BİNGÖL UNIVERSITY

Turkish Journal of Nature and Science

e-ISSN: 2149-6366
www.dergipark.gov.tr/tdfd

Research Article

Volume 15, Issue 1, Page 174-181, 2026

Received: 21.01.2025
Accepted: 10.02.2026
Published: 30.03.2026

DOI: https://doi.org/10.46810/tdfd.1623422

Doxorubicin-Loaded Niosomal Transporters Demonstrate Enhanced Cytotoxicity Against Brain Cancer Cells: An In Vitro Study

Özge ÇAĞLAR YILDIRIM[1*]

[1] Erzurum Technical University, Faculty of Science, Molecular Biology and Genetics Department, Erzurum, Türkiye

**Corresponding author: ozge.caglar@erzurum.edu.tr*

Abstract

The development of effective drug delivery systems is essential for improving the in vitro performance of anticancer agents in brain tumor models. In this study, doxorubicin (DOX)-loaded niosomes were prepared using the thin-film hydration method and evaluated for their physicochemical properties and in vitro cytotoxic potential. The prepared formulations were characterized in terms of particle size, polydispersity index, zeta potential, morphology, encapsulation efficiency, and drug release profile. The cytotoxic effects of free DOX, empty niosomes, and DOX-loaded niosomes were assessed in SHSY-5Y and U87-MG cell lines using the MTT viability assay. The results demonstrated that DOX-loaded niosomes exhibited enhanced cytotoxic effects compared to free DOX in both cell models. A dose-dependent reduction in cell viability was observed, particularly in SHSY-5Y cells. However, IC_{50} values could not be determined for all groups within the tested concentration range. The findings indicate that niosomal encapsulation improves the in vitro cytotoxic performance of DOX and represents a promising nanocarrier approach for preliminary screening in brain cancer cell models. Further mechanistic and in vivo studies are required to fully elucidate the therapeutic potential of this delivery system.

Keywords

Doxorubicin,
Niosomes,
Drug carrier systems,
Brain cancer,
Neuroblastoma,
Glioblastoma,

BİNGÖL ÜNİVERSİTESİ

Türk Doğa ve Fen Dergisi

e-ISSN: 2149-6366
www.dergipark.gov.tr/tdfd

Araştırma Makalesi

Cilt 15, Sayı 1, Sayfa 174-181, 2026

Alınış: 21.01.2025
Kabul: 10.02.2026
Basım: 30.03.2026

DOI: https://doi.org/10.46810/tdfd.1623422

Doksorubisin Yüklü Niyozomal Taşıma Sistemleri Beyin Kanseri Hücrelerine Karşı Artmış Sitotoksik Etki Gösterir: İn Vitro Bir Çalışma

Özge ÇAĞLAR YILDIRIM[1*]

[1] Erzurum Teknik Üniversitesi, Fen Fakültesi, Moleküler Biyoloji ve Genetik Bölümü, Erzurum, Türkiye

**Sorumlu yazar: ozge.caglar@erzurum.edu.tr*

Özet

Beyin tümörü modellerinde antikanser ajanların in vitro performansını iyileştirmek için etkili ilaç dağıtım sistemlerinin geliştirilmesi hayati önem taşımaktadır. Bu çalışmada, ince film hidrasyon yöntemi kullanılarak doksorubisin (DOX) yüklü niyozomlar hazırlanmış ve bunların fizikokimyasal özellikleri ile in vitro sitotoksik potansiyeli değerlendirilmiştir. Hazırlanan formülasyonlar, partikül boyutu, polidispersite indeksi, zeta potansiyeli, morfoloji, kapsülleme verimliliği ve ilaç salım profili açısından karakterize edilmiştir. Serbest DOX, boş niyozomlar ve DOX yüklü niyozomların sitotoksik etkileri, MTT canlılık testi kullanılarak SHSY-5Y ve U87-MG hücre hatlarında değerlendirilmiştir. Sonuçlar, DOX yüklü niyozomların her iki hücre modelinde de serbest DOX'a kıyasla daha güçlü sitotoksik etkiler sergilediğini göstermiştir. Özellikle SHSY-5Y hücrelerinde, hücre canlılığında doza bağlı bir azalma gözlemlenmiştir. Bununla birlikte, test edilen konsantrasyon aralığı içinde tüm gruplar için IC50 belirlenememiştir. Bulgular, niyozomal kapsüllemenin DOX'un in vitro sitotoksik performansını iyileştirdiğini ve beyin kanseri hücre modellerinde ön tarama için umut verici bir nanotaşıyıcı yaklaşımı olduğunu göstermektedir. Bu ilaç verme sisteminin terapötik potansiyelini tam olarak aydınlatmak için daha fazla mekanistik ve in vivo çalışma gereklidir.

Anahtar kelimeler

Doksorubisin,
Niyozomlar,
İlaç taşıyıcı sistemler,
Beyin kanseri,
Nöroblastom,
Glioblastom

1. INTRODUCTION

Cancer is a complex disease characterized by uncontrolled cell growth and impaired apoptotic mechanisms. This leads to tumor formation and tissue invasion. Its development is influenced by genetic, environmental and lifestyle factors. Current treatment strategies, including chemotherapy, radiotherapy and surgery, are chosen according to the type of cancer, the stage of the disease and the characteristics of the patient. However, treatment resistance and systemic side effects remain major challenges, emphasizing the necessity for more effective therapeutic approaches [1].

Brain cancers, including neuroblastoma and glioblastoma, are among the most challenging malignancies to treat worldwide due to their aggressive nature and poor prognosis. This study used SHSY-5Y and U87-MG cell lines as in vitro models of neuroblastoma and glioblastoma, respectively. While these models do not represent the blood–brain barrier (BBB), the BBB remains a significant challenge for the effective delivery of drugs in clinical settings [2]. The global burden of brain tumors is significant: glioblastoma patients have a median survival of 12–18 months, with an overall five-year survival rate of less than 10%. Depending on geographic region and cancer stage, the average annual treatment cost per patient ranges from $50,000 to $150,000, representing a substantial economic burden [3]. Therefore, developing innovative therapeutic strategies, such as nano-drug delivery systems, is crucial for improving treatment outcomes and reducing healthcare costs worldwide.

Recent years have seen the emergence of nanotechnology-based drug delivery systems as a promising approach for the treatment of brain cancer. The small size and unique surface properties of nanoparticles mean that they have been proposed as potential carriers for crossing the blood–brain barrier, thereby enabling more efficient delivery of chemotherapeutic agents to brain tissues. Moreover, the functionalization of nanoparticles with tumor-specific ligands allows for targeted therapy, ensuring selective drug accumulation in cancer cells while minimizing damage to healthy tissues. The ability to regulate the release of drugs over time is a key advantage of these systems, as it helps to maintain a consistent therapeutic concentration within the target tissue, thereby enhancing treatment efficacy and reducing the occurrence of systemic side effects [4]. In addition, nanotechnology-based carriers have the capacity to effectively address the heterogeneous nature of brain tumors and enable the co-delivery of multiple drugs within a single system, with the potential to improve therapeutic outcomes. Consequently, nanotechnology is regarded as one of the most innovative strategies in the treatment of brain cancer, with the aim of overcoming current therapeutic limitations and enhancing both patient survival rates and quality of life [5].

Niosome-based drug delivery systems have emerged as promising drug delivery platforms due to their biocompatibility, stability and ability to encapsulate hydrophilic and hydrophobic drugs. Niosomes are non-ionic surfactant-based vesicles that offer structural advantages similar to liposomes, but with improved chemical stability and cost effectiveness compared to liposomes. Their ability to enhance drug bioavailability, reduce drug toxicity and provide controlled drug release has made them an attractive option for advanced drug delivery. In addition, niosomes can be surface-modified with ligands to achieve active targeting, improving the efficacy of treatments, particularly for brain-related diseases such as Alzheimer's and brain cancer, where crossing the blood-brain barrier is a major challenge [6].

In this study, we aimed to perform a preliminary in vitro evaluation of doxorubicin-loaded niosomes prepared by the thin-film hydration method in SHSY-5Y and U87-MG cell models using MTT-based viability analysis.

2. MATERIAL AND METHOD

2.1. Materials

Doxorubicin (DOX) was purchased from Sigma Aldrich/Germany. Cholesterol, Tween 40, Tween 80 were obtained Sigma Aldrich. Chloroform and dimethyl sulfoxide (DMSO) were purchased from Merck. DMEM (Dulbecco's adjusted Eagle's medium), trypsin-EDTA, trypan blue, fetal bovine serum (FBS), phosphate-buffered saline (PBS), dimethylthiazol-2-yl-2,5 (MTT) and penicillin/streptomycin were purchased from Gibco.

2.2. Preparation of Niosomes

Niosomes were prepared by thin film hydration method in a round bottom flask with some minor modifications, as previously reported [7]. The compounds used in the synthesis of niosomes are listed in Table 1. A rotary evaporator (Hei Dolph Instruments, Germany) was used to evaporate the chloroform at 110 rpm, 60 °C for 1 hour. The prepared thin film was hydrated with DOX (1 mg/mL) solution in PBS at 60 °C, 120 rpm for 30 minutes. The samples were stored in a refrigerator at 4°C until the next experiment. All niosomal formulations are listed in detail in Table 1.

Table 1. Composition of niosomal formulations.

Formulations	Surfactant	Lipid: Drug molar ratio	DOX concentration (mg/mL	Surfactant: cholesterol molar ratio
NF1	Tween40	5	1	1:1
NF2	Tween40	5	1	2:1
NF3	Tween40	10	1	1:1
NF4	Tween40	10	1	2:1

2.3. Characterizations of Niosomes

Scanning electron microscopy (SEM, Quanta FEG 250) was used to determine the morphological properties of the niosomes. The particle size and polydispersity index (PDI) of the prepared niosomes were determined using a Zetasizer (Zetasizer Nano ZS, Malvern Instruments) and the zeta potential of drug loaded niosomes was taken using the Zetasizer.

2.4. Drug Entrapment Efficiency and Drug Release Study

To separate DOX-loaded niosomes formulations from the unencapsulated drug, the centrifugation was performed at 13000 rpm for 30 min at 4°C (Eppendorf, 5415 R, Germany). Free drug concentration in supernatant was determined by measuring absorbance at 490 nm using UV spectrophotometer (Shimadzu, Japan) [8]. Drug loaded efficacy were calculated as follows:

$$\%EE = \frac{Total\ amount\ of\ drug - free\ drug\ in\ the\ supernatant}{Total\ amount\ of\ drug} x100 \quad (1)$$

The dialysis membrane technique was used to determine the in vitro drug release of DOX-loaded niosomes [9]. DOX-niosomes were placed into a dialysis bag fractionated at 12000 Dalton molecular weight and then the bag was placed in a beaker containing PBS solution (pH 6.8). 0.5 mL medium was removed then the fresh PBS was added in tubes in certain time intervals (1 h, 2 h, 4 h, 8 h, 24 h). The drug release was measured by Nanodrop spectrophotometer for the sample.

2.5. In Vitro Toxicity of Niosomes

The cytotoxicity of prepared niosomes were evaluated on human dermal fibroblasts (HDF) cell lines. For this purpose, MTT assay was performed on HDF cell culture [10]. Briefly, the cells were cultured in 48-well plate with $5x10^4$ cells per well for 24 hours, and the prepared niosomes were added to each well in triplicate at different concentrations (0-400 µg/mL) and the culture plate was incubated with niosomes for 24 hours. The medium was discarded from all the wells then washed with 200 µL of PBS three times. Then, the MTT regent was added into per wells and the samples were incubated for 3 hours in dark at 37 °C. End of incubation period, formed formazan crystals were dissolved with 100 µL of DMSO. Finally, the plates were analyzed using a microplate reader at 570 nm.

2.6. Anticancer Activity of DOX Loaded Niosomes

The anti-cancer activity of DOX-loaded niosomes was researched on the SHSY-5Y neuroblastoma and U87-MG glioblastoma cell lines. The cells (10^4 cells/well) were seeded 48-well plate and incubated for 24 h at 37°C 5% CO_2. Then DOX-loaded niosomes, free DOX and free niosomes were added into each well at various concentrations (0-400 µg/mL). The tested concentration range (0–400 µg/mL) was selected based on previously published studies on doxorubicin-based nanocarriers and preliminary dose-screening experiments performed to cover both sub-toxic and highly cytotoxic conditions [11]. The samples were placed at culture incubator for 24 h at 37 °C 5% CO_2. In the study, untreated cells were used as negative control group and Triton X-100 treated cells were used as positive control group. The MTT analysis was used to determinate inhibition activities of niosomes on tumor growth in SHSY-5Y and U87-MG cell lines. After 24 hours of incubation, 20 µL of MTT working solution (5 mg/mL) was added to each sample and the culture plates were incubated for 24 hours in dark at 37 °C. Next, the formed formazan crystals dissolved in 100 µL DMSO. The plate was assessment using a microplate reader at 570 nm.

2.7. Statistical Analysis

Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Differences between groups were considered statistically significant at $p < 0.05$.

3. RESULTS

Niosomes were successfully synthesized by thin film hydration technique. The average particle size and morphological properties of niosomes were determined by SEM imaging. The images obtained showed that the particle size was in the range of 150-250 nm. Moreover, the prepared nanoparticles did not have a uniform size. SEM images of niosomes synthesized using the TFH technique are given as Figure 1, the prepared niosomes exhibited a relatively uniform spherical morphology with smooth surface characteristics. The absence of major aggregation and structural deformation indicates good physical stability of the formulation, which is essential for efficient drug delivery applications. The average particle size of NF1 was determined to be 150 nm and the size for NF2, NF3 and NF4 were 215, 230 and 254 nm respectively. Zetasiezer analysis (average particle diameter, surface energy) and encapsulation efficiency for all formulations are presented in Table 2. The study was continued with NF1 due to particle size and drug encapsulation rate. Furthermore, DOX encapsulation changed the NF1 PDI value to 0.290 and the particle size to 174 nm.

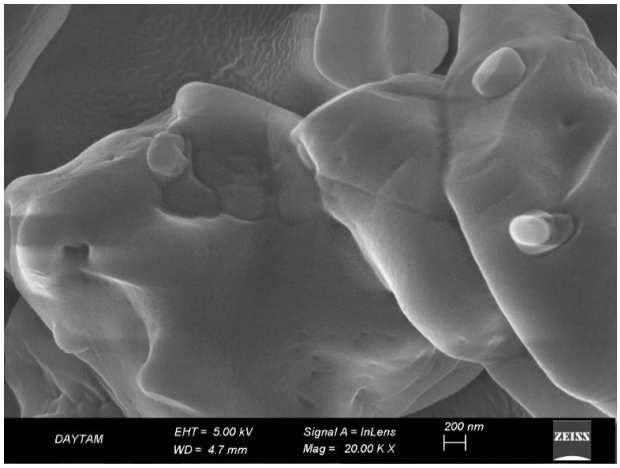

Figure 1. SEM image of niosomes prepared by using thin-layer hydration technique.

Table 2. The mean particle diameter, surface energy and encapsulation efficiency for all formulations

Formulations	Mean Particle size (nm)	Zeta potential energy (mV)	PDI	Encapsulation efficient (%)
NF1	150	-22.8	0.265	95.7
NF2	215	-14.4	0.210	46.9
NF3	230	-9.3	0.523	-
NF4	254	-5.4	0.630	-

The rate of DOX-loaded into the NF1 was determined by UV-vis spectrophotometry. The loading efficiency of DOX into niosomes was relatively high at approximately 95.7%. The dialysis membrane technique was used to evaluate the rate of DOX the time-dependent release from niosomes vesicles. DOX-loaded niosomes were placed into a dialysis bag fractionated at 12000 Dalton molecular weight and then the bag was kept in a beaker containing PBS solution (pH 6.8). The in vitro release behavior of DOX-niosomes is shown in Figure 2. Figure 2 demonstrates the cumulative release profile of DOX from the niosomal formulation. The results indicate a sustained and time-dependent drug release pattern, suggesting controlled diffusion of DOX from the vesicular structure. Such a release behavior is advantageous for maintaining prolonged therapeutic activity. The release of DOX from niosomes exhibited a regular time-dependent pattern. The release rate reached 15% within 2 hours of treatment and the cumulative release of DOX reached 30.8% over 24 hours, indicating that the prepared niosomes are an excellent carrier candidate.

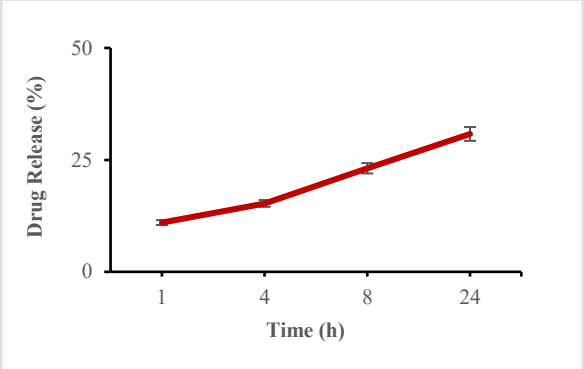

Figure 2. Rates of drug release from niosomes for DOX.

After 24 h incubation, the cytotoxicity of niosomes was evaluated on the human fibroblast cell line using the MTT assay. The MTT assay was performed at a concentration ranging from 3.12 to 400 µg/mL. When the compared to the untreated groups, the samples exposed to niosomes showed no significant toxicity at any concentration tested. Niosomes were not found to be cytotoxic in the human dermal fibroblast cell line, causing no significant reduction in cell viability even at concentrations as high as 200 µg/mL. In the highest dose application, cell viability was determined to be 73.31%. Figure 3 shows the results of the cytotoxicity analysis. No significant difference was observed between empty niosomes and the untreated control group in HDF cells at concentrations up to 200 µg/mL ($p > 0.05$). However, a slight but statistically significant decrease in cell viability was observed at 400 µg/mL ($p < 0.05$).

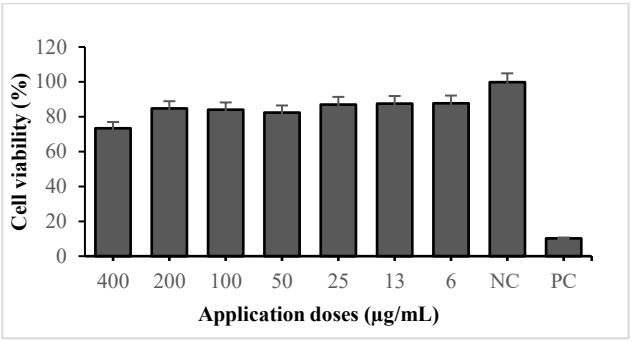

Figure 3. *In vitro* cytotoxicity of empty NF1 on HDFa cell culture for 24 hours.

The cytotoxic potential of DOX-loaded niosomes (NF1-DOX) was assessed in U87-MG and SHSY-5Y cell lines employing the MTT colorimetric viability assay. The anti-cancer activity of the drug-loaded niosomal system was assessed over a wide concentration range (3.12–400 µg/mL). The NF1-DOX treatment resulted in a marked, dose-dependent reduction in cell viability in both the SHSY-5Y and U87-MG cell lines. Overall, NF1-DOX demonstrated significantly higher levels of cytotoxic activity in comparison to both free DOX and untreated control groups, particularly at concentrations ≥50 µg/mL ($p < 0.05$). The cytotoxic effect of NF1-DOX was more pronounced in SHSY-5Y cells than in U87-MG cells ($p < 0.05$). In SHSY-5Y cells, NF1-DOX treatment reduced cell viability to below 50% at higher concentrations, whereas IC_{50} values could not be reached in U87-MG cells or in free DOX-treated groups within the examined concentration range. Furthermore, NF1-DOX induced significantly lower cell viability compared to empty niosomes at concentrations ≥12.5 µg/mL in SHSY-5Y cells ($p < 0.05$). The findings indicate an enhanced anti-cancer efficacy of the niosomal DOX formulation, particularly in neuroblastoma cells. The results of the proliferation assays are presented in Figure 4 and Figure 5 for the U87-MG and SHSY-5Y cell lines, respectively.

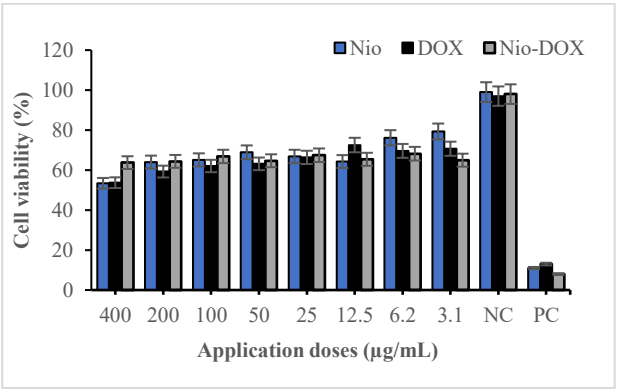

Figure 4. *In vitro* cytotoxicity of free niosomes, free DOX, and NF1-DOX on U87-MG cell culture, n = 3.

Table 3. Cell viability values (%) of U87-MG and SHSY-5Y cells after treatment with empty niosomes, free DOX, and NF1-DOX (Values are expressed as mean ± standard deviation (SD) from three independent experiments (n = 3). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. NC: negative control; **PC:** positive control. Differences were considered statistically significant at $p < 0.05$.).

	Concentration (µg/mL)	Niosome (%)	Free DOX (%)	NF1-DOX (%)
U87-MG	400	53.39±2.20	53.70±2.60	63.73±3.18
	200	64.04±2.01	59.26±3.35	64.35±3.20
	100	65.12±4.40	62.04±6.40	66.82±3.34
	50	68.90±2.24	63.12±7.10	64.66±2.23
	25	66.82±4.37	66.36±3.20	67.44±3.45
	12.5	64.27±1.12	72.53±4.75	65.38±2.90
	6.2	76.16±2.35	69.60±2.60	68.21±4.30
	3.1	79.32±3.8	70.68±4.45	64.97±3.55
	NC	99.00±1.20	97.00±2.70	98.00±2.80
	PC	11.0±5.20	13.00±2.36	8.00±3.20
SHSY-5Y	400	58.78±2.90	55.82±2.75	35.44±2.20
	200	60.78±3.21	67.48±3.35	40.22±3.15
	100	69.50±3.45	75.82±3.85	36.28±2.25
	50	65.68±3.25	75.95±3.90	41.11±3.65
	25	60.79±3.10	68.73±3.39	32.56±2.19
	12.5	59.81±2.95	78.66±3.75	42.00±3.10
	6.2	56.81±2.85	56.24±2.96	38.83±2.60
	3.1	70.25±3.60	64.22±3.20	44.17±2.60
	1.6	59.58±2.97	71.18±4.48	53.67±2.60
	NC	98.50±2.15	97.00±3.50	98.90±2.90
	PC	8.00±4.20	12.00±3.20	11.00±3.35

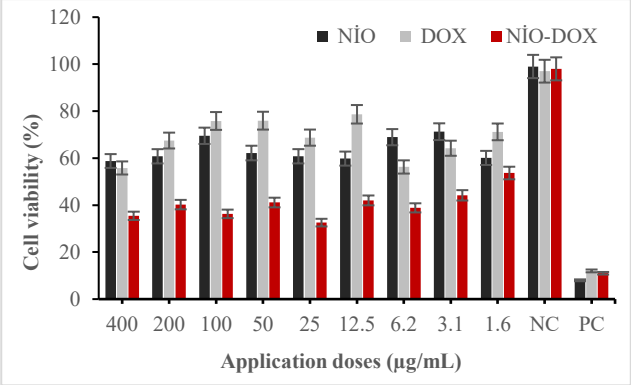

Figure 5. In vitro cytotoxicity of free niosomes, free DOX, and NF1-DOX on SHSY-5Y cell culture, n = 3.

4. DISCUSSION AND CONCLUSION

The present study evaluated the anticancer activity of DOX-loaded niosomes in two different cancer cell lines, SHSY-5Y and U87-MG. The results demonstrate the potential of niosomal delivery systems to enhance the in vitro cytotoxic performance of DOX in brain cancer models. In addition, the toxicity profile of the formulation was assessed in healthy cells.

Niosomes are vesicular systems composed of non-ionic surfactants such as Tweens and Spans and represent promising alternatives to liposomes [12]. They protect drug molecules from premature degradation and improve pharmacological performance. Recent studies have focused on the development of niosomal formulations to overcome poor water solubility, enhance bioavailability at target sites, and reduce systemic toxicity [13]. Moreover, niosomes have been reported to facilitate drug transport across the blood–brain barrier and to improve drug stability and accumulation in brain tissues [14].

The preparation method plays a crucial role in determining niosome size, encapsulation efficiency, and bilayer structure [15–18]. The thin-film hydration (TFH) technique is a simple and reproducible method commonly used for producing mono- and multilamellar niosomes [19]. In the present study, TFH enabled successful synthesis of stable niosomes. The lipid-to-surfactant ratio significantly influenced vesicle size and physicochemical properties, with NF1 exhibiting optimal characteristics, including small particle size and high surface stability Here we used the TFH technique, and niosomes were successfully synthesized. The lipid/nonionic surfactant ratio is an important parameter on the average size of niosomes vesicles and on the biological and physical properties of the encapsulated substance. Especially NF1 was successful with its small vesicle size and high surface energy.

Morphological characterization by SEM revealed size variations among different formulations, mainly due to differences in surfactant–lipid and lipid–drug ratios. The amphiphilic nature of DOX contributed to vesicle size changes following encapsulation, likely due to increased water retention and multilayer formation [20]. Zetasiezer analysis indicated a PDI of 0.290 and a zeta potential of −22.8 mV after drug loading, confirming successful encapsulation and colloidal stability [21], where the increased vesicle size may indicate multilayer niosomes [22]. The zeta potential depends on the Brown motion of the particles and the increasing potential energy increases the stability in polydispersity. Furthermore, the high encapsulation efficiency of NF1 (95%) was consistent with previously reported data [23].

These results confirm the successful synthesis of stable and homogeneous niosomes with high drug-loading capacity [24–26]. Although a slight increase in PDI was observed after DOX loading, this did not significantly affect the release profile, which remained regular and time-dependent [27].

The anticancer activity of NF1-DOX was demonstrated in SHSY-5Y and U87-MG cells using proliferation assays. SHSY-5Y cells are widely used as in vitro neuroblastoma models [28]. Previous studies have shown that DOX induces apoptosis through mitochondrial pathway activation [29]. NF1-DOX may exert cytotoxic effects consistent with previously reported apoptotic mechanisms. DOX-induced DNA intercalation has been reported as a major mechanism of cell death, and the stronger response observed in SHSY-5Y cells may be associated with cell-specific biological characteristics [30]. Despite the fact that doxorubicin-loaded nanocarriers, including vesicular systems, have been the subject of investigation in various cancer models, studies specifically focusing on niosomal doxorubicin in

neuroblastoma in vitro models remain limited. In this context, the present work makes three contributions: firstly, it optimizes Tween40: cholesterol-based niosomes with high encapsulation efficiency; secondly, it provides a controlled release profile under the tested conditions; and thirdly, it performs a direct comparison of NF1-DOX with free DOX and empty niosomes in both SHSY-5Y and U87-MG cell lines under the same experimental setup. The present findings are consistent with previous reports demonstrating that nanocarrier-mediated delivery can enhance doxorubicin efficacy in glioblastoma models by improving intracellular accumulation and retention. For instance, ApoE-directed polymersomal DOX exhibited potent antitumor activity in an orthotopic U87-MG model, thereby substantiating the notion that formulation and carrier design exert a substantial influence on DOX performance in brain tumor settings. In addition, other DOX-loaded nanoparticle platforms, including polymeric and magnetic systems, have been reported to improve therapeutic outcomes in glioma models, further highlighting the benefit of nano-enabled delivery strategies. The comparatively weaker response observed in U87-MG cells may be indicative of the intrinsic chemoresistance of glioblastoma cells, where limited intracellular drug accumulation and adaptive survival pathways can attenuate the toxicity of free DOX. In this context, nanocarrier-based delivery has been proposed as a practical approach to partially overcoming such barriers by facilitating endocytic uptake and sustained intracellular exposure. Mechanistically, the enhanced toxicity of NF1-DOX compared to free DOX can be attributed to carrier-assisted cellular internalization and prolonged intracellular drug availability. In a manner analogous to other vesicular nanocarriers, niosomal encapsulation has been demonstrated to promote endocytic uptake and reduce rapid extracellular drug loss. This, in turn, has been shown to increase the effective intracellular DOX concentration and strengthen cytotoxic responses. However, it is noteworthy that the cytotoxic effect of free DOX was relatively low compared to some literature reports.

The relatively low level of cytotoxic effect of free DOX observed in the present study in comparison to that reported in some literature sources may be attributed to a number of factors. It is evident that both the SHSY-5Y and U87-MG cell lines are characterized by an inherent resistance to chemotherapeutic agents, a phenomenon attributable to the over-expression of drug efflux transporters, such as P-glycoprotein, which serves to restrict the accumulation of drugs within the intracellular environment [30,31]. Furthermore, the relatively brief incubation period (24 hours) and the presence of serum proteins in the culture medium may reduce the bioavailability and cellular uptake of free DOX [32]. Conversely, niosomal encapsulation facilitates cellular internalization via endocytic pathways and enables sustained intracellular drug release, thereby enhancing cytotoxic efficacy [14,33].

The present study did not directly investigate the cellular uptake of DOX-loaded niosomes using fluorescence or flow cytometry techniques. This represents a limitation of the current work. However, the significantly enhanced cytotoxic effects of NF1-DOX in comparison with free DOX, in conjunction with the observed dose-dependent responses, indirectly suggest effective intracellular delivery of DOX mediated by the niosomal carrier system. Future studies will focus on detailed cellular uptake and intracellular trafficking analyses using fluorescence and confocal microscopy.

The present study demonstrates that doxorubicin-loaded niosomes prepared by the thin-film hydration method exhibit promising in vitro cytotoxic effects in SHSY-5Y and U87-MG cell lines. The formulation showed favorable physicochemical properties and sustained drug release behavior. Although the present study is limited to MTT-based viability assays, the findings suggest that niosomal delivery systems may serve as useful platforms for preliminary screening in brain cancer research. Further mechanistic and in vivo studies are required to validate the therapeutic potential of this system [34]. Therefore, future studies should focus on the evaluation of novel delivery systems and combination therapies in both in vitro and in vivo models.

REFERENCES

[1] Sarkar S, Horn G, Moulton K, Oza A, Byler S, Kokolus S, Longacre M. Cancer Development, Progression, and Therapy: An Epigenetic Overview. International Journal of Molecular Sciences. 2013; 14(10):21087-21113

[2] Debela DT, Muzazu SGY, Heraro KD, Ndalama MT, Mesele BW, Haile DC, et al. New approaches and procedures for cancer treatment: Current perspectives. SAGE Open Med. 2021 12:9:20503121211034366.

[3] Ostrom QT, Cioffi G, Waite K, Kruchko C, Barnholtz-Sloan JS. CBTRUS statistical report: Primary brain and other central nervous system tumors diagnosed in the United States in 2014-2018. Neuro Oncol. 2021; 4;25(12 Suppl 2):iv1-iv99. doi: 10.1093/neuonc/noad149.

[4] Sahu T, Ratre YK, Chauhan S, Bhaskar LVKS, Nair MP, Verma HK. Nanotechnology-based drug delivery system: Current strategies and emerging therapeutic potential for medical science. J Drug Deliv Sci Technol. 2021;63:102487.

[5] Yasamineh S, Yasamineh P, Ghafouri Kalajahi H, Gholizadeh O, Yekanipour Z, Afkhami H, et al. A state-of-the-art review on the recent advances of niosomes as a targeted drug delivery system. Int J Pharm. 2022; 25:624:121878.

[6] Pandey S, Tripathi P, Gupta A, Yadav JS. A comprehensive review on possibilities of treating psoriasis using dermal cyclosporine. Drug Deliv Transl Res. 2022;12(7): 1541-1555.

[7] Durak S, Rad ME, Yetisgin AA, Sutova HE, Kutlu O, Cetinel S, et al. Niosomal drug delivery systems for ocular disease—recent advances and future prospects. Nanomaterials. 2020;10(6):1191.

[8] Yıldırım ÖÇ, Arslan ME, Öner S, Cacciatore I, Di Stefano A, Mardinoglu A, et al. Boron nitride nanoparticles loaded with a boron-based hybrid as a

promising drug carrier system for Alzheimer's disease treatment. Int J Mol Sci. 2022;23(15):8249.
[9] Faheela MK, Malathi S, Monica Susai Mary S, Narayana Kalkura S. In-vitro characterization of pluronic P123 based niosome for targeted delivery of doxorubicin. Mater Today Proc. 2022; 58:795–801
[10] Türkez H, Yıldırım ÖÇ, Öner S, Kadı A, Mete A, Arslan ME, et al. Lipoic acid conjugated boron hybrids enhance wound healing and antimicrobial processes. Pharmaceutics. 2023;15(1):149.
[11] Arslan ME. Anticarcinogenic properties of malic acid on glioblastoma cell line through necrotic cell death mechanism. MANAS J Eng. 2021;9(1):22-29.
[12] Azmin MN, Florence AT, Handjani-Vila RM, Stuart JFB, Vanlerberghe G, Whittaker JS. The effect of non-ionic surfactant vesicle (niosome) entrapment on the absorption and distribution of methotrexate in mice. J Pharm Pharmacol. 1985;37(4):237-242.
[13] Zhu X, Yu Z, Feng L, Deng L, Fang Z, Liu Z, et al. Chitosan-based nanoparticle co-delivery of docetaxel and curcumin ameliorates anti-tumor chemoimmunotherapy in lung cancer. Carbohydr Polym. 2021; 268:118237.
[14] Gharbavi M, Amani J, Kheiri-Manjili H, Danafar H, Sharafi A. Niosome: A promising nanocarrier for natural drug delivery through blood-brain barrier. Adv Pharmacol Sci. 2018;11: 6847971.
[15] Mabrouk M, Das DB, Salem ZA, Beherei HH. Nanomaterials for Biomedical Applications: Production, Characterisations, Recent Trends and Difficulties. Molecules. 2021;26(4):1077.
[16] Moammeri A, Chegeni MM, Sahrayi H, Ghafelehbashi R, Memarzadeh F, Mansouri A, Akbarzadeh I, Abtahi MS, Hejabi F, Ren Q. Current advances in niosomes applications for drug delivery and cancer treatment. Mater Today Bio. 2023 Oct 21;23:100837.
[17] Khan R, Irchhaiya R. Niosomes: A potential tool for novel drug delivery. J Pharm Investig. 2016;46(3):195-204.
[18] Karim K, Mandal A, Biswas N, Guha A, Chatterjee S, Behera M, et al. Niosome: A future of targeted drug delivery systems. J Adv Pharm Technol Res. 2010;1(4):374-380.
[19] Burrini N, D'Ambrosio M, Gentili M, Giaquinto R, Settimelli V, Luceri C, et al. Niosomes functionalized with a synthetic carbohydrate binding agent for mannose-targeted doxorubicin delivery. Pharmaceutics. 2023;15(1):235.
[20] 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. 2015; 1:56:393-400.
[21] Xu H, Xu X, Li S, Song WL, Yu DG, Bligh SWA. The effect of drug heterogeneous distributions within core-sheath nanostructures on its sustained release profiles. Biomolecules. 2021; 11(9):1330.
[22] Targhi AA, Moammeri A, Jamshidifar E, Abbaspour K, Sadeghi S, Lamakani L, et al. Synergistic effect of curcumin-Cu and curcumin-Ag nanoparticle loaded niosome: Enhanced antibacterial and anti-biofilm activities. Bioorg Chem. 2021; 115:105116.
[23] Esmaeili Rad M, Egil AC, Ozaydin Ince G, Yuce M, Zarrabi A. Optimization of curcumin loaded niosomes for drug delivery applications. Colloids Surf A Physicochem Eng Asp. 2022; 654(1):129921.
[24] Sahab-Negah S, Ariakia F, Jalili-Nik M, Afshari AR, Salehi S, Samini F, et al. Curcumin loaded in niosomal nanoparticles improved the anti-tumor effects of free curcumin on glioblastoma stem-like cells: An in vitro study. Mol Neurobiol. 2020; 57(8):3391-3411.
[25] Hemati M, Haghiralsadat F, Yazdian F, Jafari F, Moradi A, Malekpour-Dehkordi Z. Development and characterization of a novel cationic PEGylated niosome-encapsulated forms of doxorubicin, quercetin and siRNA for the treatment of cancer by using combination therapy. Artif Cells Nanomed Biotechnol. 2019; 47(1):1295-1311.
[26] 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.
[27] Basheer HA, Alhusban MA, Zaid Alkilani A, Alshishani A, Elsalem L, Afarinkia K. Niosomal delivery of celecoxib and metformin for targeted breast cancer treatment. Cancers (Basel). 2023; 15(20):5004.
[28] Hoffmann LF, Martins A, Majolo F, Contini V, Laufer S, Goettert MI. Neural regeneration research model to be explored: SH-SY5Y human neuroblastoma cells. Neural Regen Res. 2023;18 (6):1265-1266.
[29] Pilco-Ferreto N, Calaf GM. Influence of doxorubicin on apoptosis and oxidative stress in breast cancer cell lines. Int J Oncol. 2016;49(2):753-762.
[30] Persano F, Gigli G, Leporatti S. Natural compounds as promising adjuvant agents in the treatment of gliomas. Int J Mol Sci. 2022; 23(6):3360.
[31] Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: Role of ATP-dependent transporters. Nat Rev Cancer. 2002;2(1):48-58.
[32] Anand U, Mukherjee S. Binding, unfolding and refolding dynamics of serum albumins. Biochim Biophys Acta Gen Subj. 2013;1830(12):5394-5404.
[33] Malhotra M, Tomaro-Duchesneau C, Saha S, Kahouli I, Prakash S. Development and characterization of chitosan-PEG-TAT nanoparticles for the intracellular delivery of siRNA. Int J Nanomedicine. 2013;8:2041-2052.
[34] Barani M, Mirzaei M, Torkzadeh-Mahani M, Lohrasbi-Nejad A, Nematollahi MH. A new formulation of hydrophobin-coated niosome as a drug carrier to cancer cells. Mater Sci Eng C. 2020;113:110975.