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Original Article

Synthesis and evaluation of Escitalopram-loaded niosomes on colon cancer cell lines

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ABSTRACT

Introduction: It has been approved that selective serotonin reuptake inhibitors (SSRIs) may exhibit anti-proliferative or cytotoxic effects on several types of cancers. The aim of the present study was to evaluate the cytotoxic effects of a newly formulated niosome of Escitalopram oxalate on a colorectal cancer cell line.

Methods: The niosomes were prepared using a thin layer hydration method, resulting in particles with a size range between 150 – 450 nm and spherical morphology. Moreover, its permeability release showed 25% in 4 hours. The cytotoxicity evaluation was performed using a quantitative colorimetric MTT assay.

Results: The cell viability of colon cancer cells after treatment with niosomes and pure escitalopram reduced to 28.3 ± 0.83 % and 24.07 ± 0.56%, respectively. However, the cytotoxicity assay of escitalopram-loaded niosomes suggested that the anti-proliferative effect of the niosomal formulation of escitalopram was dose and incubation time-dependent.

Conclusion: These results confirm the potential of the anti-proliferative activity of escitalopram-loaded niosomes. Further application to an in vivo model is needed to study various pharmacokinetic and pharmacodynamics parameters to establish its complete therapeutic potential.

Keywords:
Escitalopram
Cell viability
Niosome
MTT assay

Introduction

Nanomedicines offer substantial advantages for targeted delivery to low accessible target sites with high selectivity, such as the BBB (blood brain barrier), BCB (blood cerebrospinal fluid barrier) and BTB (blood tumor barrier) (Lockman et al, 2002; Yang, 2010).

Escitalopram is an FDA-approved anti-depressant drug used for the treatment of major depression (Llorca et al, 2005; Taylor et al, 2008). This drug undergoes extensive metabolism in the liver and its oral bioavailability is approximately 80% (Burke and Kratochvil 2002; Montgomery et al, 2001). Escitalopram has shown a

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beneficial effect in the treatment of depression, it also has a low therapeutic index, making it a suitable candidate for loading in nanoparticles (Cipriani et al, 2009; Kumud 2016; Rajput et al, 2016). Escitalopram has demonstrated anti-proliferative activity and the induction of apoptosis in various cancer cells, including non-small cell lung cancer cells (Yuan et al, 2018), glioma cells such as C6 cells (Dikmen et al, 2011) and U-87MG cells (Chen et al, 2018), as well as in metastatic breast cancer cell line, MDA-MB-231 (Patel, 2013).

Niosomes are nanoparticulated delivery systems composed of non-ionic surfactants, offering many advantages such as biodegradablity, relatively low toxicity, stability and cost-effectiveness. They are considered a promising alternative to liposomes (Kazi et al, 2010; Sankhyan and Pawar, 2012). These nanostructures can be applied for the delivery of both hydrophilic and lipophilic drugs as they can entrap active ingredients in the aqueous layer or vesicular membrane (Sankhyan and Pawar, 2012). Nisomes have been explored for brain delivery of anticancer chemotherapeutics (Bragagni et al, 2012; Ingallina et al, 2016), as well as topical delivery for skin cancers (Alvi et al, 2011). They have shown promising results for oral delivery of chemotherapeutic agents, with successful localized therapy for breast cancer using tamoxifen (Shaker et al, 2015), and confirmed potential in oral delivery of paclitaxel (Bayindir and Yuksel, 2010). Niosomes can be utilized for various routs of administration, including oral, parenteral, dermal, transdermal, mucosal and local applications (Ge et al, 2019). Moreover, niosomes have proposed as promising carriers for efficient drug delivery in the cancer treatment due to their smaller size, offering a possibility of enhanced permeability and retention in tumor tissue (Tavano et al, 2016).

Accordingly, escitalopram-loaded noisomes are expected to have improved pharmacological effects, especially for rectal administration in colon cancer treatment. In this study, we focused on evaluating the *in vitro* cytotoxicity of escitalopram (alone) and escitalopram-loaded niosomes on different cancer cell lines, like human colon cancer cell lines (SW48) and human colorectal adenocarcinoma cell line (HT29), in comparison to human liver hepatocellular cell line (HepG2), human embryonic kidney (HEK293t) and human Fetal Lung Fibroblast Cells (MRC-5). This study focused on the response of colon cancer cell lines since some SSRI antidepressants have recently shown potential as candidates for colon cancer treatment (Chubak et al, 2011; Gil-Ad et al, 2008; Jang et al, 2019).

Material and Methods

Materials

Escitalopram oxalate was obtained from Tehran Daru Co. (Tehran, Iran) as a gift. Cholesterol, SDS (Sodium dodecyl sulfate), Span60 (Sorbitan monostearate), and Tween 60 (Polysorbate 60) were obtained from Sigma Aldrich (USA). PBS (phosphate-buffered saline) from BIO BASIC (Canada) was obtained. Methanol, Chloroform, Amicon (Ultra-15 Membrane, MWCO 30000 Da) were purchased from Merck (Germany). Water was filtered using Milli-Q at each step (Millipore, Darmstadt, Germany). SW48, HepG2, HEK293t, HT29, MRC5, human fibroblast and fat cell lines were purchased from Pasture Institute, Tehran, Iran. Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serums (FBS), Trypsin EDTA were supplied by Gibco (Thermo Fisher Scientific, Germany). MTT was purchased from Sigma-Aldrich Chemicals (Bornem, Belgium).

Preparation of Niosomes

To prepare the niosomes, the thin layer hydration method was utilized (Ghafelehbashi et al, 2019). In summary, cholesterol and surface-active agent (1:2 molar ratios) were dissolved in chloroform and methanol (10 mL, 2:3 v/v). Then, 15 mg of escitalopram was added to the solution. The solvent was separated using a rotary evaporator (Heidolph Instruments, Germany) for 30min at 150 rpm at 60 ºC. Then, to form the niosomal formulation, the remaining dried thin films were hydrated with 10 ml of PBS (PH=7.4) at 60 ºC at 120 rpm for 30 min. Eventually, the samples were sonicated (Hielscher up-50Hultrasonic processor, Germany) for 7 min and then stored in a refrigerator at 4°C. To investigate the effect of vesicle composition on niosomal properties, different molar ratios of span60: tween 60: cholesterol were used (Table 1).

Determination of Particle Size

The size and polydispersity index (PDI) of escitalopram-loaded vesicles were determined using a dynamic light scattering nanoparticle size analyzer at a temperature of 25°C with a 45 mm focus lens and a beam length of 2.4 mm (Malvern Instrument Ltd. Malvern, UK).

TABLE 1: The percentage of surfactant compounds in the preparation of niosomal formulations

Niosomal formulation	Surfactants: cholesterol % molar ratio	Surfactant blend	
		Tween 60 (%)	Span 60 (%)
E1	1:1	0	100
E2	1:1	50	50
E3	1:1	100	0
E4	2:1	0	100
E5	2:1	50	50
E6	2:1	100	0

Morphology Evaluation of Niosomes

To analyze the morphology of escitalopram-loaded niosomes, a sample was placed on the FE-SEM holder and coated with 100 Å thick layer of gold for 3 min under argon at a pressure of 0.2 atm. The investigation was conducted using FESEM with the NOVA NANOSEM 450 FEI model at an accelerating voltage of 15 kV.

Encapsulation Efficiency

To analyze the encapsulation efficiency (EE) of escitalopram, the ultrafiltration method was used. Accordingly, 0.5 ml of the niosomal formulations was placed in the internal chamber of an Ultracel-30K Millipore filters assembly (Millipore Corporation, Billerica, MA) and centrifuged at 4000g for 45 min at 4℃ (Eppendorf® 580R centrifuge, Germany). The escitalopram loaded in noisome remained in the inner chamber while free escitalopram passed through the filter membrane to the outer chamber. Finally, the amount of free escitalopram was measured using UV-Visible spectrophotometry at 238 nm. The following equation was used to calculate EE:

$$Encapsulation\ efficiency(EE\%) = \frac{initial\ Escitalopram\ added(mg) - free\ Escitalopram(mg)}{initial\ Escitalopram\ added(mg)} \times 100$$

Release of Escitalopram from Niosomes

The Escitalopram release profiles were investigated in PBS buffer (pH 7.4) using a dialysis membrane (MWCO 12 KD). A total of 2 ml of escitalopram-loaded niosomes and free forms were dialyzed into 50 ml of PBS buffer solution for 72 h at 37°C. Then, at specific time intervals (0, 0.5, 1, 2, 4, 8, 24, 48 and 72 h), 1 ml of PBS was replaced with the same volume of PBS. To determine the concentrations of released escitalopram, UV-Visible spectrophotometry was used. The Kinetic mechanism of niosomal sample releases was analyzed using zero-order, first-order, Higuchi and the Korsmeyer-Peppas kinetic models.

Storage Stability of Niosomal Liquid Form

To examine any drug leakage from the niosomes during storage, the stability of the niosomes of escitalopram was evaluated. Each formulation of niosome was kept at 4°C and 25°C for 2 months.

Cell culturing and experimental model

Various cell lines including SW48, HepG2, HEK293t, HT29, MRC5, human fibroblast and fat cells, were studied. The cell lines were cultured in DMEM media with 5% FBS (Fetal bovine serum) at a temperature of 37°C with 5% CO2. The cells grew in the flask within 48 hours and were then trypsinized and seeded at a concentration of 5×105 cell/well in 96-well plates. They were incubated for 24 hours under the same media and conditions. After 24 hours, the cells were under a microscope and if their morphology was normal, niosomes and pure escitalopram were added to the plates for 24 hours and 48 hours of incubation time.

MTT Assay

The MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5 - diphenyl-2H-tetrazolium bromide) assay was performed to check cell viability. This method monitored the mitochondrial activity of viable cells and showed the cell viability quantitatively. SW48, HepG2, HEK293t, HT29, MRC5, human fibroblast and fat cells were treated with Escitalopram at different concentrations, niosome

TABLE 2: Size, entrapment efficiency and polydispersity index of E1-E6 niosomal formulations loaded with escitalopram

Formulation	Size (nm)	EE** (%)	PDI*
E1	256.8	92.55	0.358
E2	188.6	97.36	0.395
E3	380.3	90.84	0.261
E4	224	90.04	0.313
E5	276.8	95.18	0.282
E6	419.1	87.36	0.244

* Polydispersity index

** Entrapment Efficiency

blank (without escitalopram) and escitalopram-loaded nanoparticles with an optimized formulation and different concentrations. Each sample was added to wells in triplicates and incubated for 24 and 48 hours. Then, MTT reagent (20µl) was added and kept for incubation (4 hours). Then, the reaction between MTT reagent and dehydrogenase enzymes were formed formazan crystals which were further dissolved with Dimethyl sulfoxide (DMSO) solution. The absorbance was measured using a multi-detection micro plate reader at a wavelength of 570 nm. 5-FU was used as an anti-cancer drug and positive control in this study.

Statistical Analysis

The experiments were performed in sets of triplicate and the statistical data were expressed as the mean value of ± standard deviation. All the experimental data were analyzed using one-way or two-way analysis of variance (ANOVA) and p-value<0.05 was considered a significant level.

Results

Preparation of Niosomes and Niosomal Formulation of Escitalopram

Different molar ratios of surfactants and cholesterol were used to prepare niosomal dispersions with a lipid phase (see Table 1). Based on the results, different formulations of the surfactant mixture (span 60, tween 60) and cholesterol influenced the average size and encapsulation efficiency of the vesicles. It was confirmed that the entrapment efficiency for niosomes using a combination of span 60 and tween 60 was higher than those prepared using only one of them. Also, the combination of span and tween resulted in niosomes with highly stable membranes (Table 2). The physicochemical properties of the nonionic surfactants are shown in Table 2. The Advantages of non-ionic surfactants, such as spans and tweens, include improved stability, widespread compatibility and formulation flexibility. However, the high hydrophilicity of tween 60 surfactant weakens its strength in the niosome membrane. The presence of span 60, with more hydrophobicity and in combination with tween 60 at a 1:1 mole ratio, can lead to condensed niosomal films (Sadeghi et al, 2020). Systems with extensive hydrophilic-lipophilic balance (HLB) ranges are produced by mixing spans and tweens at different ratios (Bharti et al, 2012; Taymouri and Varshosaz 2016).

Generally, multiple factors like the amount of drug and cholesterol, centrifugation force, and alkyl chain length of non-ionic surfactants, affect the entrapment efficiency of drugs in the niosomal suspension (Charnvanich et al, 2010). Niosomes with long alkyl chain (C18) surfactants showed higher entrapment efficiency and greater stability than those with shorter length (Manosroi et al, 2003). The centrifugation process may also increase the risk of damaging niosomal vesicles due to high centrifugation forces, resulting in reduced entrapment efficiency. Cholesterol concentration also plays a crucial role in the entrapment efficiency of escitalopram, as it depends on factors such as increasing cholesterol content, hydrophobicity, and bilayer stability, while decreasing permeability (Bernsdorff et al, 1997). However, a significant increase in cholesterol concentration may compete with the drug for packing space into the bilayer, resulting in the drug remaining unentrapped (Balakrishnan et al, 2009). To achieve a formulation with high entrapment efficiency, an optimal molar ratio of cholesterol: surfactants (1:1) is recommended (Rochdy Haj-Ahmad et al, 2015). As mentioned, the size of the vesicles also influences the entrapment efficiency (Table 2). HLB value

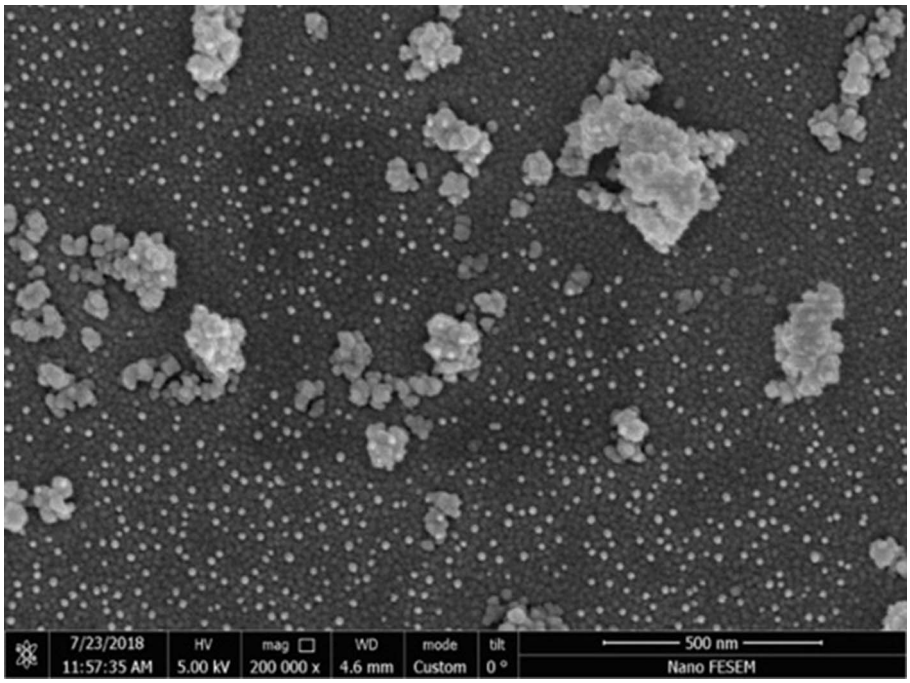


FIGURE 1. Field-Emission SEM image of E2 formulation composed of different weight ratios of surfactants (span 60: tween 60): cholesterol

of surfactants is an important factor affecting the size and distribution of bilayer vesicles (Rochdy Haj-Ahmad et al, 2015). An increase in the HLB value of surfactant results in the formation of larger vesicles (Akbari et al, 2015). Among all formulations, E3 and E6 formulations, with the highest HLB value, had the largest size (Yoshioka et al, 1994).

PDI is used to measure the heterogeneity of the sizes of molecules or particles in a mixture, estimating the width distribution or size heterogeneity (Moghassemi et al, 2015). The PDI value is calculated between 0 and 1; a lower PDI value indicates a more homogeneous suspension (Waddad et al, 2013). As shown in Table 2, all formulations have the same PDI values, indicating that the particle size was homogenous in all formulations.

Microscopic Examination of Niosomal Preparations

Field Emission SEM (FESEM) was used to observe the morphology of the prepared niosomes. Figure 1 shows the FESEM image of E2 formulation. Niosome preparation usually results in multi-lamellar vesicles with the Thin-film hydration method (Hope et al, 1986). These vesicles exhibit higher entrapment efficiency and less drug release (Akbari et al, 2015; El-Sayed et al, 2017). SEM measures the exact diameter of each particle, while the Zetasizer Nano ZS system measures the size, molecular weight, dispersion, and zeta potential of the nanoparticles. Thus, the features of niosomes obtained by SEM were much smaller than those measured by the Nano Zetasizer.

Release Assay of Escitalopram from Niosomal Formulation

The release rate of drugs from vesicular systems is crusial and should be optimized. The release profile of escitalopram from niosome was investigated in PBS buffer (pH 7.4) with E2 and E5 formulations, as they exihibited better size, particle size distribution, and entrapment efficiency than other formulations (Figure 2).

To determine the percentage of released escitalopram at each time period, the total amount of escitalopram was considered in both formulations. The release of escitalopram followed a biphasic process, with an initial relatively rapid release phase followed by a slower extended-release phase. The initial rapid phase resulted from the desorption of escitalopram from the outer surface of niosomes, while the slower phase was primarily related to the diffusion of escitalopram through the vesicles (Manosroi and Bauer 1989). A similar biphasic release process was observed in the release study of ketoprofen from niosomes, with an initial rapid drug leakage observed for the first 6 hours (approximately 50-60%),

TABLE 3: Stability study of escitalopram-loaded niosomes (E2) stored at 4 ± 2 °C and 25± 2 °C after 2 months

Time of storage	4°C			25°C		
(day)	Size (nm)	PDI	EE (%)	Size(nm)	PDI	EE (%)
0	192.30±3.44	0.402±0.00	97.07±0.31	192.30±3.44	0.402±0.007	97.07±0.31
14	212.7±6.4	0.426±0.006	94.88±1.05	225.43±4.65	0.441±0.008	93.94±1.79
30	279.73±8.25	0.452±0.008	93.1±0.66	298.37±8.05	0.473±0.006	91.44±1.23
60	362.2±7.45	0.501±0.009	90.77±0.58	420.67±11.02	0.534±0.008	88.70±1.48

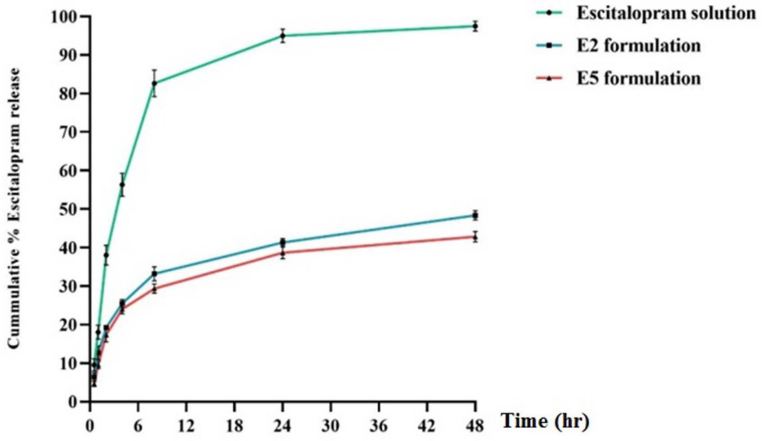


FIGURE 2. In vitro drug release profiles of escitalopram from E2 and E4 niosome formulations.

followed by a slower phase for the next 6 hours (approximately 20%) (Raslan 2013). This finding is consistent with the release study of flurbiprofen from span 40 and span 60 niosomes (Mokhtar et al, 2008). In our study, the initial rapid phase was the same in both formulations, indicating that about 25% of escitalopram was released during the first 4 hours.

The lamellarity of vesicles plays a role in the retention of entrapped drugs, and the thin-film hydration method produces Multi Lamellar Vesicles (MLV). The release rate can vary and depends on several factors, such as the methods of preparation, lamellarity, vesicle size, type and amount of surfactant, cholesterol content, type of drug, and membrane fluidity (Akbari et al, 2015; Ruckmani and Sankar, 2010; Weiner 1989).

Storage Stability of Niosomal Formulations

The size, PDI and percentage of escitalopram remaining in the E2 niosomal formulation were investigated at 4°C and 25°C for 60 days (Table 3). A stable vesicular suspension has a stable size, PDI and drug level, with no precipitation of ingredients (Uchegbu and Vyas, 1998).

Comparing the results of samples stocked at 4°C and 25°C (Table 3) showed that the size of samples stored at 4°C remained more stable than the others. In E2 formulation, the size and PDI of escitalopram increased and EE percentage of escitalopram reduced during 60 days. So, there was a notable difference between samples stored at 4°C and 25°C in terms of size (Balasubramaniam et al, 2002). Experiments usually show an increase in the size of vesicles during storage due to fusion (Lawrence et al, 1996) or aggregation (Seras-Cansell et al, 1996). According to thermodynamic theory, smaller niosomes have more surface energy (Moazeni et al, 2010).

The decrease in EE percentage may be attributed to the higher fluidity of lipid vesicles at higher temperatures, leading to drug leakage (Pardakhty et al, 2011). The high fluidity intensifies vesicle fusion, and during fusion, some vesicles rupture, causing drug leakage. In addition, at high temperatures, the bilayer thickness decreases, and the rate of diffusion across bilayer membrane increases due to the fatty acid chains of the surfactants adopting an irregular configuration (Balasubramaniam et al, 2002).

Cytotoxicity Analysis of Escitalopram and Its Niosomal Formulations

The results obtained from MTT assay after treatment

TABLE 4: Cell viability at different concentrations of 5-FU in different cell lines (%)

Cell lines		SW48	Fibroblast
5-FU concentrations	250 µg/ml	58.1%	92.1%
	100 µg/ml	75.4%	82.3%
	10 µg/ml	81.5%	83.6%
	1 µg/ml	100.0%	86.2%

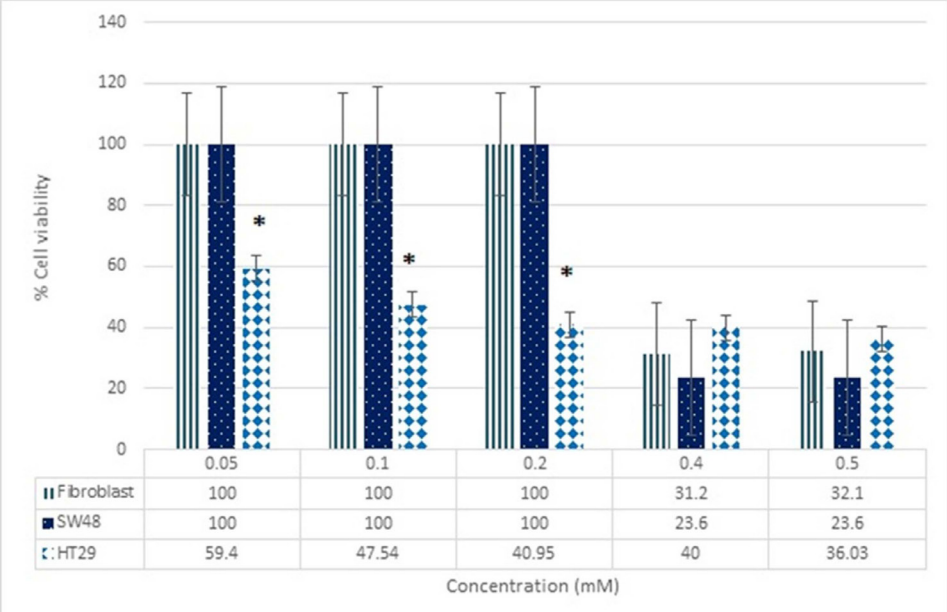


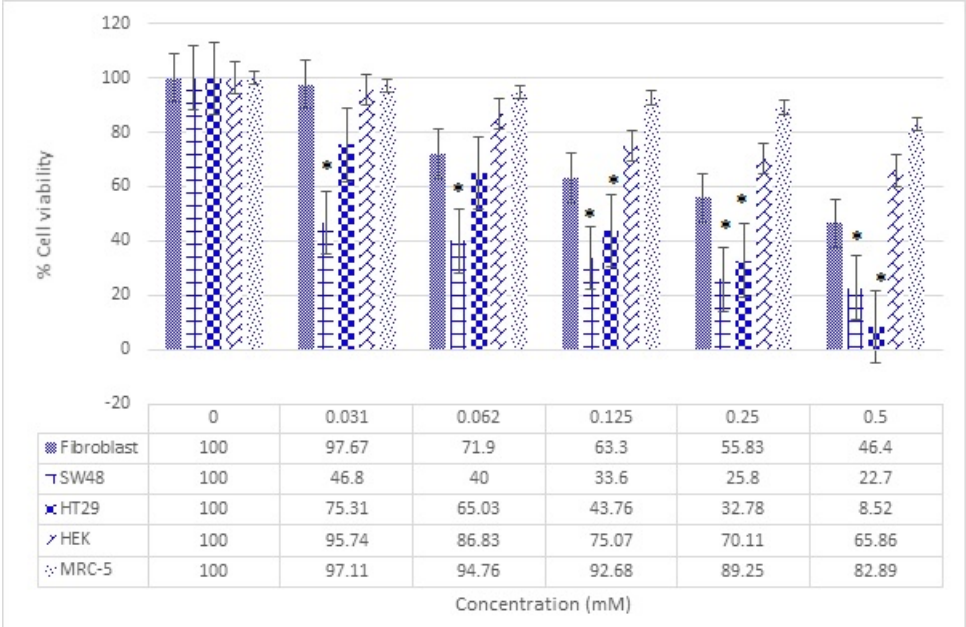
FIGURE 3. MTT assay of escitalopram at different concentrations on fibroblast cells and two colon cancer cell lines (SW48 and HT29) after 48 hours. * *p*-value<0.05 considered significant (comparison between the effects on fibroblast and on cancer cell lines).

with escitalopram (alone) (Figure 3), suggest a direct relationship between the escitalopram concentration and cell viability. As the drug concentration increases, there is a significant increase in cell toxicity. As seen in figure 3, escitalopram in the concentration of 0.2 mM did not reduce the cell viability of SW48 and fibroblast cells during the 48-hour MTT assay, but it significantly reduced the cell viability of HT29 cells to less than 50% in the same time.

Doses greater than 0.2 mM of escitalopram have significant cytotoxic effects on all cell lines, including normal and cancerous cells. Therefore, doses higher than 0.2 mM cannot be considered suitable for cancer treatment. The IC50 values (efficient concentration that causes a 50% decrease in cell viability) were calculated from the respective dose-response curves through regression analysis using Sigma Plot Software (v.8). In Figure 3, it is shown that escitalopram had IC50=0.3761mM on SW48 cells, and IC50=0.2407 mM on Fibroblast cells and IC50=0.0332 mM on HT29 cells. On the other hand, niosome-loaded escitalopram had IC50=0.37 mM on fibroblast, and IC50=0.048 mM on SW48 cells and IC50=0.11 mM on HT29 cells.

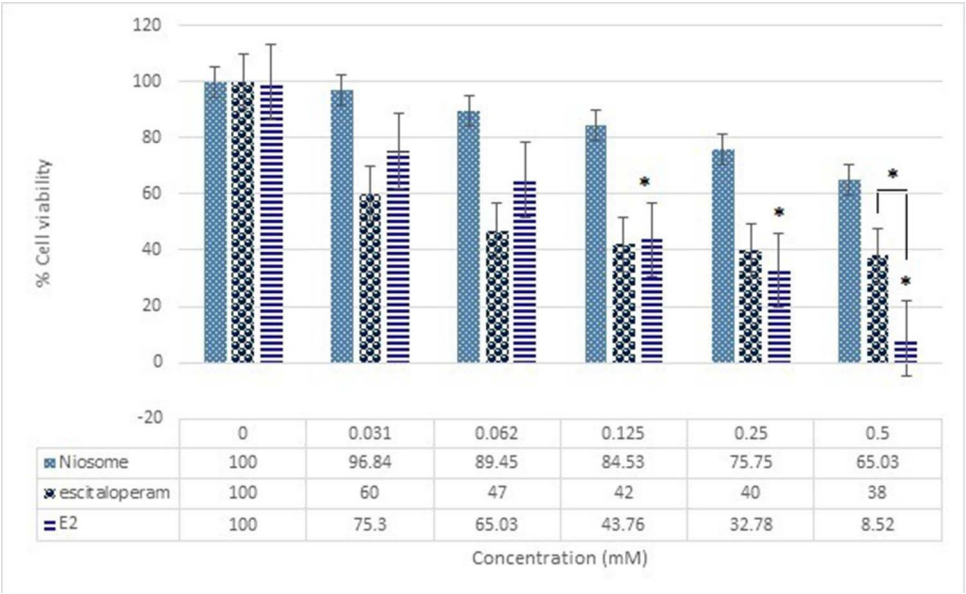
Discussion

However, in the analysis of the niosomal formulations of escitalopram and niosomes (without drug load), comparable drops in cell viability were not observed. Niosome structures without escitalopram loading showed lower cell toxicity than the niosomal formulation of escitalopram in a dose-dependent manner, as niosomes with escitalopram reduced the cell viability to 28.3 ± 0.68. But, the pure drug in concentration of 250mM reduced the cell viability to 24.07 ± 0.35%. Previous experiments revealed that escitalopram significantly inhibits the proliferation of A549 and H460 cell lines and significantly increases the sub-G1 population and caspase-3 activity in these cancer cells (10). It was previously demonstrated that escitalopram can decrease cell viability up to 88.2 ± 0.35 % in NB41A3 cell lines, and this effect was dose and incubation time dependent (8). These findings propose that escitalopram could be a



	0	0.031	0.062	0.125	0.25	0.5
Fibroblast	100	97.67	71.9	63.3	55.83	46.4
SW48	100	46.8	40	33.6	25.8	22.7
HT29	100	75.31	65.03	43.76	32.78	8.52
HEK	100	95.74	86.83	75.07	70.11	65.86
MRC-5	100	97.11	94.76	92.68	89.25	82.89

FIGURE 4. MTT assay of escitalopram-loaded niosomes (E2) at different concentrations on various cell lines after 48 hours. * *p*-value <0.05 was considered significant (comparison between the effects on fibroblast and on cancer cell lines).



	0	0.031	0.062	0.125	0.25	0.5
Niosome	100	96.84	89.45	84.53	75.75	65.03
escitaloperam	100	60	47	42	40	38
E2	100	75.3	65.03	43.76	32.78	8.52

FIGURE 5. The in vitro cytotoxicity analysis of different concentrations of escitalopram and E2 on HT29 cell line after 48 hours. *p-value <0.05 was considered as significant (comparison between the free niosomes and E2 formulation). The effect of E2 formulation on HT29 cell line compared to free escitalopram at the concentration of 0.5 mM was also significant.

promising therapeutic agent for the treatment of colon cancer due to its antiproliferative effects.

The MTT assay, which evaluated the effect of escitalopram on SW48 and fibroblast cells during a 24-hour incubation, showed a significant decrease in cell viability at a concentration of 0.2 mM after 24 hours. After 48 hours of incubation, cell viability showed a significant decrease compared to the 24-hour incubation, up to a concentration of 0.4 mM. However, at concentrations of 0.5 mM and 1mM, cell viability showed an increasing trend at 48 hours compared to the 24-hour test (data not shown). Following these results, 48-hour incubation after treatment was selected as the optimal time for cytotoxicity assays. Evaluation of the effect of escitalopram on colon cancer cell lines confirmed its inhibitory effect on cell proliferation, especially on HT29 cell line. The best effect of escitalopram as an anti-proliferative agent on HT 29 cells was observed at concentrations less than

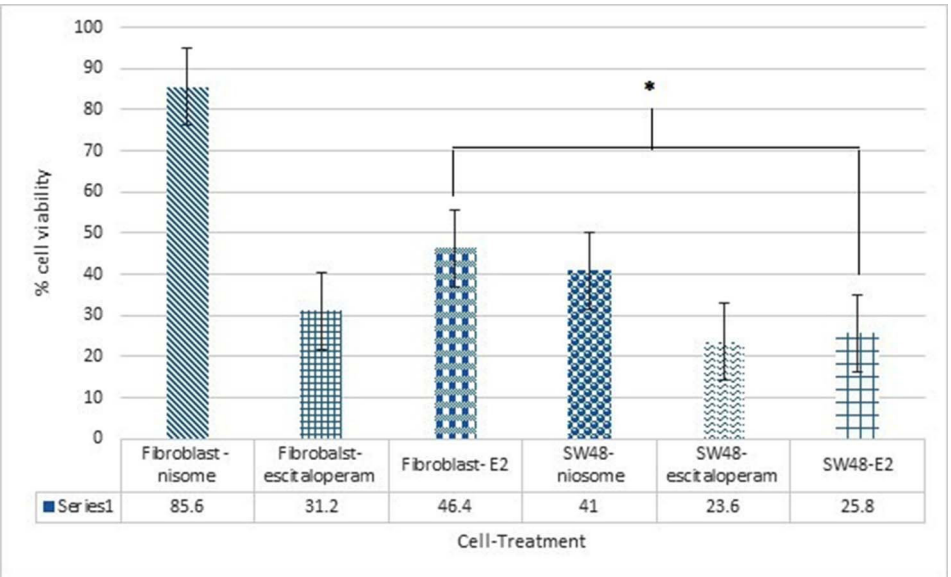


FIGURE 6. Cytotoxicity assay of samples on SW48 cell lines compared to fibroblast cells after 48 hours of treatment. Niosomes without drug loading (niosome), Escitalopram drug only (escitalopram), Escitalopram-loaded nanoparticle formulation (E2); **p*-value <0.05 was considered as significant.

0.2mM, where it did not have a cytotoxic effect on normal fibroblast cells.

Cytotoxicity of several niosomal formulations of escitalopram (Table 2) was evaluated on SW48, HT29, Hek, HepG2, and human fat cells. Among the formulations, only E2 showed low toxicity on normal cells, making it the best candidate for further assays due to its higher reported release of escitalopram. However, based on the results obtained, new formulations with even less toxicity on normal cells should be designed and evaluated in future studies.

According to Figure 4, the niosomal formulation of escitalopram had the most potent anti-proliferative effect on HT29, and this effect significantly increased in a dose-dependent manner. However, at high doses, this niosomal formulation could also reduce the cell viability of normal fibroblasts cells. Therefore, we cannot suggest this formulation at doses greater than 0.2 mM.

5-Flurouracil (5-FU), an approved chemotherapeutic agent, was used to compare the anti-proliferative effects of escitalopram on cell viability with a routinely used anti-cancer drug. The results of the MTT assay for 5-FU are reported in table 4. The cytotoxic effect of E2 formulation on SW48 cell lines at a low concentration (0.031 mM) that did not show cytotoxic effect on fibroblast cells, was found to be comparable to 250 µg/ml of 5-FU.

The effect of the E2 formulation of escitalopram was compared to the niosome free sample and free escitalopram as the active ingredient. The results are shown in Figure 5, which demonstrates that at low concentrations (less than 0.125 mM), most of the anti-proliferative effect of the E2 formulation is attributed to the effect of its active ingredient, escitalopram. However, with increasing concentration, the cytotoxic effect of the delivery system (niosome) contributes to an increased cytotoxic effect of the E2 formulation compared to escitalopram alone. In addition, a synergistic effect of escitalopram and the niosome formulation was observed on the HT29 cell line after 48 hours of incubation with a high concentration (0.5 mM). This means that the reduction in cell viability after treatment with the E2 formulation was grater than the numerical summation of the anti-proliferative effects of niosome and escitalopram when used seprately.

Comparison of the cytotoxic effects of noisomes, escitalopram, and the E2 formulation on fibroblast cells and the cancerous SW48 cell line (Figure 6), revealed that escitalopram, on its own, had a significant effect on the cell viability of normal cells such as fibroblast cells. However, in the noisome formulation, its cytotoxic effect mildly decreased. Accordingly, using noisome structures as a delivery vehicle can be a promising strategy for utilizing escitalopram as a chemotherapeutic agent. In addition, the cell viability of the SW48 cell line decreased about 2-fold more compared to fibroblast cells.

Conclusion

Nanoparticles have been used to protect drugs in the systemic circulation and deliver them at a controlled and sustained rate to the target site. Due to escitalopram oxalate's high hepatic metabolism, a nanoparticle-based formulation was chosen to increase its efficacy and reduce adverse effects on the liver. The preparation of escitalopram-loaded niosomes was successfully optimized, achieving a drug concentration of 1.5 mg/ml.

Further, *in vitro* cytotoxicity effect of niosomes was evaluated on SW48, HT29, skin fibroblast cell lines, Hek293t, HepG2, MRC-5, and Human Fat cell lines. MTT assay results showed that niosomes were not toxic to the human fibroblast and fat cell lines as normal cells, and they had no significant effect on MRC-5 cell lines during the test period (except at high concentrations). The escitalopram and its niosomal formulations showed significant cytotoxicity against colon cancer cell lines (SW48 and HT29). our study suggests that the niosomal formulation of escitalopram can be considered an anti-proliferative agent for colon cancer cell lines. If the formulation can be improved to result in a less toxic nanosystem on normal cells, it could be further evaluated in *in vivo* models, particularly using rectal formulations, to study various pharmacokinetic and pharmacodynamic parameters and fully establish its therapeutic potential.

Conflict of interest

The authors report no conflict of interest.

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