RESEARCH ARTICLE

Development & Formulation of Taxifolin Loaded Niosomes for its Anticancer Effect on MCF-7 Breast Cancer

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ABSTRACT

In order to increase Taxifolin's solubility and bioavailability, this study set out to create and assess niosomes of Taxifolin powder by the thin film hydration approach. A nonionic surfactant called span 60 and a membrane stabiliser called cholesterol were utilised. Particle size distribution, zeta potential, and light scattering analysis were all examined. Niosomes demonstrated a mean particle size between 106.5± 7.42 nm and 115.8 ±11.61 nm, with particle size increasing as the surfactant and cholesterol molar ratios were raised. The percentage yield was found to be in range 44 ± 0.235% to 81 ± 0.216%. The optimized batch (FC6) had zeta potential of -27.7 ± 2.37 mV and 92.78 ± 3.14 %entrapment efficiency. Taxifolin loaded niosomes demonstrated sustained release of Taxifolin.The anticancer potential of compound was evaluated using an *in vitro* cytotoxicity assay on MCF-7 cell lines. Finally, associated to free Taxifolin, cytotoxic impact of Taxifolin loaded niosomes on MCF-7 cell line was found to be substantially improved by the MTT assay. Result demonstrated that solubility and bioavailability of naturally derived Taxifolin phytochemicals can be increase by loading it into niosomes, thus enhance their anticancer efficacy.

Keywords: Taxifolin, Phytochemical, Niosomes, MCF-7 Breast Cancer Cell.

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INTRODUCTION

The greater safety and biocompatibility of bioactive substances produced from natural sources make them the preferred choice over manufactured medications.[1] Herbal remedies made from plants have a long history of use as a remedy. Natural medicines, like Taxol have recently gained popularity as an alternative therapy and have received clinical approval for management of cancer patients.[2,3] Plumbagin, a yellow dye that is officially generated from naphthoquinone, is another example. Multiple cancer cell lines have shown that plumbabin induces cell death and cell cycle arrest, including melanoma, breast, and lung cancers.[4] Phytochemicals have a limited therapeutic window, are poorly soluble in water, have a low bioavailability, penetrate cells poorly, have a hepatic disposition, and are quickly absorbed by normal tissues, all of which reduce their effectiveness in fighting cancer. In order to increase selectivity, efficacy, safety, dose reduction, and patient compliance, drug delivery structures based on nanocarriers have been established for a variety of natural bioactives.[5] Various chemicals produced from edible plants have been associated with cancer treatment and chemoprevention.[6,7] Among these organic substances naturally occurring polyphenolic chemicals, including flavones, isoflavones, flavanones, flavonols, chalcones, and anthocyanins, are rich in flavonoids, a family of phytochemical agents. The potential of dietary flavonoids to prevent and treat cancer has been thoroughly investigated in both laboratory and clinical research.[8] Flavonoids have been demonstrated to have a variety of pharmacological actions and have a chemical structure with quercetin. The compound taxifolin, formally known as dihydroquercetin, has potent antioxidant properties.[9,10] Comparable to quercetin, which has been shown to trigger cell death, Taxifolin shares many of the same characteristics. Current medical practice makes extensive use of taxifolin for its antioxidant, anticancer, and anti-virus capabilities, among others. This compound has also found widespread application in the food and health care industries.[11] Taxifolin have low solubility made it difficult to absorb and metabolize in the body, resulting in a considerable reduction of its bioavailability and effectiveness.[12] The dissolving rate and drug solubility were found to be correlated with precise surface area of materials, by smaller drug particles having a larger effective area contacting the media.[13] To increase Taxifolin's solubility and bioavailability, it is essential to use nanotechnology to produce uniformly small niosomes as nanocarrier. Drug delivery involves use of several nanocarriers, with niosomes being one of the most effective. A vesicular nanoparticle known as a niosomes is made up of helper lipids like cholesterol and non-ionic surfactants that can make the bilayer stiffer.[14] Niosomes possess a great deal of potential to specifically target tumor cells and exhibit a regulated release within them. Due to their remarkable attributes such as Uncomplicated fabrication, affordability, excellent stability, and the capability to encase both

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Table 1: Optimization on the basis of Surfactant and Cholesterol.

Sr. No.	FC	Taxifolin: Span 60: Cholesterol; (% w/w/w)	% Practical Yield	Average Particle Size (nm)	Zeta Potential (mV)	% EE	PDI
1.	F1	1:1:1	44 ± 0.235%	106.5± 7.42	-23.8± 1.32	53.51 ± 3.31	0.094 ± 0.083
2.	F2	1:1:2	57 ±0.169	108.7 ± 11.84	-17.7 ± 1.74	64.51 ± 3.31	0.103 ± 0.12
3.	F3	1:2:1	62 ± 0.261	105.2± 8.78	-18.4 ± 1.32	73.32 ± 3.73	0.213 ± 0.086
4.	F4	1:2:2	69 ± 0.198	100.6± 10.57	-25 ± 2.74	76.68 ± 2.87	0.420 ± 0.08
5.	F5	1:3:3	75 ± 0.283	112.1± 9.42	-20.7 ± 1.46	81.34± 3.27	0. 226 ± 0.089
6	F6	1:4:4	81 ± 0.216%	115.8 ±11.61	-27.7 ± 2.37	92.78 ± 3.14	0.191 ± 0.98

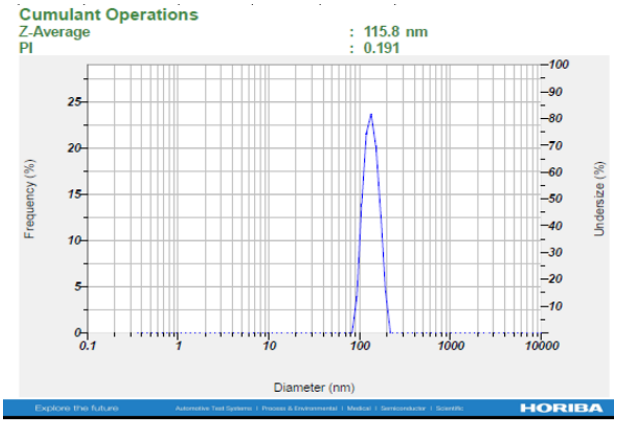

Figure 1: Particle size of FC6formulation.

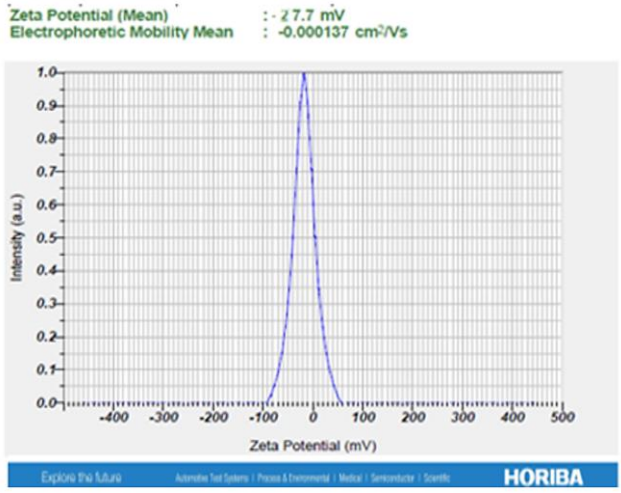

Figure 2: Zeta potential of FC 6formulation.

hydrophobic and hydrophilic drugs niosomes are considered a viable alternative to other delivery systems, especially liposomes.[15,16]

MATERIAL AND METHOD

Material

Taxifolin powder was obtained from Yucca Enterprises Mumbai, Cholesterol and Span 60 was obtained from Loba Chemie. All other materials were of analytical grade. The MCF-7 cell lines were bought from NCCS, Pune, India.
MTT reagent, Dimethylsulfoxide, were procured from Sigma-Aldrich Chemical Private Ltd India.

Method of preparation

Thin film hydration method was used to create Taxifolin-encapsulated niosomes, as previously reported. In round-bottom flask with chloroform, exact molar ratios of Taxifolin, span 60 & cholesterol (1:4:4), and DCP were dissolved. Following that, a thin film was created and the chloroform was evaporated by rotary evaporator set to 60°C and 100 rpm for 30 minutes (Labline PBV 7D). The resulting lipid film was hydrated for one hour at 60°C using 10 milliliters of pH 7.4 phosphate buffer in order to produce niosomes formulations. To produce niosomes with a consistent size distribution, the resultant milky solution was collected and consequently sonicated on ultrasonic bath for 30 min at 50°C.[17] This suspension is then allowed to freeze overnight at -20 °C refrigerator. To obtain powder, the frozen niosomes was then subjected to lyophilize. The formed niosomes was used for evaluated.[18,19]

Characterization of niosomes

Determination of percentage yield

The percentage yield can be used to calculate how much product is produced once the process is finished. The percentage yield of all batches of formulations was calculated by dividing measured weight of formulations with theoretical weight and multiplying with 100 by subsequent equation.[20]

Percentage Yield = (Practical Yield/ Theoretical Yield) ×100

Analysis of PDI, Particle size, and zeta potential

PDI, particle size, and zeta potential were measured, which employs dynamic light scattering technology. Prior to analysis, the formulations were diluted and transferred into a cuvette, where assessment were conducted at 25°C ± 5°C. Zeta potential was assessed using a specialized cuvette with an applied potential of 3.3 mV. For determining electrophoretic mobility, the niosomes formulations were placed in a zeta potential cuvette.[21,22]

% Entrapment efficiency

Entrapment efficiency represents the proportion of the active component encapsulated within the niosomes. Entrapment efficiency of niosomal formulation was evaluated using a centrifugation technique. Niosomes were isolated through ultracentrifugation at 18,000 rpm for 25 minutes, and entrapment efficiency was assessed by computing difference between total drug captured inside niosomes and unencapsulated drug available in supernatant. Quantity of free active constituent in supernatant was assessed spectrometrically at a wavelength of 288 nm. Entrapment efficiency (EE) % was then derived using expression below.[23]

% Entrapment Efficiency = (Total drug −Free drug in supernatant/ Total drug) ×100

Table 2: Model, equation, and regressions of Taxifolin release from niosomes.

Model	Equation	R^2
Zero-Order	y = 3.2208x + 30.224	R^2 = 0.662
First Order	y = 0.0459x + 1.1984	R^2 = 0.3168
Higuchi	y = 19.397x + 9.6295	R^2 = 0.9005
Kors-peppas	y = 0.933x + 0.9459	R^2 = 0.5341

DSC analysis

DSC was utilized to evaluate the thermal behavior and physical form of pure Taxifolin, the physical mixture, and the optimized formulation. The analysis was performed under nitrogen flow within a temperature 25°C to 260°C, by heating increment of 10°C per minute, on samples weighing 3-5 mg that were placed in aluminium pans.[24,25]

***In vitro* drug release**

An *in vitro* drug release experiment was executed to investigate release profiles of free Taxifolin and Taxifolin-loaded niosomes. Dialysis bag method was employed for this dissolution study. Separate dialysis bags, each containing either free Taxifolin or lyophilized Taxifolin-loaded optimized formulation were soaked overnight in phosphate buffer (pH 7.4). After sealing both ends of bags, they were connected to paddles of a dissolution test instrument (Electrolab Dissolution Tester TDL-08L, India) containing phosphate buffer (pH 7.4) as dissolution fluid. Wholemethod was upheld at 37 ± 0.5℃ by a paddle rotational frequency of 100 rpm. At predefined time recesses, samples were taken out and exchangedby an equal volume of newly prepared medium to preserve saturated state. Quantity of drug released from formulation was measured using a UV spectrometrically at 288 nm.[26,27]

Kinetic release study

The *in vitro* drug release data was evaluated using many kinetic models. Following are the models used to determine the niosome-release kinetics of Taxifolin: the Zero-order, First-order, Higuchi, and Korsmeyer-Peppa versions (KP).Determination coefficient (r^2) for each model was calculated to identify the optimal model forrelease data.[28,29]

***In vitro* cytotoxicity (MTT assay) for anticancer activity**

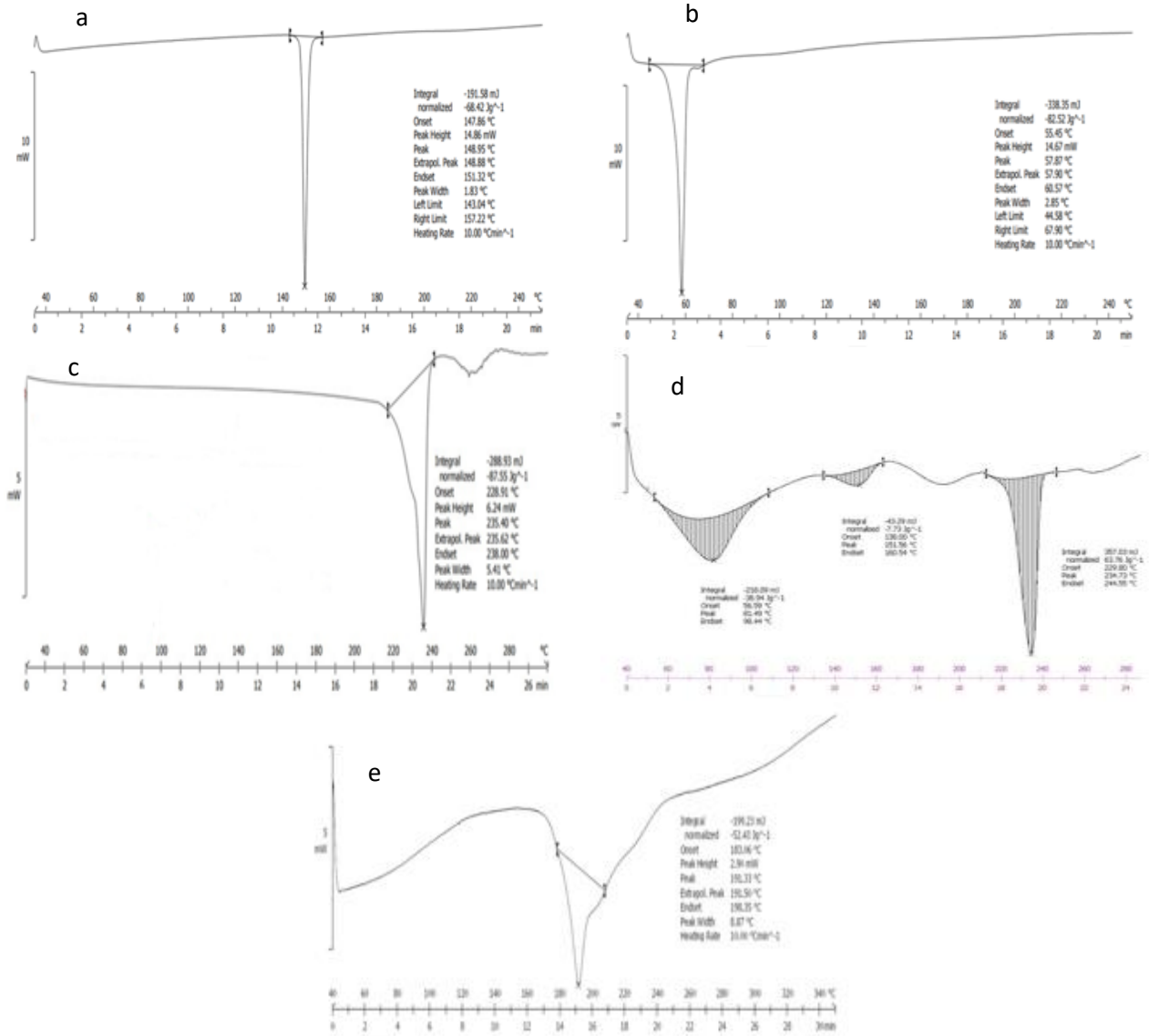

Figure 3: DSC thermogram of a.cholesterol, b. span 60, c. Taxifolin, d. physical mixture, e. Taxifolin niosomes formulation (FC6).

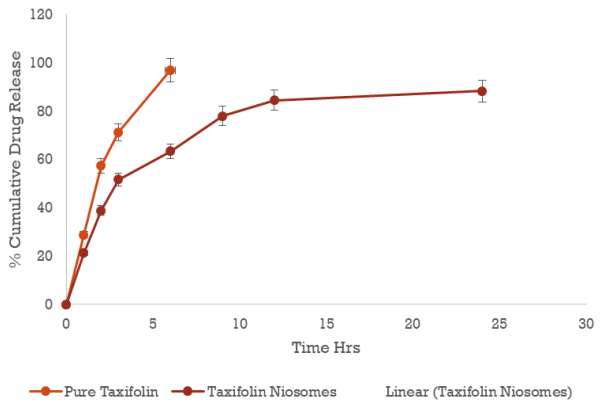

Figure 4: Taxifolin and Taxifolin niosomes *In vitro release* profiles in PBS 7.4 at 37 °C.

Preparation of cells

Non-essential amino acids and 10% foetal bovine serum were added to the MEM in which MCF-7 cells were cultured. The cells were sustained at 37°C in a 5% CO_2 environment, with entire medium being altered each 2 to 3 days.

MTT assay procedure

The cytotoxic effects of the niosomes formulation were assessed using the MTT assay with MCF-7. The MCF-7 cell line, sourced from NCCS, Pune, India, was grown in 96-well plates and humidified incubator with 5% CO2 for 24 hours, achieving a near-confluent monolayer. Culture medium was then detached, and cells were subjected to seven concentrations levels of Taxifolin and Taxifolin-loaded niosomes (10, 25, 50, 100, 250, 500, and 1000 µg/ml). Cisplatin was used as a reference standard. Cells cultured without any test substances served as the control. Following a 24-hours incubation and 5% CO2, cells were inspected under an inverted microscope for any morphological changes or signs of cell death.[30] Following this observation, 100 µl of freshly made medium and 10 microlitres of MTT dye were used to replace the original media.[31] After that, the plates needed to be incubated for four hours at 37°C with 5% CO2. Afterwards, 100 µl of a solubilisation solution was added to each well, and plates were thereafter left at 37°C with 5% CO2 for an additional hour.[32] The absorbance of formazan crystals at 570 nm was measured using a microplate reader to assess the cell survival percentage.[33,34] Next, the percentage was calculated utilising formulas that have been published.[35,36]

Percent Cell Survival (%) = (Absorbance of Test/ Absorbance of Control) * 100

RESULTS AND DISCUSSION

Determination of practical yield

% yield of FC6 formulation was highest as compared to other formulations. The percentage yield was found to be in range 44 ± 0.235% to 81 ± 0.216%.

Analysis of size of particle, PDI and zeta potential

Optimization of niosomes formulations was achieved by adjusting surfactant to cholesterol molar ratio to maximize entrapment efficiency, target size of particle, zeta potential, and particle size distribution. Span 60 to cholesterol ratio was refined to identify optimal formulation. Particle size measurements, conducted using dynamic light scattering with a Horiba SZ 100, ranged from 106.5 ± 7.42 nm to 115.8 ± 11.61 nm. An increase in particle size was noted with higher concentrations of cholesterol and Span 60, likely due to the development of a more robust bilayer membrane.Using the PDI, we were able to determine how homogeneous the formulation was; values below 0.1 indicated very high homogeneity, while values above 0.3 indicated very high heterogeneity. The optimised formula (FC6) showed a PDI of 0.191±0.98, which means it was stable and homogeneous enough. The zeta potential was used to assess the stability of niosomes formulations. Typically, a zeta potential close to ±30 mV ensures stability for longer duration. The optimized batch exhibited a zeta potential of -27.7±2.37 mv. High negative zeta potential values generally reflect stable formulations and minimize aggregation (Figure 1 and Figure 2). The inclusion of Taxifolin in niosomes resulted in lower zeta potential values, contributing to increased formulation stability.[37]

Determination of % entrapment efficiency

The entrapment efficiency data across various formulation codes (FC1 to FC6) indicate that FC6 stands out as the optimized formulation, achieving the highest entrapment efficiency.The optimised FC6 has an entrapment efficiency of 92.78% with a margin of error of 3.14%. The incorporation of hydrophobic medicines into niosome bilayers through electrostatic interactions has been demonstrated in numerous studies. Exceptional entrapment of Taxifolin in niosomes is due to its hydrophobic properties, which enhance its affinity for and incorporation into the bilayers. This high drug loading is advantageous for systemic administration, ensuring a substantial release of taxifolin at the locus of action. In comparison, other study reported a loading efficiency of 69% for lawsone-encapsulated niosomes with a particle size of 0.3microns (Table 1). The Taxifolin-loaded niosomes developed in this study not only demonstrate superior loading efficiency but also exhibit a reduced particle size relative to the sizes as reported earlier.[38,39]

DSC analysis

The DSC thermogram technique was used to confirm the physical state of optimized niosomes formulation. DSC thermogram ofcholesterol, span 60, Taxifolin, its physical mixtureand optimized batch Taxifolin niosomes (FC6) was recorded and displayed in figure 3a, 3b, 3c, 3d, and 3e respectively. DSC analysis of pure Taxifolin, a prominent endothermic peak at 235.40°C was seen, demonstrating its purity. In the physical mixture unique endothermic peaks of the individual components were evident. DSC analysis of the optimized batch (FC6) was found to be 198.35 °C. It indicates that Taxifolin is dispersed in molecular level to excipients or Taxifolin is in amorphous form. It was also observed that the thermogram showed no additional peaks of available free Taxifolin, indicating that there had not been any precipitation and that the Taxifolinhad been completely encapsulated in the vesicles.[40]

***In vitro* release study**

Diffusion bag release studies were executed in a phosphate buffer with a pH of 7.4 at 37 °C to determine *in vitro* release of both free Taxifolin and niosomes loaded with Taxifolin. Figure 4 shows that there is a substantial alteration in cumulative % release between free Taxifolin and Taxifolin niosomes formulation. Free Taxifolin was able to completely release its drug content within 6 hours when it was allowed to freely diffuse in solution form. Nevertheless, the release of Taxifolin from niosomes showed a biphasic pattern, beginning by a burst release of 21.24% during the first hour and continuing with sustained release for up to 24 hours. The release rate of Taxifolin from niosomes was much lower than that of free Taxifolin, remaining at 88.18 ± 0.012% for the entire 24 hours.

Niosome bilayer loading was facilitated by Taxifolin's hydrophobic properties. Since there is no shell to allow for prolonged release, the release of free Taxifolin was extremely rapid; nearly all of the drug was liberated within 6 hours. Our formulation's Taxifolin release rate was much lower than that of free Taxifolin. These results demonstrated that niosomes can release Taxifolin in a controlled and sustainable manner. A key component of effective cancer treatment, according to research, is the prolonged release of medicines from niosomes. The release kinetics of paclitaxel-encapsulated niosomes were studied earlier. Following 17 hours, their formulation had a 95.6% drug release rate. Drug packed niosomes exhibit a persistent release pattern, as demonstrated by the aforementioned research investigations.[41]

Kinetic release study

By comparing *in vitro* release data with several release kinetics models, drug release mechanism from niosomes was anticipated. Kinetic studies showed that R^2 Value of Higuchi Model was highest than that of others, i.e. (R^2> 0.9005) near to one suggesting the Higuchi Kinetic Model was the best fit release model followed (Table 2). It indicates drug release from insoluble matrix.[42]

***In vitro* cytotoxicity (MTT assay) for anticancer activity**

The release that occurs in a controlled laboratory setting by employing the MTT assay, the researchers examined the anticancer effects of Taxifolin-loaded niosomes *in vitro* and contrasted them with free Taxifolin in solution, setting of human breast cancer MCF-7 cells, taxifolin niosomes and conventional cisplatin are examined. Because niosomes are more soluble and interact better with malignant cells, loading hydrophobic medicines into them can increase the toxicity of the resulting formulations. The results shown in figure 5 and figure 6 indicate that taxifolin-loaded niosomes have better anticancer activity than free taxifolin in solution and standard cisplatin. It is clear that the toxicity of the medicine increased when the amounts of Taxifolin were raised. The loading of Taxifolin into niosomes resulted in a notable increase in cytotoxicity. Taxifolin niosomes had a

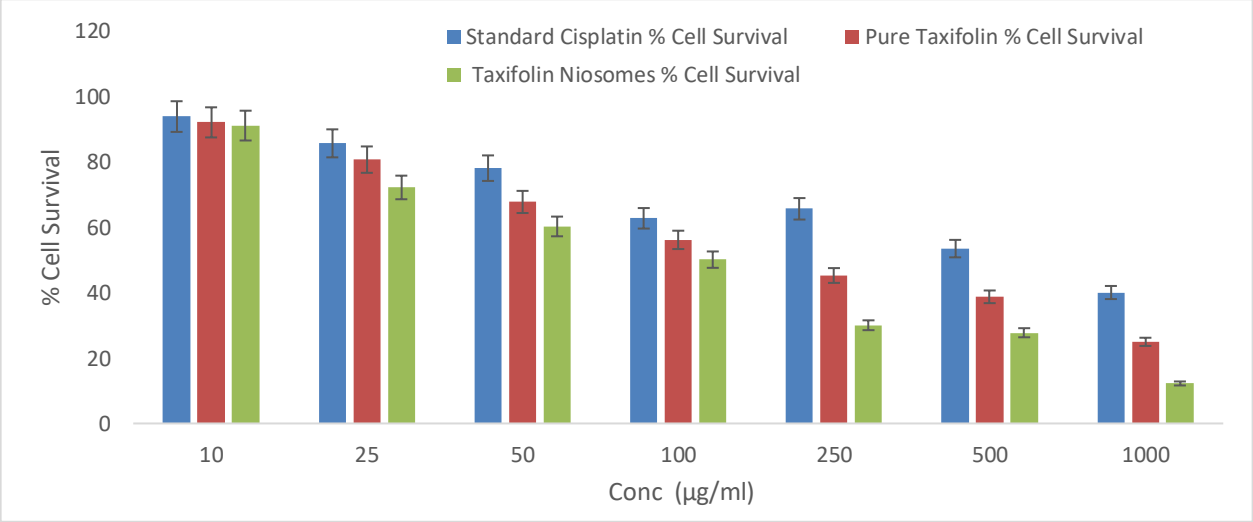

Figure 5: MTT cytotoxicity assay of cisplatin, pure Taxifolin, and Taxifolin niosomes (FC6) was determined at various concentrations (µg/ml) in MCF7 cells.

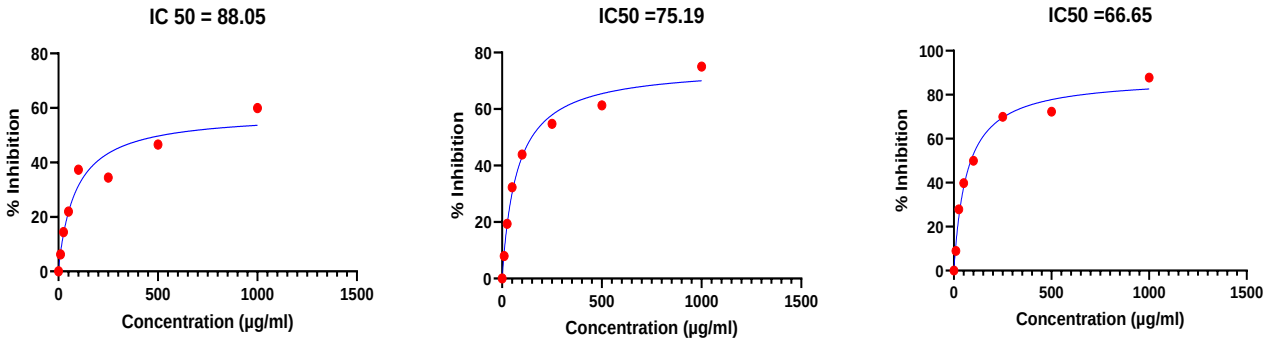

Figure 6: The bar graph indicating % cell survival of standard cisplatin, free Taxifolin and Taxifolin niosomes formulation against MCF-7 cell line.

viability of 12.23% at a concentration of 1000 µg/ml, compared to 24.95% for free Taxifolin. A drug's IC50 value is concentration at which 50% of cell growth is inhibited. The inhibitory concentration (IC50) of Standard Cisplatin, Pure Taxifolin and Taxifolin Niosomes were determined using the MTT assay. IC50 of Standard Cisplatin, Pure Taxifolin in MCF-7 cells was found to be 88.08 ug/ml and 75.19 ug/mlcorrespondingly. An IC50 of 66.65 ug/ml for Taxifolin optimised niosomes formulation (FC6) demonstrated that the cytotoxic effect of Taxifolin loaded formulations was significantly greater than that of free Taxifolin. The results of this work provide credence to theory that niosomes, by virtue of their capacity to encapsulate phytochemical components and improve their efficacy with time, could be a useful nanocarrier for cancer treatment.[43]

CONCLUSION

It was shown that the cytotoxic effect of Taxifolin loaded formulations was much larger than that of free Taxifolin, with an IC50 of 66.65 ug/ml for the Taxifolin optimised niosomes formulation (FC6). In conclusion, this study's findings support the idea that niosomes are a capable nanocarrier for cancer management due to their capability to excellently encapsulate phytochemical components and enhance their efficacy over time.

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REFERENCES

1. Bothiraja C, Kapare HS, Pawar AP, Shaikh KS. Development of plumbagin-loaded phospholipid–Tween® 80 mixed micelles: formulation, optimization, effect on breast cancer cells and human blood/serum compatibility testing. Therapeutic delivery. 2013;4(10):1247-1259. https://doi.org/10.4155/tde.13.92
2. Barani M, Mirzaei M, Torkzadeh-Mahani M, Nematollahi MH. Lawsone-loaded Niosome and its antitumor activity in MCF-7 breast Cancer cell line: a Nano-herbal treatment for Cancer. DARU Journal of Pharmaceutical Sciences. 2018;26:11-17.
3. Nicolaou KC, Yang Z, Liu JJ, Ueno H, Nantermet PG, Guy RK, Claiborne CF, Renaud J, Couladouros EA, Paulvannan K, Sorensen EJ. Total synthesis of taxol. Nature. 1994; 17;367(6464):630-634.
4. Castro FA, Mariani D, Panek AD, Eleutherio EC, Pereira MD. Cytotoxicity mechanism of two naphthoquinones (menadione and plumbagin) in Saccharomyces cerevisiae. PloS one. 2008 22;3(12):e3999. DOI:10.1371/journal.pone.0003999
5. Jin SE, Jin HE, Hong SS. Targeted delivery system of nanobiomaterials in anticancer therapy: from cells to clinics. BioMed research international. 2014;2014(1):814208.http://dx.doi.org/10.1155/2014/814208
6. Sakpakdeejaroen I, Somani S, Laskar P, Mullin M, Dufès C. Transferrin-bearing liposomes entrapping plumbagin for targeted cancer therapy. Journal of interdisciplinary nanomedicine. 2019;4(2):54-71. doi: 10.1002/jin2.56.
7. Ahmad R, Srivastava S, Ghosh S, Khare SK. Phytochemical delivery through nanocarriers: A review. Colloids and Surfaces B: Biointerfaces. 2021; 1;197:111389.doi: https://doi.org/10.1016/j.colsurfb.2020.111389
8. Jeetah R, Bhaw-Luximon A, Jhurry D. Nanopharmaceutics: phytochemical-based controlled or sustained drug-delivery systems for cancer treatment. Journal of biomedical nanotechnology. 2014;1;10(9):1810-1840. https://doi.org/10.1166/jbn.2014.1884.
9. Chen X, Gu N, Xue C, Li BR. Plant flavonoid taxifolin inhibits the growth, migration and invasion of human osteosarcoma cells. Molecular medicine reports. 2018;17(2):3239-3245. doi: 10.3892/mmr.2017.8271.
10. Kim YJ, Choi SE, Lee MW, Lee CS. Taxifolin glycoside inhibits dendritic cell responses stimulated by lipopolysaccharide and lipoteichoic acid. Journal of Pharmacy and Pharmacology. 2008;60(11):1465-1472., https://doi.org/10.1211/jpp.60.11.0007
11. Kang MJ, Eum JY, Park SH, Kang MH, Park KH, Choi SE, Lee MW, Kang KH, Oh CH, Choi YW. Pep-1 peptide-conjugated elastic liposomal formulation of taxifolin glycoside for the treatment of atopic dermatitis in NC/Nga mice. International journal of pharmaceutics. 2010; 15;402(1-2):198-204. doi: 10.1016/j.ijpharm.2010.09.030.
12. Zu Y, Wu W, Zhao X, Li Y, Wang W, Zhong C, Zhang Y, Zhao X. Enhancement of solubility, antioxidant ability and bioavailability of taxifolin nanoparticles by liquid antisolvent precipitation technique. International journal of pharmaceutics. 2014;25;471(1-2):366-376.doi: 10.1016/j.ijpharm.2014.05.049.
13. Shikov AN, Pozharitskaya ON, Miroshnyk I, Mirza S, Urakova IN, Hirsjärvi S, Makarov VG, Heinämäki J, Yliruusi J, Hiltunen R. Nanodispersions of taxifolin: Impact of solid-state properties on dissolution behavior. International Journal of Pharmaceutics. 2009;30;377(1-2):148-152.doi: 10.1016/j.ijpharm.2009.04.044.
14. Rasenack N, Müller BW. Micron-size drug particles: common and novel micronization techniques. Pharmaceutical development and technology. 2004;1;9(1):1-3. doi: 10.1081/pdt-120027417.
15. Singh B, Sharma P, Bose S, Thakur J, Saini G, Vyas M. Niosomes: The current and future perspectives in drug delivery system. Research Journal of Pharmacy and Technology. 2021;14(7):3961-3966.http://dx.doi.org/10.52711/0974-360X.2021.00687

16. Kumar P, Verma N. An overview on niosomes: As an auspesious drug delivery system on the bases of application. Research Journal of Pharmacy and Technology. 2021; 14(5):2896-2902. http://dx.doi.org/10.52711/0974-360X.2021.00508
17. Avinash V, Umashankar MS, Damodharan N. Niosomes: A more promising tool to load poorly penetrating drug through skin for the treatment of Acne vulgaris. Research Journal of Pharmacy and Technology. 2020;13(6):3035-3040.http://dx.doi.org/10.5958/0974-360X.2020.00536.3
18. Hajizadeh MR, Maleki H, Barani M, Fahmidehkar MA, Mahmoodi M, Torkzadeh-Mahani M. In vitro cytotoxicity assay of D-limonene niosomes: an efficient nano-carrier for enhancing solubility of plant-extracted agents. Research in pharmaceutical sciences. 2019 Oct 1;14(5):448-458.doi: 10.4103/1735-5362.268206.
19. Agarwal S, Mohamed MS, Raveendran S, Rochani AK, Maekawa T, Kumar DS. Formulation, characterization and evaluation of morusin loaded niosomes for potentiation of anticancer therapy. RSC advances. 2018;8(57):32621-32636.doi: 10.1039/c8ra06362a.
20. Hajizadeh MR, Parvaz N, Barani M, Khoshdel A, Fahmidehkar MA, Mahmoodi M, Torkzadeh-Mahani M. Diosgenin-loaded niosome as an effective phytochemical nanocarrier: Physicochemical characterization, loading efficiency, and cytotoxicity assay. DARU Journal of Pharmaceutical Sciences. 2019;1;27:329-339.doi: 10.1007/s40199-019-00277-0.
21. Dhole R, Patil U, Jadhav N. Stabilization of hydrochlorothiazide nanocrystals using fibroin. Marmara Pharm. J. 2019;1;23:997-1008.10.35333/jrp.2019.64.
22. Joshi S, Sharma L, Tare M, Baheti D, Dama G, Tare H. The Nutritional Needs of Mothers and Babies: A Review. International Journal of Pharmaceutical Quality Assurance. 2023; 14(2):421-425.
23. Kumar YA, Setty CM. Formulation and evaluation of maltodextrin based doxorubicin HCl proniosomes. Research Journal of Pharmacy and Technology. 2019;12(6):2776-2780.http://dx.doi.org/10.5958/0974-360X.2019.00466.9
24. Olii AT, Nugroho AK, Martien R, Riyanto S. Effect of Ratio Span 60-Cholesterol on the Characteristic of Niosomes Vitamin D3. Research Journal of Pharmacy and Technology. 2022;15(12):5551-5554. http://dx.doi.org/10.52711/0974-360X.2022.00937
25. Gaikwad DS, Chougale RD, Patil KS, Disouza JI, Hajare AA. Design, development, and evaluation of docetaxel-loaded niosomes for the treatment of breast cancer. Future Journal of Pharmaceutical Sciences. 2023;24;9(1):43.https://doi.org/10.1186/s43094-023-00494-0
26. Khan DH, Bashir S, Khan MI, Figueiredo P, Santos HA, Peltonen L. Formulation optimization and in vitro characterization of rifampicin and ceftriaxone dual drug loaded niosomes with high energy probe sonication technique. Journal of Drug Delivery Science and Technology. 2020;1; 58:101763. https://doi.org/10.1016/j.jddst.2020.101763.
27. Tyagi R, Waheed A, Kumar N, Ahad A, Bin Jardan YA, Mujeeb M, Kumar A, Naved T, Madan S. Formulation and Evaluation of plumbagin-loaded niosomes for an antidiabetic study: Optimization and in vitro evaluation. Pharmaceuticals. 2023;17;16(8):1169.doi: 10.3390/ph16081169.
28. Suriyaprakash Tn, Parthiban S, Prabu Sl, Sumathi A. Formulation and evaluation of paclitaxel niosome for its improved anti-cancer activity. ACTA Pharmaceutica Sciencia. 2011; 53(3).
29. Mohanty C, Acharya S, Mohanty AK, Dilnawaz F, Sahoo SK. Curcumin-encapsulated MePEG/PCL diblock copolymeric micelles: a novel controlled delivery vehicle for cancer therapy. Nanomedicine. 2010;1;5(3):433-449.https://doi.org/10.2217/nnm.10.9
30. Katrolia A, Chauhan SB, Shukla VK. Formulation and evaluation of metformin hydrochloride-loaded curcumin–lycopene niosomes. SN Applied Sciences. 2019;1:1-6.https://doi.org/10.1007/s42452-019-1768-6
31. Negi P, Aggarwal M, Sharma G, Rathore C, Sharma G, Singh B, Katare OP. Niosome-based hydrogel of resveratrol for topical applications: An effective therapy for pain related disorder (s). Biomedicine & Pharmacotherapy. 2017;1;88:480-7. https://doi.org/10.1016/j.biopha.2017.01.083
32. Graidist P, Martla M, Sukpondma Y. Cytotoxic activity of Piper cubeba extract in breast cancer cell lines. Nutrients. 2015;10;7(4):2707-2718. https://doi.org/10.3390/nu7042707
33. Riss TL, Moravec RA, Niles AL, Duellman S, Benink HA, Worzella TJ, Minor L. Cell viability assays. Assay guidance manual . 2016;1.
34. Van Meerloo J, Kaspers GJ, Cloos J. Cell sensitivity assays: the MTT assay. Cancer cell culture: methods and protocols. 2011:237-245.https://doi.org/10.1007/978-1-61779-080-5_20.
35. Kumar S, Baldi A, Sharma DK. In vitro antioxidant assay guided ex vivo investigation of cytotoxic effect of phytosomes assimilating taxifolin rich fraction of Cedrus deodara bark extract on human breast cancer cell lines (MCF7). Journal of Drug Delivery Science and Technology. 2021;1;63:102486. 10.1016/j.jddst.2021.102486.
36. Wang CF, Fan L, Tian M, Qi XS, Liu JX, Feng JB, Du SS, Su X, Wang YY. Radiosensitizing effect of schinifoline from Zanthoxylum schinifolium Sieb et Zucc on human non-small cell lung cancer A549 cells: A preliminary in vitro investigation. Molecules. 2014;1;19(12):20128-20138. doi: 10.3390/molecules191220128.
37. Ertus EB, Gulbahce-Mutlu E, Alpa S, Ozturk A. Production and biological properties of nano porous glass microparticles for anticancer drug carrier. Journal of Applied Pharmaceutical Science. 2024;14.http://doi.org/10.7324/JAPS.2024.168308
38. Beconi MG, Howland D, Park L, Lyons K, Giuliano J, Dominguez C, Munoz-Sanjuan I, Pacifici R. Pharmacokinetics of memantine in rats and mice. PLoS

Currents. 2011;15;3. https://doi.org/10.1371/currents.RRN1291
39. Mane V, Killedar S, More H, Tare H. Preclinical study on camellia sinensis extract-loaded nanophytosomes for enhancement of memory-boosting activity: optimization by central composite design. Future Journal of Pharmaceutical Sciences. 2024;1;10(1):66.
40. Ahmad S, Shaikh TJ, Patil J, Meher A, Chumbhale D, Tare H. Osmotic Release Oral Tablet Formulation, Development, and Evaluation of an Anti-epileptic Drug. International Journal of Drug Delivery Technology. 2023; 13(1):305-312.
41. Ahmad S, Mujawar T, Batewal B, More P, Gaikwad A, Chumbhale D, Tare H. RP-UHPLC and HPTLC method development and validation for analysis of andrographolide from herbal hepatoprotective formulation. International Journal of Pharmaceutical Quality Assurance. 2023; 14:96-104.
42. Dragicevic N, Maibach HI. Liposomes and Other Nanocarriers for the Treatment of Acne Vulgaris: Improved Therapeutic Efficacy and Skin Tolerability. Pharmaceutics. 2024 Feb 22; 16(3):309.
43. Mane V, Killedar S, More H, Nadaf S, Salunkhe S, Tare H. Novel Phytosomal Formulation of Emblica officinalis Extracts with Its In Vivo Nootropic Potential in Rats: Optimization and Development by Box-Behnken Design. Journal of Chemistry. 2024 ;(1):6644815. https://doi.org/10.1155/2024/6644815.