0712-y
70. Vadlamudi C, Sevukarajan M. Niosomal drug delivery system-a review. Indo Am J Pharm Res. 2012;2(9):1222-8.
71. Moammeri A, Chegeni MM, Sahrayi H, Ghafelehbashi R, Memarzadeh F, Mansouri A, et al. Current advances in niosomes applications for drug delivery and cancer treatment. Mater Today Bio. 2023;23:100837. https://doi.org/10.1016/j.mtbio.2023.100837
72. Ayers D, Nasti A. Utilisation of nanoparticle technology in cancer chemoresistance. J Drug Deliv. 2012;2012:265691. https://doi.org/10.1155/2012/265691
73. Ma P, Dong X, Swadley CL, Gupte A, Leggas M, Ledebur HC, et al. Development of idarubicin and doxorubicin solid lipid nanoparticles to overcome Pgp-mediated multiple drug resistance in leukemia. J Biomed Nanotechnol. 2009;5(2):151-61. https://doi.org/10.1166/jbn.2009.1021
74. Li B, Xu H, Li Z, Yao M, Xie M, Shen H, et al. Bypassing multidrug resistance in human breast cancer cells with lipid/polymer particle assemblies. Int J Nanomedicine. 2012;7:187-97. https://doi.org/10.2147/IJN.S27864
75. Kang KW, Chun MK, Kim O, Subedi RK, Ahn SG, Yoon JH, et al. Doxorubicin-loaded solid lipid nanoparticles to overcome multidrug resistance in cancer therapy. Nanomedicine. 2010;6(2):210-3. https://doi.org/10.1016/j.nano.2009.12.006
76. Gokhale P, Radhakrishnan B, Husain S, Abernethy D, Sacher R, Dritschilo A, et al. An improved method of encapsulation of doxorubicin in liposomes: pharmacological, toxicological and therapeutic evaluation. Br J Cancer. 1996;74(1):43-8. https://doi.org/10.1038/bjc.1996.313

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