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ORIGINAL ARTICLE

Evaluation of Dabrafenib Incorporated Nanosponges on Cell Lines

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ABSTRACT
The aim of this research is to formulate, assess, and evaluate the cytotoxic effects of dabrafenib-loaded nanosponges for cancer treatment. Dabrafenib loaded nanosponges were synthesized via a lyophilization method and the resultant nanosponges were assessed for both qualitative and quantitative criteria. The MTT experiment was conducted to assess the cytotoxicity of the nanosponges and their capacity to inhibit cell proliferation, measured spectrophotometrically based on mitochondrial activity in the A431 cell lines. The dabrafenib-loaded nanosponges were effectively synthesized, and the assessed qualitative and quantitative parameters fell within the acceptable range. The in vitro anticancer activity was assessed on A431 cell lines using the produced nanosponges, yielding IC50 value of 73.03 µg/ml, indicating significant anticancer efficacy. This outcome indicates that the formulated dabrafenib-loaded nanosponges were characterized well and exhibit minimal cytotoxicity towards the A431 cell lines.
***Keywords:** Nanosponges, dabrafenib, toxicity, cell line, cancer, IC_{50}*

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INTRODUCTION

Nanosponges are advanced hyper-cross-linked structures made of solid nanoparticles with colloidal dimensions and nanoscale cavities [1]. β-CD-based nanosponges are a prominent choice due to continues drug discharge, improved stability, substantial carrier capacity, & ability to encapsulate both hydrophilic and hydrophobic compounds, thereby improving bioavailability by modifying the pharmacokinetic characteristics of the drug [2, 3]. Comprehensive studies have been undertaken on nanosponges, including drug transport, fabrication techniques, and documented applications [4, 5]. Consequently, it is essential to design non-toxic formulations capable of delivering dabrafenib to the target location and facilitating its progressive release to reduce nonspecific biodistribution and toxicity resulting from drug overdose [6].

Dabrafenib (Tafinlar) is a BRAF inhibitor, a category of targeted medicine known as a signal transduction inhibitor, which diminishes tumor size and prolongs survival in individuals with advanced melanoma [7]. The United States Food and Drug Administration authorized it in 2013. The average response duration increases from 5.6 to 9.5 months when a BRAFi is administered alongside a MEK inhibitor. Dabrafenib mesylate salt (DBF.MS) is a BCS Class II medication characterized by low solubility and high permeability, available for acquisition [8, 9].

Cells subjected to nanosponges experience alterations in shape, growth rate, apoptosis, and cellular disintegration [10]. Consequently, the assessment of cell viability must be mandatorily conducted for all chemical of potential significance in investigational pharmacology. The assessment of variations in mitochondrial action can be conducted by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) [11]. Targeted cancer therapies aim to minimize damage to healthy cells, offering a more precise and effective approach compared to conventional treatments like chemotherapy and radiotherapy. Advancements in cancer-targeted nanotechnologies benefit clinical diagnosis and

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treatment (12). Another proven technique for increasing drug solubility, dissolving rate, and enhancing the bioavailability of medications with limited water solubility is the production of nanosponges (13). The present research entails an *in vitro* cytotoxicity assessment of dabrafenib nanosponges. This investigation aims to formulate, evaluate, and measure cytotoxicity of dabrafenib-loaded nanosponges for cancer therapy.

MATERIAL AND METHODS

Materials

Dabrafenib in its pure form was obtained through Hetero Drugs Ltd., located in Hyderabad, India. Sigma Aldrich, USA, provided β-Cyclodextrin (β-CD), Diphenyl carbonate, & Glutaraldehyde. Solvents obtained from S.D. Fine Chemicals, Hyderabad. Fetal Bovine Serum [#RM10432], DMEM [#AL007A], D-PBS [#TL1006], & EMEM [#AL047S] got sourced from HiMedia. MTT Reagent [# M5655] & DMSO [# PHR1309] got sourced through Sigma. The 96-well plate utilized for cell culture got sourced from Corning, USA. Cell lines acquired from National Center for Cell Sciences in Pune, India, & cultured in Eagle's minimum essential medium.

Method

Drug loaded nanosponges

β-Cyclodextrin-based nanosponges were synthesized by diphenyl carbonate as crossconnecting agent and an ultrasoundsupported method, into which dabrafenib was incorporated through lyophilization. The synthesized drug-loaded nanosponges were filtered and freeze-dried for subsequent examination [4].

Description of drug loaded nanosponges

The size, polydispersity index, & zeta potential got measured using dynamic light scattering. The morphology of both plain and dabrafenib-loaded nanosponges was examined via scanning electron microscopy, together with the drug & excipients. Compatibility testsgot conducted using Fourier Transform Infrared Spectroscopy & Differential Scanning Calorimetry. *In vitro*discharge kinetics were assessed via the dialysis bag technique. Stability studies were executed following ICH stability protocols. *Ex vivo* studies utilized an inverted intestinal pouch, and single-pass intestinal perfusion was also evaluated.

In-vitro cytotoxic analysis

MTT assessgot conducted to assess cytotoxicity of dabrafenib-loaded nanosponges and their capacity to inhibit cell proliferation, measured spectrophotometrically based on mitochondrial activity in living A431 cell line [14].

Cell line preparation

Cell line got removed through liquid nitrogen packing& rapidly softened to room heat. Vial fillings got combined with 9ml of whole medium & centrifuged at 125g; 5 min. Following centrifugation, supernatant got removed, & the pellet got combined to 10ml of whole medium, then placed in T-25 flask &nurtured at 37°C at 5% CO_2. Upon achieving approximately 80% cell confluence, cells got centrifuged at 125 g; 5min; the pellet got resuspended in 15 mL of whole media & distributed into T-75 flasks. Upon achieving approximately 80-90% cell confluence, the cells in flask got utilized for test [15, 10].

MTT ASSAY

Two hundred microliters of cell suspension (in whole culture media+10% FBS) were inoculated into a 96-well plate (20,000 cells/well), having no the test material, & permitted to incubate for 24h. Following 24h of development, the consumed media in wells were substituted by suitable doses of Dabrafenib test formulation & incubated for 48h; 37°C; 5% CO_2 environment. Subsequent to development period, plates got extracted from the incubator; the wasted media gotcastoff, and MTT got added to attain final point of 0.5 mg per ml (sterilized through a 0.2μm filter). Plates got enveloped in aluminum foil to prevent light contact& incubated for three hours. Following incubation, the MTT reagent got discarded, & 100 μl of DMSO got introduced. Absorbance quantified using spectrophotometer (Tecan™ Infinite 200Pro; 570 nm [16, 17].

The vitality percentage of cells in untreated (-ve control) group got established at 100%, & viability percentages of cells in treatment group were assessed in relation to –ve control [18]. Percentage viability got graphed against concentration & assessed for dose to response associations. A suitable model got fitted to study I_{max} and IC_{50} based on the dose-response relationships.

Percentage viability:

$$\% \, Viability = \frac{100 \ X \ OD570e}{OD570b}$$

OD570e=Optical Density of test dilutions; OD570b=Optical Density of -ve control

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RESULTS AND DISCUSSION

Drug-encapsulated nanosponges

Several researchers endeavored to design innovative drug release methods by nanosponges to reduce harmfulness, enhance firmness& specificity, improve bioavailability, & provide continued liberation of diverse hydrophilic & hydrophobic medications. Based on physicochemical characteristics of polymer & medication for encapsulation, many procedures were used for manufacture of nanosponges, with lyophilization technique selected for synthesis of dabrafenib-loaded nanosponges.

Evaluation of nanosponges

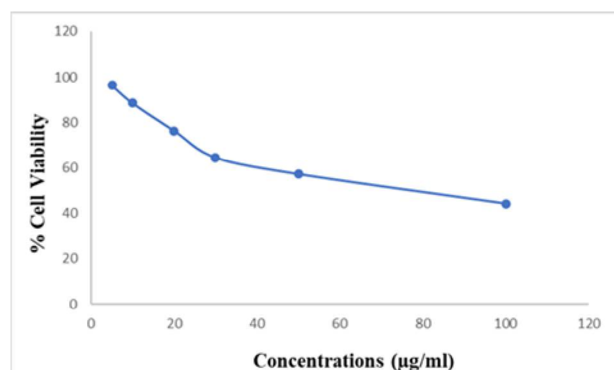
Evaluation of dabrafenib-loaded nanosponges was conducted. Size, zeta potential, & polydispersity index of dabrafenib-loaded nanosponges got measured at 0.282 ± 0.0044nm, -24.13 ± 4.02 mV. The dynamic light scattering approach correlates effectively with scanning electron microscopy measurements of dabrafenib-loaded nanosponges. Lack of distinctive peak of the drug in Fourier transformed infrared spectra & absence of an endothermic steak in the thermogram of dabrafenib-loaded nanosponges suggest that the drug may be encapsulated inside the β-cyclodextrin matrix. After 48 hours, the release profiles of the optimized β-cyclodextrin nanosponges and the free medication are 88.48 ± 7.4 and 29.66 ± 5.66, respectively. Stability assesses demonstrated that dabrafenib nanosponges constitute a stable formulation. The formulation demonstrated superior performance, with an apparent permeability increase of 2.11, 1.696, and 2.60-fold across several colonic segments (duodenum, jejunum, and ileum), respectively, indicating higher drug absorption in the form of nanosponges due to improved mucosal permeability. The effective permeability, determined from steady-state drug concentrations in the perfusate, exhibited a significant increase, rising from $0.069 \times 10^{-4}$to 0.42×10^{-4} cm/s.

In-vitro cytotoxic assess

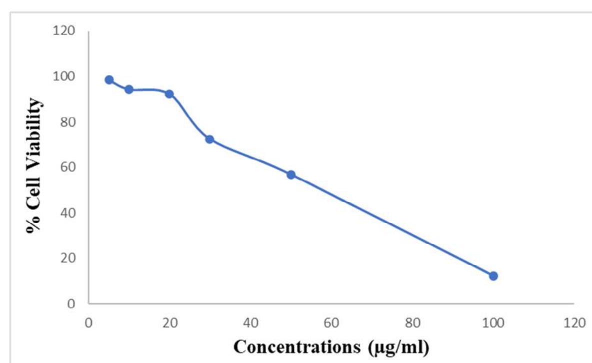
These experiments provide valuable insights into the merits and drawbacks of model for broadcast& mechanistic evaluation of possibly harmful chemicals. Diverse range of morphological & biochemical marker exists for acquiring data at cellular & molecular level to identify chemical-induced disturbance [18]. Cell line model is relatively simple to execute under lab circumstances. Cellular test in oncological investigate primarily involves human cell line. This system is optimal for management and reproducibility [19]. Various quantities of dabrafenib-loaded nanosponges were evaluated for toxicity using cell viability assays on the A431 (Table 1; Figure 1) using the MTT method to determine IC50 values.

Table 1: Cytotoxicity of dabrafenib pure drug and dabrafenib loaded nanosponges on A431 cell line

Concentrations (µg/ml)	Dabrafenib Pure Drug		Dabrafenib Loaded Nanosponges	
	% Mean Viability	IC50 (µg per ml)	% Mean Viability	IC50 (µg per ml)
Blank	-	57.53	-	73.03
Vehicle Control	100.00±0.00		100.00±0.00	
5.00	96.46±1.67		98.37±1.54	
10.00	88.54±2.36		94.36±1.84	
20.00	76.32±1.24		92.26±2.57	
30.00	64.42±1.98		72.54±2.69	
50.00	57.18±1.24		56.87±2.54	
100.00	44.17±1.13		12.36±1.65	



Cytotoxicity of Dabrafenib Pure Drug on A431 cell line

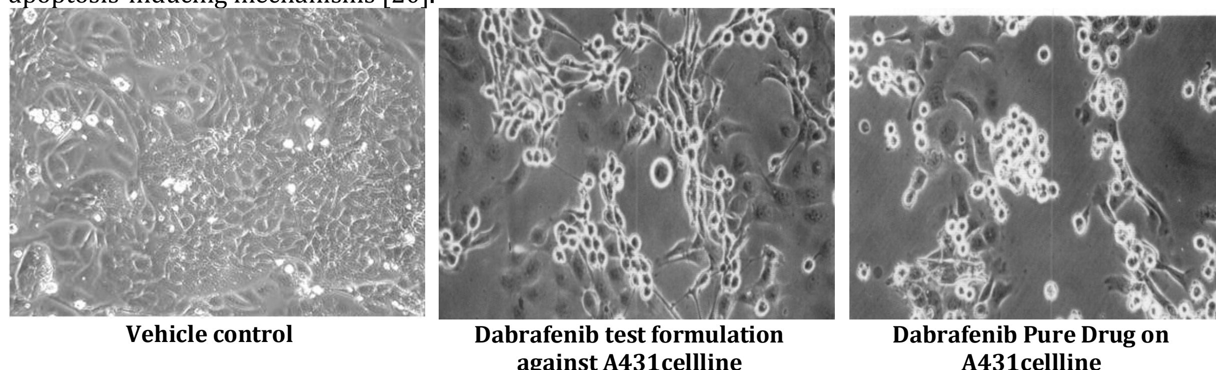


Cytotoxicity of Dabrafenib nanosponges on A431 cell line

Figure 1: Cytotoxicity of Dabrafenib Pure Drug and Dabrafenib nanosponges on A431 cell line

The morphology of the A 431 cell lines was examined following treatment with various concentrations of dabrafenib and dabrafenib-loaded nanosponges (Figure 2). Significant deformations and modifications

were noted on the cell surface, likely resulting from the influence of the examined nanosponges. Pure medicines demonstrated a diminished impact on cell morphology. The increase in dabrafenib-loaded nanosponges concentration correlated with changes in cell shape and apoptotic alterations. The anticancer effect of dabrafenib-loaded nanosponges is linked to their membrane-disrupting and apoptosis-inducing mechanisms [20].



Vehicle control **Dabrafenib test formulation against A431cellline** **Dabrafenib Pure Drug on A431cellline**

Figure 2: Morphological variations of A431 treated with Dabrafenib nanosponges

The MTT assess is colorimetric method whichassesses cellular metabolic action. The A431 got utilized to evaluate cytotoxic probable of a test medication for preliminary testing of apoptosis. Biological machinery of the MTT assess entails the NAD(P)H-dependent cellular oxidoreductase enzyme, which transforms yellow tetrazolium MTT [3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] in the insoluble (E, Z)-5-(4,5-dimethylthiazol-2-yl)-1,3-diphenylformazan (formazan). The formulation including the aforementioned safe chemicals exhibited little cytotoxicity in protection assessment utilizing MTT assess. It got verified that cell viability (%) of pretreatment media by the engineered preparation exceeded 97% (<3% cell mortality at each concentration). Such outcome demonstrates that formulated microemulsion exhibits negligible cytotoxicity towards the A431 cell-lines.

CONCLUSION

Nanodrug release systems recently garnered significant interest for the site targeted release of diverse anti-cancer agents, encompassing both synthetic molecules and ordinary items. Improved formulation exhibited optimal particle size for tumor accumulation. Nanosponges demonstrated a restricted size distribution, indicating homogeneous monodispersity of the formulation. The MTT experiment demonstrated a significant cytotoxic consequence of formulation. All of such demonstrated clinical importance of dabrafenib-loaded nanosponges. The findings indicate that the formulated dabrafenib-loaded nanosponges exhibit negligible cytotoxicity towards the A431 cell lines.

CONFLICTS OF INTEREST

No conflict of interest.

AUTHOR CONTRIBUTION

K.S.C.R., J.P.K., and DB conducted the research, executed the project, and authored the work plan, review, and revisions. All writers concur with the submission and publishing. All writers reviewed & consented to published form of work.

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