

Research article

Co-encapsulation of thymoquinone with docetaxel enhances the encapsulation efficiency into PEGylated liposomes and the chemosensitivity of MCF7 breast cancer cells to docetaxel



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ABSTRACT

Combinatorial therapeutic strategies to eradicate tumors can be superior to a single therapeutic modality. Docetaxel (DT) has been approved for the treatment of local or metastasized breast cancer alone or in combination with other chemotherapeutic agents. Thymoquinone (TQ) originated from the seeds of *Nigella Sativa* plant has been reported to possess *in vitro* and *in vivo* antitumor activity against variety of tumors. In the current study, we have investigated the synergistic anticancer efficacy of a novel combination of DT and TQ on MCF7 breast cancer cell line using MTT cell viability assay. Moreover, this study describes for the first time the co-encapsulation of DT and TQ into PEGylated liposomes. The results showed that the combination of DT and TQ resulted in significant synergistic cytotoxicity compared to DT and TQ alone. Moreover, DT and TQ have been successfully co-encapsulated into PEGylated liposomes with higher encapsulation efficiency compared to DT and TQ alone. In conclusion, DT and TQ combination poses a synergistic effect and may aid in decreasing the required doses of DT. Also, the co-encapsulation of DT and TQ into PEGylated liposomes can provide a promising DT and TQ delivery system into cancer cells.

1. Introduction

Cancer is a global health problem and considered one of the main causes of morbidity and mortality worldwide [1]. Tumor cells are characterized by their high degree of heterogeneity, plasticity, and acquiring/ inheriting resistance to anti-cancer drugs [2]. During the last decades, several strategies have been developed to enhance therapeutic potency and bypassing tumor recurrence [3]. These strategies include i) the development of targeted therapies, ii) engineering drug delivery systems, and iii) using combinatorial therapeutic modalities [4, 5]. There is firm evidence showing the importance of using combinatorial therapeutic approaches that can target the hallmarks of cancer when treating tumors [6]. Tumor cells can adapt to single therapeutic modality by

switching different signaling pathways, which provide higher chances of tumor survival and relapse [7, 8].

Regimens based on anthracyclines (doxorubicin, daunomycin, and epirubicin) and taxanes (paclitaxel and docetaxel) are among the most frequently used therapies for treating solid tumors including ovarian cancer, melanoma, colon cancer, stomach cancer, prostate cancer, lung cancer, head and neck cancers, and breast cancer [9, 10, 11, 12, 13]. Taxanes are class of diterpenes that originated from the yew tree. Taxanes interfere with cell division through interrupting microtubule function in which induces G2/M cell cycle arrest and apoptosis [14]. Docetaxel (Figure 1A) [(1S,2S,3R,4S,7R,9S,10S,12R,15S)-4-acetyloxy-1,9,12-trihydroxy-15-[(2R,3S)-2-hydroxy-3-[(2-methylpropan-2-yl)oxycarbonylamino]-3-phenylpropanoyl]oxy-10,14,17,

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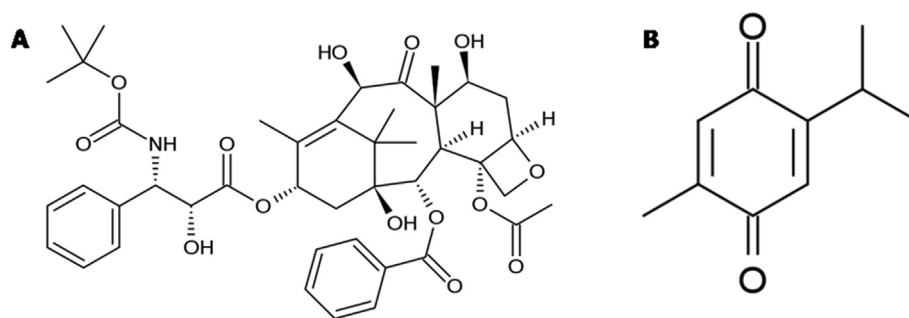


Figure 1. The chemical structure of Docetaxel (A), and Thymoquinone (B).

17-tetramethyl-11-oxo-6-oxatetracyclo[11.3.1.0^{3,10}.0^{4,7}]heptadec-13-en-2-yl] benzoate, trihydrate) is a semi-synthetic analog of paclitaxel (Taxol®) and elicits comparable antitumor to paclitaxel and other taxanes [10, 15, 16].

Thymoquinone (Figure 1B) (2-isopropyl-5-methylbenzo-1,4-quinone) is a compound originating from the seeds of *Nigella Sativa* plant (also known as black seed) [17]. Thymoquinone (TQ) exhibits different biological activities such as anti-oxidant, anti-inflammatory, and anti-tumor activities both *in vitro* and *in vivo* [18]. In particular, TQ has been reported to possess *in vitro* anti-tumor activity against variety of tumors including breast cancer, ovarian cancer, colon cancer, lung cancer, leukemia, and other cancer cell lines. Moreover, the anti-tumor efficacy of TQ has also been confirmed *in vivo* when tested against xenograft models of human cancers [18, 19]. The mechanism of TQ action is not yet well understood. Several reports have described TQ as an angiogenesis inhibitor and can regulate the pro-inflammatory and proliferative proteins such as COX-2, inducible NOS, 5-lipoxygenase, tumor necrosis factor (TNC), and cyclin D1 [20]. Moreover, TQ has been found to inhibit and modulate the activity of several signaling pathways involved in tumor progression such as Akt, NF- κ B, ERK, STAT3, p53, BCL-2, Bax, and p21 [20].

Nanoparticles have unique properties that expanded the scope of pharmacokinetics and pharmacodynamics of insoluble and/or unstable drugs [21, 22]. Amongst all nanoparticles, liposomes are widely used in the clinical application of drug delivery [23, 24]. The first generation of liposomes (conventional liposomes) was prepared by AlecBanghamat the beginning of the sixties of the last century [25]. Liposomes are spherical, self-closed vesicles with a phospholipids bilayer membrane. Moreover, liposomes have advantageous properties including their biocompatibility, low toxicity, biodegradability, and the targeting capability [26]. Due to its tiny size, liposomes have the ability to penetrate leaky blood vessels of tumor cell by passive targeting mechanism, while conjugating liposomes with antibodies, peptides or sugar components, is considered an advanced active targeting [27]. Additionally, stealth liposomes, which are liposomal formulations coated with polymers such as polyethylene glycol (PEG), were developed to prolong liposomes half-life by masking the detection and destruction of these liposomes by mononuclear phagocyte system (MPS). PEG has many useful properties, allowing it to be used widely in the clinical field, including biocompatibility, low toxicity, and good water solubility [28].

Docetaxel treatment is a key part of standard chemotherapeutic regimens of breast cancer patients. However, insensitivity to DT treatment is a challenging drawback for successful cancer management. Recent studies have shown that resistance to DT can be overcome by inhibition of AKT activity [29, 30]. On the other hand, TQ has been shown to suppress Akt pathway effectively [31, 32]. Therefore, investigation the cytotoxic effect of TQ and DT combination on breast cancers is highly important. MCF7 cell line is very well characterized and considered one of the most used breast cancer cell line model to investigate anticancer drugs. MCF7 breast cancer cell line is estrogen receptor positive (ER⁺) and progesterone receptor positive (PR⁺) and classified as luminal A molecular subtype [33].

In the current work, we hypothesized a possible synergism between DT and TQ against MCF7 breast cancer cell line. Furthermore, the current study describes the co-encapsulation of DT/TQ into PEGylated liposomes and their characterization for the encapsulation efficacy and the cytotoxic effect against MCF7 breast cancer cell line.

2. Results

2.1. The effect of single and combination drug treatment

To investigate the effect of free DT on the growth of MCF7 breast cancer cell line, MCF7 cells were treated with increasing concentrations of DT (0.75–375 nM) for 72 h. DT caused substantial growth inhibition, and the IC₅₀ value was 3.8 ± 1.1 nM (Figure 2A). Then, we evaluated the effect of free TQ on the growth of the MCF7 cells using increasing concentrations of TQ (0.78–100 μ M). The results showed growth inhibition with IC₅₀ of 40 ± 3.8 μ M (Figure 2B). These findings indicate that both DT and TQ were effective inhibitors for MCF7 proliferation. Subsequent investigation to the anti-proliferative effect of DT and TQ combination was performed. For this purpose, MCF7 cells were treated with increasing concentrations of DT (0.244–125 nM), TQ (0.39–200 μ M), and both DT/TQ single combination for 72 h. The results showed a higher decrease in the cell viability when the MCF7 cells were treated with DT/TQ combination compared to DT or TQ alone. The fraction affected (Fa) values have been determined after treatment with different concentrations of drug combinations, then the combination index (CI) and drug reduction index (DRI) were calculated for each Fa value. Figure 3A shows the Fa-CI plot of DT and TQ at fixed drug ratio and illustrate the synergistic effect (CI < 1) on MCF7 cells. Moreover, the synergism corresponding to the CI < 1 provided a favorable DRI (>1) for both drugs (Figure 3B, D). The Fa-DRI plot (shown in Figure 3B) indicates that the DT doses can be reduced when combined with TQ at different Fa values. The isobologram shown in Figure 3C indicates synergy between DT and TQ at the effective doses; ED₅₀, ED₇₅, and ED₉₀.

2.2. Liposomes preparation and characterization

The liposomes (empty and loaded) were prepared using thin film dispersion method, the mean hydrodynamic diameter of empty (385 ± 32 nm) and loaded liposomes (415 ± 17 nm) were close in values and provided Large unilamellar vesicles (LUV) [34]. The polydispersity index of all liposomes was less than 0.2 indicating almost monodisperse nanoparticles. The loaded and the unloaded liposomes showed similar surface charges since no difference in the zeta potential was noticed (1.4 ± 0.6 mV) when investigated in 1x PBS.

2.3. Encapsulation efficiency and drug loading

The encapsulation efficiency results of DT are shown in Figure 4A and B, respectively. To examine the effect TQ concentration on the DT encapsulation into liposomes, a fixed amount of DT (2 mg) and increasing

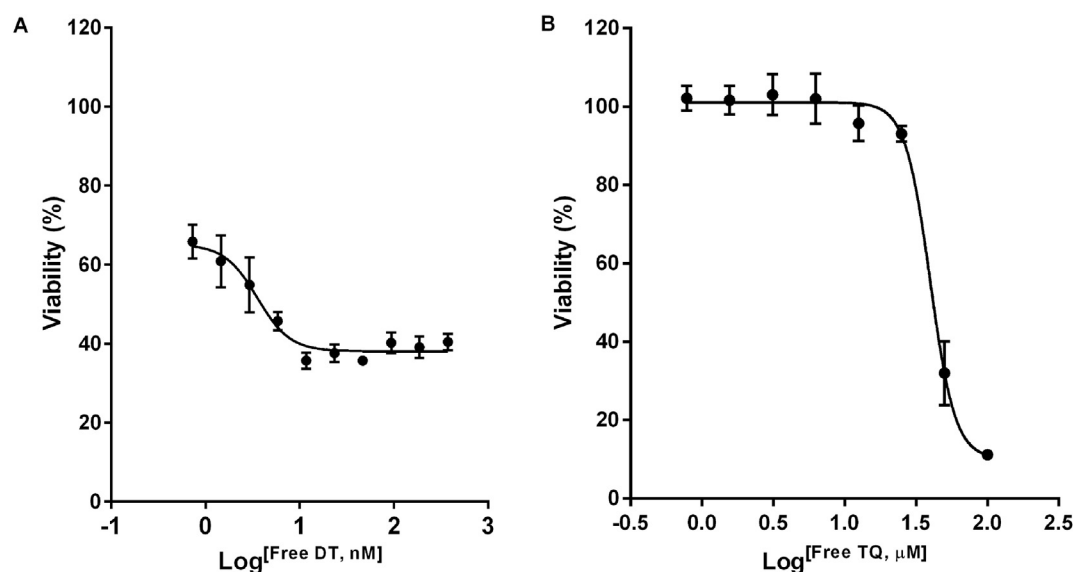


Figure 2. IC_{50} values after free drug treatment. The MCF7 cell lines were treated with different concentrations of DT and TQ to assess the cytotoxicity level. (A) The dose-response curve for MCF7 cells treated with DT and; (B) The dose-response curve for MCF7 cells treated with free TQ. All cytotoxicity values represent the average $\pm$ SD of three independent experiments.

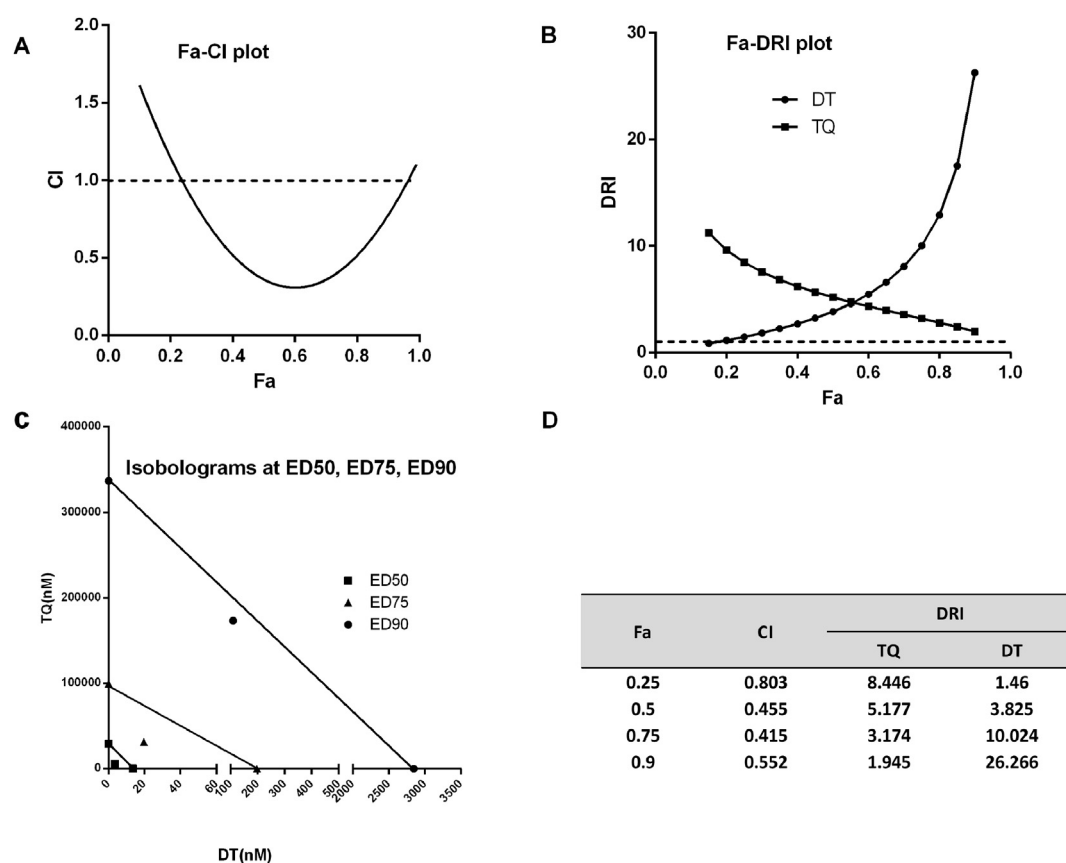


Figure 3. Diagnostic graphics generated for synergistic effect quantification. (A) The fraction affected (Fa) versus combination index (CI) plot after treatment with DT/TQ combination, that most of CI values are indeed <1 at different effect points of the drug combination, indicating a clear synergistic effect for the DT/TQ combination. (B) The Fa-DRI plot (Chou-Martin plot) for the constant ratio of DT/TQ combination. (C) Isobologram graph shows that the combination data point for effective doses (ED) ED50, ED75, and ED90 falls on the lower left side in which indicate synergism. (D) A table summarizing the CI and the DRI for 0.25, 0.5, 0.75, and 0.9 Fa points.

amounts of TQ (2.5, 5, and 7.5 mg) have been investigated. The encapsulation efficacy (%) of DT was 25 ± 9.2 , 52 ± 1.4 and 41 ± 7.8 for 2.5, 5, and 7.5 mg of TQ respectively (Figure 4A). Moreover, the drug loading of DT/lipid (wt/wt %) showed 0.35 ± 0.13 , 0.73 ± 0.01 , and 0.58 ± 0.11

for 2.5, 5, and 7.5 mg of TQ respectively (Figure 4C). These results indicate that the optimal amount of TQ providing best encapsulation efficacy of DT is 5 mg. Furthermore, we have investigated the impact of the increasing amount of DT (2, 4, and 6 mg) using a fixed amount of TQ

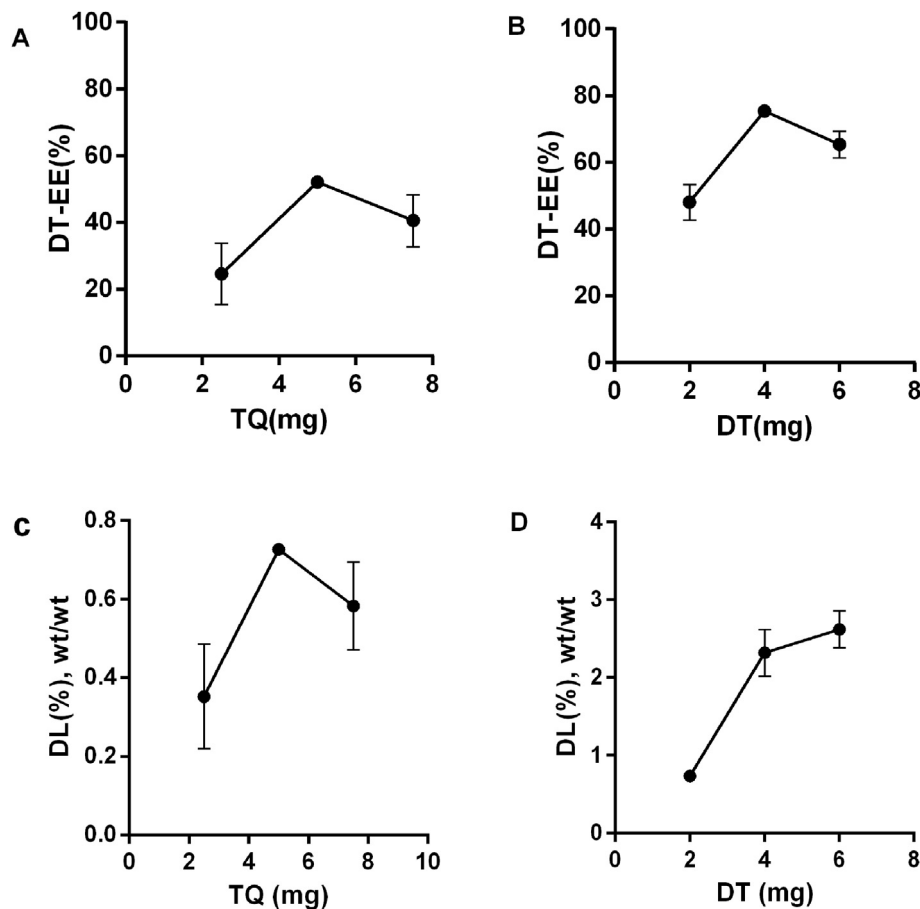


Figure 4. The encapsulation efficacy (EE) and the drug loading (DL) of both DT and TQ. (A) EE% of DT with an increased amount of TQ. (B) The EE% of DT with an increased amount of DT. (C) The DL of DT with an increased amount of TQ. (D) The DL of DT with an increased amount of DT. All values represent the average $\pm$ SD of three independent experiments.

(5 mg) on the encapsulation efficacy and drug loading of DT. The encapsulation efficacy (%) of DT was 48 ± 5.3 , 75 ± 1.5 , and 65 ± 4 for 2, 4, and 6 mg of DT respectively (Figure 4B). The drug loading of DT/lipid (wt/wt %) was 0.73 ± 0.02 , 2.3 ± 0.3 , and 2.6 ± 0.24 for 2, 4, and 6 mg of DT respectively (Figure 4D).

2.4. The *in vitro* anti-proliferative effect of DT and TQ loaded liposomes

The cytotoxic activity of DT (lipDT), TQ (LipTQ), and DT-TQ (LipDT/TQ) loaded liposomes against the MCF7 breast cancer cell line was evaluated using cell viability assay (MTT). The MCF7 cells treated with increased concentrations of loaded liposomes for 72 h and the IC_{50} has been calculated. The LipDT showed IC_{50} of 0.54 ± 0.2 nM (Figure 5A), which represents ~7-fold increase in activity compared to free DT (3.8 ± 1.4 nM) ($P = 0.0162$) (Figure 2A). Interestingly, the IC_{50} of DT in combination with TQ (LipDT/TQ) (0.91 ± 0.1 nM) (Figure 5C) was lower than the IC_{50} of free DT ($P = 0.0235$) and slightly higher compared to LipDT IC_{50} ($P = 0.0457$). The IC_{50} of TQ loaded liposomes (432 ± 171 μ M) (Figure 5B) showed higher IC_{50} compared to free TQ (40.1 ± 3.6 μ M) (Figure 2B). However, the IC_{50} of TQ in LipDT/TQ liposomes decreased to 1.5 ± 0.25 μ M (Figure 5D) (288 fold decrease compared to LipTQ and 26 fold compared to free TQ). The empty liposomal vehicle showed no significant toxicity at tested equal drug-loaded liposomes.

3. Discussion

Breast cancer is associated with a high incidence of mortality and morbidity rates among women worldwide. In 2018, the estimated newly

diagnosed breast cancer cases are about 2.1 million and account for 25% of cancers among women and 11.6 % of all cancers among both genders [35]. The developments in the antineoplastic agents led to a significant improvement in breast cancer treatment. The use of combination therapy is a keystone in cancer therapy and produced better therapeutic efficacy when compared to monotherapeutic modality [4, 36]. Many studies were performed to evaluate the combination of different chemotherapeutic agents with DT against different cancers. For example, a study conducted by Budman et al, 2002 looked into several combinations including vinorelbine, dexrazoxane, cis-retinoic acid, disulfiram, doxorubicin, epirubicin, and dexrazoxane [37]. The *in vitro* results of that study showed synergistic combinations of DT with epirubicin, 9-cis-retinoic acid, dexrazoxane, and vinorelbine when tested on the MCF7 breast cancer cell line [37]. Different studies showed a synergistic effect when DT is combined with doxorubicin or aneustat against prostate cancer [38, 39], cisplatin against osteosarcoma [40], or ceramide against breast cancer [41]. Moreover, the synergetic combinations of TQ with different anticancer agents have been investigated against different cancers such as breast and pancreatic cancers [42, 43, 44, 45]. For example, the combination of TQ with melatonin, resveratrol, paclitaxel showed a synergistic anticancer effect against breast cancer [43, 44, 45]. In the current study, we have investigated the cytotoxic effect of DT/TQ combination on MCF7 breast cancer cells, followed by exploring the encapsulation of DT/TQ combination into PEGylated liposomes and studying the cytotoxic effect DT/TQ loaded liposomes on MCF7 cells (summarized in Table 1).

There are few studies that have reported the synergetic effect of DT and TQ against cancer. For example, a study performed by Dirican et al,

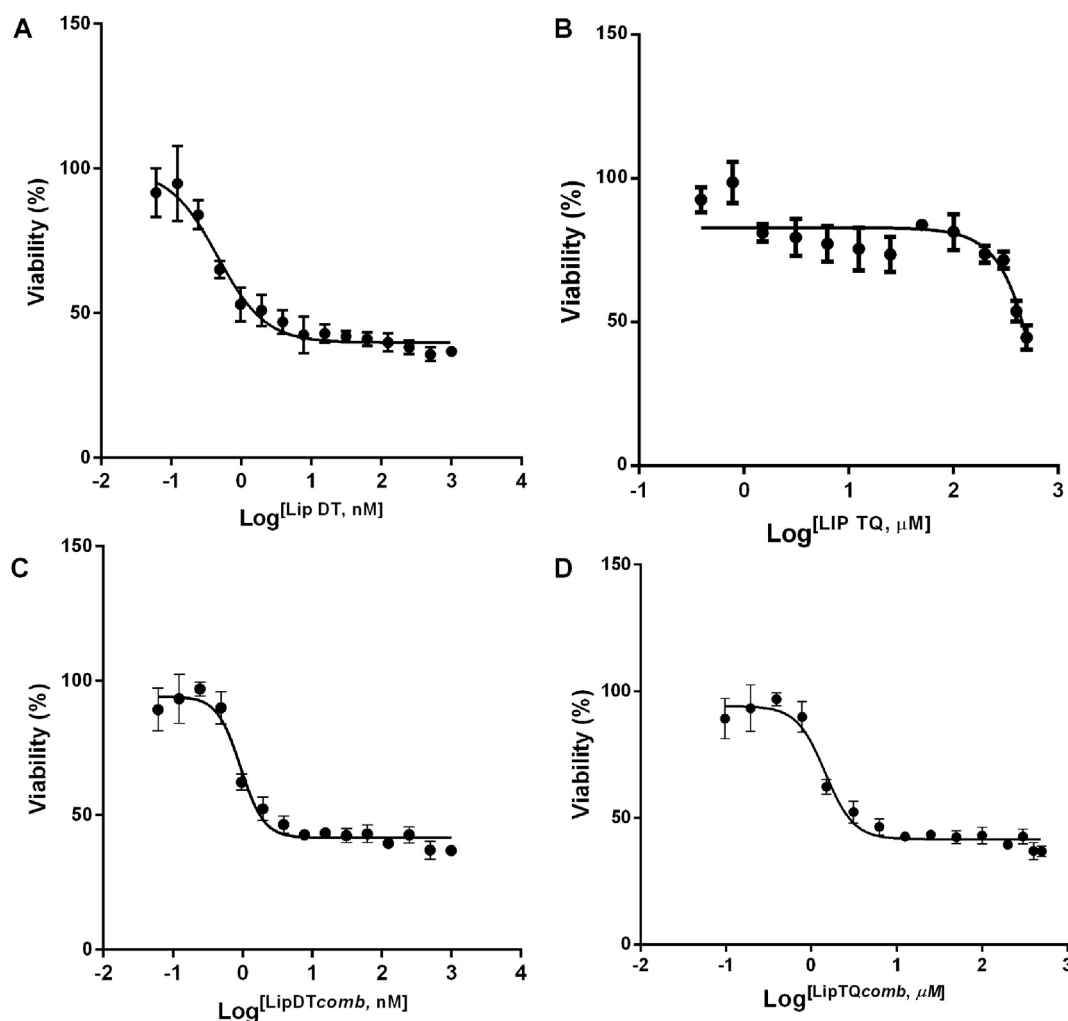


Figure 5. IC₅₀ values after treatment with drug-loaded liposomes. The MCF7 cell lines were treated with DT and TQ loaded liposomes to assess the cytotoxicity levels (A) The dose-response curve for MCF7 cells treated with LipDT and; (B) The dose-response curve for MCF7 cells treated with LipTQ; (C) The dose-response curve for MCF7 cells treated with LipDT in combination with TQ; (D) The dose-response curve for MCF7 cells treated with LipTQ in combination with DT. All cytotoxicity values represent the average $\pm$ SD of three independent experiments.

2015 showed a synergistic effect between DT and TQ against DU-145 human prostate cancer cells [46]. The authors have also explained that the cytotoxic effect induced by DT and TQ combination can be through the blocking of the PI3K/Akt signaling pathway [46]. In this work, we have studied the cytotoxic effect of TQ and DT combination on breast cancer cell line (MCF-7) at fixed drug concentration ratio, and we have been able to demonstrate a synergistic effect for this combination ($CI < 1$). The calculation of CI was based on the Chou-Talalay method which does not only take into account the “potency” but also the “shape” of dose-effect curves of each drug and their combinations [47].

Thymoquinone and docetaxel are known as effective anticancer agents and can exert lethal effects on cancer cells through different mechanisms that all end with inhibiting cell division [10, 48, 49]. Docetaxel has been reported to be safer than paclitaxel [16]. However, docetaxel suffers from low water solubility and induce serious adverse effects such as neutropenia and preferable nephropathy and

hypersensitivity [15, 48, 50]. Moreover, TQ is water-insoluble and suffers from low bioavailability. Therefore, developing a proper drug delivery system using biocompatible and biodegradable materials such as liposomes is of high interest. Docetaxel and thymoquinone encapsulation into liposomes have been described by several reports [50, 51, 52]. The encapsulation of these drugs into liposomes have successfully enhanced the solubility and therapeutic efficacy, and reduced toxicity [50, 51, 53]. However, there are no studies on the co-encapsulation of both drugs into liposomes. In this study, we have successfully co-encapsulated both drugs into PEGylated liposomes. Interestingly, the co-encapsulation of TQ and DT enhanced the encapsulation efficacy of DT into liposomes. Such phenomena can be explained by enhancing liposomes stability through increasing rigidity of the membrane. Our group has previously reported the encapsulation of TQ into liposomes and the results showed increased liposomes stability [47]. This can be attributed greatly to the lipophilicity of TQ which makes it stack with the lipid used (especially the hydrophobic tails). Since TQ is a small molecule compared to the phospholipids used, it is expected that it increased the backing and stacking of the molecules and hence decreasing the permeability of the bilayer. Indeed it was evident from NMR data that TQ will remain attached to the lipids used in the formation of the liposomes even after the disruption of the liposomes [51].

Following successful encapsulation of TQ and DT into liposomes, we have investigated the cytotoxic effect of liposomes loaded with each drug

Table 1. Summary of IC₅₀ values for single and combination of DT and TQ.

Treatment	DT (nM)	TQ (μ M)
Free drug alone	3.8 ± 1.4	40.3 ± 3.8
Free drug in combination	1.7 ± 0.7	8.4 ± 5.5
Drug-loaded liposomes (Single drug)	0.54 ± 0.2	432 ± 171
Drug-loaded liposomes (Combination drug)	0.91 ± 0.1	1.5 ± 0.25

alone or in combination. Interestingly, the IC₅₀ of DT-loaded liposomes in combination was slightly higher in combination compared to the DT/TQ loaded liposomes. This result can be explained by the higher stability of the liposomes when loaded with TQ. This result supported by the IC₅₀ obtained by the TQ loaded liposomes. The IC₅₀ for TQ was around 22 times higher when loaded into liposomes compared to free TQ, and this result supports the high stability of liposomes loaded with TQ.

4. Conclusions

In conclusion, our study showed for the first time a synergetic effect DT/TQ combination against MCF7 breast cancer cells proliferation *in vitro*. Moreover, the encapsulation of both DT/TQ into PEGylated liposomes has been successfully achieved. This liposomal drug-formulation represents a promising drug delivery system for further development. It would be interesting to investigate the encapsulation of both drugs into different liposomal and nanoparticles formulations. Moreover, further work to functionalize liposomes with targeting ligands to enhance therapeutic potency will be of high interest. *In vivo* investigation of antitumor efficacy using xenograft models of human breast cancers will add a clearer image about the therapeutic efficacy of these liposomal preparations.

5. Materials and methods

5.1. Materials

The lipids DPPC (1, 2-dihexadecanoyl-sn-glycero-3-phosphocholine), DSPE-PEG2000(1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethyleneglycol)-2000]), and Cholesterol were purchased from Avanti Polar Lipids (Alabaster, USA). Docetaxel and Thymoquinone were obtained from Sigma-Aldrich (USA). The MTT kit was from Promega (USA) and other chemicals obtained from different sources.

5.2. Cytotoxicity assay

The cytotoxicity assay was performed using MTT reagent (Promega, USA). 1×10^4 cells/well were seeded into 96-well plate and incubated at 37 °C for 24 h in a humidified CO₂ incubator. Then, the cells were treated with different concentrations of TQ, DT, or DT/TQ combination and incubated at 37 °C for 72 h in a humidified CO₂ incubator. After incubation, 15 µL of MTT reagent has been added and incubated at 37 °C for 4 h in CO₂ incubator followed by the addition of 100 µL of stop solution for 1 h in dark followed by reading the absorbance at 570 nm using 96-well plate reader. Cytotoxicity assay for the drug-loaded liposomes was performed using the same protocol described above.

5.3. Combination index

The combination index (CI) was calculated for combined DT and TQ using Calcsyn software (BioSoft, UK). The CI < 1 indicates synergism; CI = 1 indicates additive effect, and CI > 1 indicates antagonism. The mean IC₅₀ for DT was 1.89 nM, which was calculated from the tested concentration range (0.25–500 nM). The cytotoxic effect of DT increased with concentration from 0.25 to 125 nM after which the fraction of affected cells was stable at 60% at maximal concentration tested of 500 nM. Dose reduction index (DRI) was calculated using the same software. DRI is a measure of how much the dose of each drug in a synergistic combination may be reduced at a given effect level compared with the doses of each drug alone.

5.4. Liposomes preparation

Liposomes-loaded with DT and TQ were prepared by lipid thin-film hydration method [54]. Lipid solutions composed of DPPC, cholesterol, and DSPE-PEG in a 64:30:6 molar ratio was mixed with DT (4 mg) and TQ

(5 mg) in 4 mL of chloroform. The thin-film then was prepared by evaporation of chloroform using rotary evaporator for 30 min at 37 °C under reduced pressure. Then, the lipid film was hydrated with 5–6 mL of 1X PBS buffer (pH 7.4) with vigorous shaking.

5.5. Encapsulation efficiency and drug-loading in liposomes

To determine the encapsulation efficiency and drug loading, the free drug has been removed using dialysis tubing, 20 kDa molecular weight cut-off membrane. Liposomes were dialyzed against three changes of 400 mL of HEPES buffer (pH 7.4) using plate shaker at 150 rpm and overnight incubation at 37 °C. Following dialysis, the liposomes were ruptured using acetonitrile and water bath sonication, followed by centrifugation at 12,000 rpm. The supernatants then were analyzed using HPLC (Shimadzu, Japan) for the drug quantification. For HPLC analysis, C18 column has been used, the mobile phase composed of water: acetonitrile (25:75 volume ratio), the flow rate 1 mL/min and the detection wavelength at 290 nm. The encapsulation efficiency (EE%) and drug loading (DL%) were calculated by Eqs. (1) and (2) as follows:

$$EE(\%) = \frac{\text{Drug in the esupernatent}}{\text{Total drug added}} \times 100 \quad (1)$$

$$DL(\%) = \frac{\text{Total amount of drug}}{\text{Total amount of lipids}} \times 100 \quad (2)$$

5.6. Liposomes' size and charge

The mean hydrodynamic diameter of the liposomes was determined by Dynamic Light Scattering (Microtrac NPA 152, USA). Samples were prepared in PBS buffer (pH 7.4) and analyzed at a backscattering angle of 173°. The polydispersity index (PDI) has been used as an indicator of size distribution. The zeta potential has been measured using the same apparatus.

5.7. Statistical analysis

The statistical analyses were performed using standard two-tailed student's t-test. All values expressed as mean ± SD and the significant difference considered when p-value is less than 0.05.

Declarations

Author contribution statement

F. Odeh, S. Ismail: Conceived and designed the experiments; Analyzed and interpreted the data; Performed the experiments; Contributed reagents, materials, analysis tools or data.

R. Naffa, H. Azzam: Performed the experiments; Analyzed and interpreted the data.

I. Mahmoud, A. Al Bawab: Analyzed and interpreted the data.

W. Alshaer: Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

- [1] S. McGuire, World Cancer Report 2014. Geneva, Switzerland: World Health Organization, International Agency for Research on Cancer, WHO Press, Adv. Nutr. 7 (2016) 418–419.
- [2] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, *Cell* 144 (2011) 646–674.
- [3] G. Housman, S. Byler, S. Heerboth, K. Lapinska, M. Longacre, N. Snyder, S. Sarkar, Drug resistance in cancer: an overview, *Cancers (Basel)* 6 (2014) 1769–1792.
- [4] R. Bayat Mokhtari, T.S. Homayouni, N. Baluch, E. Morgatskaya, S. Kumar, B. Das, H. Yeger, Combination therapy in combating cancer, *Oncotarget* 8 (2017) 38022–38043.
- [5] W. Alshaer, H. Hillaireau, E. Fattal, Aptamer-guided nanomedicines for anticancer drug delivery, *Adv. Drug Deliv. Rev.* 134 (2018) 122–137.
- [6] J. Li, Y. Wang, Y. Zhu, D. Oupicky, Recent advances in delivery of drug-nucleic acid combinations for cancer treatment, *J. Control. Release* 172 (2013) 589–600.
- [7] S. Lal, A. Mahajan, W. Ning Chen, B. Chowbay, Pharmacogenetics of target genes across doxorubicin disposition pathway: a review, *Curr. Drug Metabol.* 11 (2010) 115–128.
- [8] H.M. Zalloum, M.O. Taha, Development of predictive *in silico* model for cyclosporine-and aureobasidin-based P-glycoprotein inhibitors employing receptor surface analysis, *J. Mol. Graph. Model.* 27 (2008) 439–451.
- [9] K.A. Lyseng-Williamson, C. Fenton, Docetaxel: a review of its use in metastatic breast cancer, *Drugs* 65 (2005) 2513–2531.
- [10] K. Gelmon, The taxoids: paclitaxel and docetaxel, *Lancet (London, England)* 344 (1994) 1267–1272.
- [11] S. AbuHammad, M. Zihlif, Gene expression alterations in doxorubicin resistant MCF7 breast cancer cell line, *Genomics* 101 (2013) 213–220.
- [12] D.A. AlQudah, M.A. Zihlif, M.O. Taha, Ligand-based modeling of diverse arylalkylamines yields new potent P-glycoprotein inhibitors, *Eur. J. Med. Chem.* 110 (2016) 204–223.
- [13] C.J. Lovitt, T.B. Shelper, V.M. Avery, Doxorubicin resistance in breast cancer cells is mediated by extracellular matrix proteins, *BMC Canc.* 18 (2018) 41.
- [14] D.L. Morse, H. Gray, C.M. Payne, R.J. Gillies, Docetaxel induces cell death through mitotic catastrophe in human breast cancer cells, *Mol. Cancer Ther.* 4 (2005) 1495.
- [15] M.A. Izquierdo, M. Garcia, J.L. Ponton, M. Martinez, V. Valenti, M. Navarro, M. Gil, F. Cardenal, R. Mesia, X. Perez, R. Salazar, J.R. Germa-Lluch, A phase I clinical and pharmacokinetic study of paclitaxel and docetaxel given in combination in patients with solid tumours, *Eur. J. Cancer (Oxford, England)* 42 (2006) 1789–1796.
- [16] D.S. Grant, T.L. Williams, M. Zahaczewsky, A.P. Dicker, Comparison of antiangiogenic activities using paclitaxel (taxol) and docetaxel (taxotere), *Int. J. Cancer* 104 (2003) 121–129.
- [17] M.M. Abukhader, Thymoquinone in the clinical treatment of cancer: fact or fiction? *Pharmacogn. Rev.* 7 (2013) 117–120.
- [18] F. Forouzanfar, B.S. Bazzaz, H. Hosseinzadeh, Black cumin (*Nigella sativa*) and its constituent (thymoquinone): a review on antimicrobial effects, *Iranian J. Basic Med. Sci.* 17 (2014) 929–938.
- [19] M.A. Randhawa, M.S. Alghamdi, Anticancer activity of *Nigella sativa* (black seed) - a review, *Am. J. Chin. Med.* 39 (2011) 1075–1091.
- [20] S. Banerjee, S. Padhye, A. Azmi, Z. Wang, P.A. Philip, O. Kucuk, F.H. Sarkar, R.M. Mohammad, Review on molecular and therapeutic potential of thymoquinone in cancer, *Nutr. Cancer* 62 (2010) 938–946.
- [21] J. Shi, P.W. Kantoff, R. Wooster, O.C. Farokhzad, Cancer nanomedicine: progress, challenges and opportunities, *Nat. Rev. Cancer* 17 (2017) 20.
- [22] A. Wicki, D. Witzigmann, V. Balasubramanian, J. Huwyler, Nanomedicine in cancer therapy: challenges, opportunities, and clinical applications, *J. Control. Release* 200 (2015) 138–157.
- [23] U. Bulbake, S. Doppalapudi, N. Kommineni, W. Khan, Liposomal formulations in clinical use: an updated review, *Pharmaceutics* 9 (2017) 12.
- [24] W. Alshaer, H. Hillaireau, J. Vergnaud, S. Ismail, E. Fattal, Functionalizing liposomes with anti-CD44 aptamer for selective targeting of cancer cells, *Bioconjug. Chem.* 26 (2015) 1307–1313.
- [25] T.M. Allen, P.R. Cullis, Liposomal drug delivery systems: from concept to clinical applications, *Adv. Drug Deliv. Rev.* 65 (2013) 36–48.
- [26] M. Voinea, M. Simionescu, Designing of 'intelligent' liposomes for efficient delivery of drugs, *J. Cell Mol. Med.* 6 (2002) 465–474.
- [27] S. Tran, P.-J. DeGiovanni, B. Piel, P. Rai, Cancer nanomedicine: a review of recent success in drug delivery, *Clin. Transl. Med.* 6 (2017) 44.
- [28] G. Bangale, K. Rajesh, G. Shinde, Stealth liposomes: a novel approach of targeted drug delivery in cancer therapy, *Int. J. Pharma. Sci. Res.* 5 (2014) 750–759.
- [29] Y. Yasumizu, A. Miyajima, T. Kosaka, Y. Miyazaki, E. Kikuchi, M. Oya, Dual PI3K/mTOR inhibitor NVP-BEZ235 sensitizes docetaxel in castration resistant prostate cancer, *J. Urol.* 191 (2014) 227–234.
- [30] G. Dey, R. Bharti, A.K. Das, R. Sen, M. Mandal, Resensitization of Akt induced docetaxel resistance in breast cancer by 'Iturin A' a lipopeptide molecule from marine bacteria *Bacillus megaterium*, *Sci. Rep.* 7 (2017) 17324.
- [31] S. Rajput, B.N. Kumar, K.K. Dey, I. Pal, A. Parekh, M. Mandal, Molecular targeting of Akt by thymoquinone promotes G(1) arrest through translation inhibition of cyclin D1 and induces apoptosis in breast cancer cells, *Life Sci.* 93 (2013) 783–790.
- [32] B. Iskender, K. Izgi, H. Canatan, Novel anti-cancer agent myrtoicommulone-A and thymoquinone abrogate epithelial-mesenchymal transition in cancer cells mainly through the inhibition of PI3K/AKT signalling axis, *Mol. Cell. Biochem.* 416 (2016) 71–84.
- [33] S. Comsa, A.M. Cimpean, M. Raica, The story of MCF-7 breast cancer cell line: 40 years of experience in research, *Anticancer Res.* 35 (2015) 3147–3154.
- [34] V.P. Torchilin, Recent advances with liposomes as pharmaceutical carriers, *Nat. Rev. Drug Discov.* 4 (2005) 145–160.
- [35] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, *CA A Cancer J. Clin.* 68 (2018) 394–424.
- [36] J.H. Lee, A. Nan, Combination drug delivery approaches in metastatic breast cancer, *J. Drug Deliv.* 2012 (2012) 17.
- [37] D.R. Budman, A. Calabro, In vitro search for synergy and antagonism: evaluation of docetaxel combinations in breast cancer cell lines, *Breast Canc. Res. Treat.* 74 (2002) 41–46.
- [38] S. Qu, X. Ci, H. Xue, X. Dong, J. Hao, D. Lin, P.-L. Clermont, R. Wu, C.C. Collins, P.W. Gout, Y. Wang, Treatment with docetaxel in combination with Aneustat leads to potent inhibition of metastasis in a patient-derived xenograft model of advanced prostate cancer, *Br. J. Canc.* 118 (2018) 802.
- [39] E. Tsakalozou, A.M. Eckman, Y. Bae, Combination effects of docetaxel and Doxorubicin in hormone-refractory prostate cancer cells, *Biochem. Res. Int.* 2012 (2012) 832059.
- [40] H. Tao, X. Tang, L. Jin, Y. Zhao, Y. Luo, Z. Zhang, L. Cai, F. Tao, W. Guo, Synergistic effect of docetaxel combined with cisplatin on inhibiting human osteosarcoma in nude mice, *Biochem. Biophys. Res. Commun.* 505 (2018) 372–377.
- [41] L.-X. Feng, M. Li, Y.-J. Liu, S.-M. Yang, N. Zhang, Synergistic enhancement of cancer therapy using a combination of ceramide and docetaxel, *Int. J. Mol. Sci.* 15 (2014) 4201–4220.
- [42] A. Pandita, B. Kumar, S. Manvati, S. Vaishnavi, S.K. Singh, R.N.K. Bamezai, Synergistic combination of gemcitabine and dietary molecule induces apoptosis in pancreatic cancer cells and down regulates PKM2 expression, *PLoS One* 9 (2014), e107154.
- [43] L.H. Odeh, W.H. Talib, I.A. Basheti, Synergistic effect of thymoquinone and melatonin against breast cancer implanted in mice, *J. Cancer Res. Ther.* 14 (2018) S324–s330.
- [44] O.H. Alobaedi, W.H. Talib, I.A. Basheti, Antitumor effect of thymoquinone combined with resveratrol on mice transplanted with breast cancer, *Asian Pac. J. Trop. Med.* 10 (2017) 400–408.
- [45] Ç. Şakalar, K. İzgi, B. İskender, S. Sezen, H. Aksu, M. Çakır, B. Kurt, A. Turan, H. Canatan, The combination of thymoquinone and paclitaxel shows anti-tumor activity through the interplay with apoptosis network in triple-negative breast cancer, *Tumor Biol.* 37 (2016) 4467–4477.
- [46] A. Dirican, H. Atmaca, E. Bozkurt, C. Erten, B. Karaca, R. Uslu, Novel combination of docetaxel and thymoquinone induces synergistic cytotoxicity and apoptosis in DU-145 human prostate cancer cells by modulating PI3K-AKT pathway, *Clin. Transl. Oncol.: official publication of the Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico* 17 (2015) 145–151.
- [47] T.C. Chou, Drug combination studies and their synergy quantification using the Chou-Talalay method, *Cancer Res.* 70 (2010) 440–446.
- [48] F.K. Engels, A. Sparreboom, R.A. Mathot, J. Verweij, Potential for improvement of docetaxel-based chemotherapy: a pharmacological review, *Br. J. Canc.* 93 (2005) 173–177.
- [49] A.H. Rahmani, M.A. Alzohairy, M.A. Khan, S.M. Aly, Therapeutic implications of black seed and its constituent thymoquinone in the prevention of cancer through inactivation and activation of molecular pathways, evidence-based complementary and alternative medicine, *eCAM* 2014 (2014) 724658.
- [50] H. Zhang, R.-y. Li, X. Lu, Z.-z. Mou, G.-m. Lin, Docetaxel-loaded liposomes: preparation, pH sensitivity, pharmacokinetics, and tissue distribution, *J. Zhejiang Univ. - Sci. B* 13 (2012) 981–989.
- [51] F. Odeh, S.I. Ismail, R. Abu-Dahab, I.S. Mahmoud, A. Al Bawab, Thymoquinone in liposomes: a study of loading efficiency and biological activity towards breast cancer, *Drug Deliv.* 19 (2012) 371–377.
- [52] S. Pereira, R. Egbu, G. Jannati, W.T. Al-Jamal, Docetaxel-loaded liposomes: the effect of lipid composition and purification on drug encapsulation and in vitro toxicity, *Int. J. Pharm.* 514 (2016) 150–159.
- [53] W. Wu, Y. Cheng, B.-H. Guo, Q. Wu, Pharmacokinetics of liver-targeted docetaxel liposomes modified with 6-O-acyl-D-galactose esters in rabbits, *Biomed. Rep.* 2 (2014) 545–548.
- [54] A.D. Bangham, R.W. Horne, Negative staining of phospholipids and their structural modification by surface-active agents as observed in the electron microscope, *J. Mol. Biol.* 8 (1964), 660-IN610.