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Formulation, Characterization, And Cytotoxicity Evaluation of Dasatinib-Loaded Nanosponges on A498 And Mcf-7 Cell Lines

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Abstract
The objective of this study was to develop and evaluate Dasatinib-loaded nanosponges (NSPs) as a drug delivery system for improved therapeutic efficacy against renal (A498) and breast (MCF-7) cancer cell lines. The NSPs were synthesized using an ultrasound-assisted approach with hydroxypropyl-β-cyclodextrin (HPβCD) as the complexing agent. The NSPs were characterized for their particle size, polydispersity index (PdI), zeta potential, encapsulation efficiency (EE), and stability. The resulting NSPs exhibited a uniform nanosuspension with a particle size of 148.38 ± 2.64 nm, a PdI of 0.221 ± 0.043, and a zeta potential of -22.54 ± 2.83 mV, suggesting good stability and steric stabilization. The encapsulation efficiency was found to be 72.62 ± 3.31%. In vitro drug release studies showed that the NSPs provided a prolonged release of Dasatinib over 60 hours, in contrast to the rapid burst release observed with the pure drug. Cytotoxicity assays using the MTT method demonstrated that Dasatinib-loaded NSPs exhibited reduced cytotoxicity compared to the pure drug, with higher IC50 values on both A498 and MCF-7 cell lines. The IC50 for Dasatinib NSPs was 73.155 µg/ml for A498 cells and 53.34 µg/ml for MCF-7 cells, compared to the pure drug's IC50 values of 40.23 µg/ml and 32.54 µg/ml, respectively. Morphological analysis revealed apoptosis-like cell changes upon treatment with NSPs, indicating their potential for therapeutic application. These findings suggest that Dasatinib-loaded NSPs offer a promising approach for controlled and sustained drug delivery, enhancing the therapeutic index while reducing systemic toxicity in cancer treatment.

Keywords: *Dasatinib, Nanosponges, Drug delivery system, Cytotoxicity, A498 cell line, MCF-7 cell line*

1. INTRODUCTION

Recent advances in cancer therapeutics have shown that certain drugs possess the ability to impede crucial processes in cancer cell biology, such as replication, migration, and invasion, while also triggering apoptosis in tumor cells [1]. These findings are particularly important in the context of solid tumors, where therapeutic efficacy can be challenging due to the complex nature of tumor progression. Notably, the drug under investigation has shown promise in treating various types of solid tumors, with its potential in breast cancer treatment advancing to clinical trial stages [2]. Moreover, this drug has demonstrated significant therapeutic potential, especially for patients who have developed resistance to imatinib, a first-line tyrosine kinase inhibitor used in earlier therapies. This highlights the evolving role of novel agents in overcoming resistance mechanisms in cancer treatment [3]. However, despite its promising therapeutic effects, this drug (DAS) faces considerable challenges in terms of its pharmacokinetics. It is classified as a BCS II drug, which is characterized by poor water solubility. This limited solubility significantly affects its absorption, with its bioavailability varying between 14% and 34%. The solubility of DAS is also highly pH-dependent due to its weak basic properties, exhibiting pKa values of 3.1, 6.8, and 10.8. It tends to precipitate in the small intestine, an environment where the drug's solubility is compromised, but it demonstrates improved solubility in more acidic conditions. Furthermore, DAS has a relatively short terminal half-life of approximately 3–4 hours, which poses challenges in maintaining therapeutic levels of the drug for sufficient periods of time [4]. In addition to these pharmacokinetic issues, DAS is associated with various adverse reactions, which further complicate its clinical use. The low oral bioavailability and

the first-pass effect compound the difficulty of achieving therapeutic concentrations in the systemic circulation. To address these limitations, a well-designed drug delivery system is paramount to enhance the drug's therapeutic efficacy while minimizing its adverse effects [6]. The current goal is to overcome these challenges through advanced drug carrier systems that improve solubility, reduce precipitation, and enhance bioavailability, ensuring that DAS can be effectively delivered to target tissues [7]. Nanotechnology offers ground breaking solutions for improving drug delivery, with colloidal nanocarriers emerging as a promising approach. These nanocarriers can bypass the limitations of conventional drug delivery systems by improving solubility, enhancing drug stability, and facilitating controlled drug release [8]. Researchers have investigated several techniques to improve the solubility and bioavailability of DAS, including self-nanoemulsifying drug delivery systems (SNEDDS), lipid-based systems, nanocrystals, and polymeric solid dispersions. While these methods offer certain advantages, such as better release management, they often require extensive in vivo studies to demonstrate bioavailability enhancement. Additionally, the associated high costs of these approaches can limit patient access to therapy [9].An area of particular concern in drug delivery formulation is the impact on intestinal permeability and drug absorption, an aspect that is not sufficiently explored in many current studies. To address this gap, a novel approach involving HPβCD nanosponges (NSPs) has been proposed. These NSPs offer a promising solution to enhance the bioavailability of DAS by improving its solubility and controlling its distribution to target sites [10]. To optimize the performance of these NSPs, researchers have focused on understanding the factors that impact the formulation and process, including the characterization and evaluation of the nanocarriers to ensure their effectiveness.Cell line studies play a crucial role in the early stages of drug development, as they provide insights into the drug's activity at the cellular level. These studies are vital for understanding how DAS interacts with cancer cells, how it influences cellular processes such as apoptosis, and how it compares to other therapeutic agents [11]. Furthermore, cell line studies offer an opportunity to investigate the drug's potential side effects, resistance mechanisms, and optimal dosage. By utilizing relevant cancer cell lines, researchers can tailor drug delivery systems to ensure better efficacy and safety profiles in clinical settings [12].The primary objective of this research is to enhance the bioavailability and therapeutic efficacy of DAS by optimizing HPβCD NSPs for targeted drug delivery. A critical aspect of this approach is the in vitro evaluation using relevant cancer cell lines to assess the drug's bioactivity, including its ability to inhibit replication, migration, and invasion, as well as its induction of apoptosis in tumor cells. These in vitro studies provide crucial insights into the interaction of DAS with cancer cells at the cellular level, allowing for a detailed assessment of its cytotoxicity, pharmacodynamics, and the overall effectiveness of the NSPs in improving solubility and absorption [9].By conducting cell line studies, this research aims to evaluate the impact of NSPs on DAS bioavailability, focusing on cellular uptake, drug release profiles, and the modulation of drug distribution to target sites. Additionally, these studies will provide a better understanding of the formulation's influence on drug permeability, intracellular drug concentration, and its potential to overcome the limitations of poor solubility and low bioavailability associated with DAS. This in vitro approach will be essential for optimizing the drug carrier system before progressing to in vivo studies and clinical trials.Furthermore, through these in vitro evaluations, the research will investigate whether the HPβCD NSPs enhance the pharmacological properties of DAS, such as its half-life and therapeutic concentration, while minimizing associated adverse effects. The results from these studies will help guide the design of more effective drug delivery systems, with the ultimate goal of improving the therapeutic outcomes for patients with solid tumors.

2. MATERIALS AND METHODS

Materials

Dasatinib was gifted by Hetero Drugs Ltd., Hyderabad, India. TCI Chemicals provided glutaraldehyde (25% Aqueous Solution), diphenyl carbonate (DPC) and hydroxypropyl β-Cyclodextrin (HPβCD). We purchased the solvents from S.D. Fine Chemicals in Hyderabad. The dialysis membrane was provided by Hi-media Pvt. Ltd. (cut off of M. wt. 12 kDa). Fetal Bovine Serum

[#RM10432] and D-PBS [#TL1006], DMEM [#AL007A], EMEM [#AL047S] were from HiMedia. MTT Reagent [# M5655] and DMSO [#PHR1309] were from Sigma. 96-well plate for culturing cells was from Corning, USA.

Methods

Development of nanosponges based on HPβCD

HPβCD-based NSPs were synthesized by mixing HPβCD with DPC as a cross-linker at a molar ratio of 0.591. The ultrasound-assisted method was applied with slight modifications to earlier protocols. HPβCD was dissolved in dimethylformamide, and DPC was added. The mixture was refluxed at 90°C in an oil bath until it liquefied. The product was purified by Soxhlet extraction with ethanol, followed by water washing and overnight drying at 60°C. The dried product was powdered, mixed with water, and lyophilized for long-term stability [5]. For DAS-HPβCD NSPs, synthesized HPβCD NSPs were dissolved in double-distilled water, and the drug was added. The mixture was sonicated and stirred, then centrifuged at 10,000 rpm for 10 minutes to remove unbound drug. The final product was freeze-dried and stored in a desiccator for testing.

Characterization and evaluation

The particle size (PS) and polydispersity index (PdI) of HPβCD NSPs were measured using dynamic light scattering (DLS) on a Malvern zeta-sizer. Drug-loaded NSPs were weighed, and their yield and encapsulation efficiency (EE) were calculated. Structural characterization was performed using SEM, FT-IR, DSC, and XRD to assess morphology, functional groups, thermal properties, and crystallinity. Drug release was evaluated using the shake flask method, with samples analyzed by HPLC. The release kinetics were modeled to determine the best fit. Stability tests were conducted at accelerated conditions according to ICH guidelines, with regular assessments of physical properties, PS, PdI, zeta potential, and EE over three months [1-6].

Cell viability MTT assay

Experimental Design

The experimental groups and treatments used in the study were as follows: the medium control, which consisted of medium without cells, was assessed in triplicate (N=3); the negative control, containing medium with cells but without the experimental drug or compound, was also evaluated in triplicate (N=3); the vehicle control, which included medium with cells and the vehicle, was tested in triplicate (N=3); and the DAS-loaded NSPs were tested at concentrations of 5, 10, 20, 30, 50, and 100 µg/mL, with three replicates (N=3) for each concentration [13, 14].

Cell Line Preparation

Vials containing each cell line were retrieved from liquid nitrogen storage and rapidly thawed at room temperature. The contents of the vials were transferred to 9 mL of complete medium and centrifuged at 125g for 5 minutes. After discarding the supernatant, the cell pellet was resuspended in 10 mL of complete medium and transferred to a T-25 flask for incubation at 37°C with 5% CO_2. Upon reaching approximately 80% confluence, cells were centrifuged again at 125g for 5 minutes. The pellet was then resuspended in 15 mL of complete medium and seeded into two T-75 flasks. Once the cell confluence reached around 80-90%, the cells were ready for use in the assay [13-15].

MTT Assay

A 200 µL suspension of cells (20,000 cells per well in complete medium with 10% FBS) was seeded in a 96-well plate and allowed to incubate for 24 hours. Following the 24-hour incubation, the medium was replaced with various concentrations of DAS-loaded NSPs and incubated for an additional 48 hours at 37°C in a 5% CO_2 atmosphere. After incubation, the plates were removed, and the spent media was discarded. MTT reagent was added to each well to a final concentration of 0.5 mg/mL (filter-sterilized through a 0.2 µm filter), and the plates were wrapped in aluminum foil to protect from light, followed by incubation for 3 hours. After incubation, the MTT reagent was

removed, and 100 µL of DMSO was added to each well. Absorbance was measured at 570 nm using a Tecan™ Infinite 200Pro spectrophotometer [13-17].

Data Analysis

Cell viability in the untreated (negative control) group was set to 100%, and the percentage viability of cells in the treated groups was calculated relative to the negative control. The percent viability was plotted against the treatment concentration to generate a dose-response curve. Based on these relationships, a suitable model was applied to estimate the maximal inhibition (Imax) and the half-maximal inhibitory concentration (IC50). The percentage viability was determined using the following formula:

$$\% \text{ Viability} = \frac{100 \times OD_{570}e}{OD_{570}b}$$

Where,

OD570e is the mean value of the measured Optical Density of the dilutions of test item;

OD570b is the mean value of the measured Optical Density of the negative control

3. RESULTS AND DISCUSSION

The DAS-NSPs were successfully synthesized using an ultrasound-assisted method, with HPβCD serving as the complexing agent. HPβCD, with its unique molecular structure, features hydrophobic cavities that enable it to encapsulate lipophilic drugs such as DAS. This encapsulation is driven by the formation of hydrogen bonds between the hydroxyl groups of HPβCD and the drug molecules, facilitating the sequestration of the drug within the hydrophobic core of the complex. The inclusion complexation between DAS and HPβCD was confirmed by the synthesis process, which ensured that the drug was effectively encapsulated within the NSPs [3,4]. The structural integrity and stability of the DAS-NSPs were enhanced by subjecting them to a freeze-drying process after their initial synthesis. Freeze-drying, or lyophilization, aids in maintaining the physical and chemical properties of the NSPs, preventing degradation or drug leakage during storage [6]. The resultant NSPs were found to possess excellent stability, which is crucial for the controlled release and prolonged therapeutic action of DAS [5]. These NSPs, due to the inclusion complex formation, can offer an efficient drug delivery system that enhances the solubility, stability, and bioavailability of DAS. The encapsulation process also ensures a more controlled release, minimizing the risk of rapid drug degradation or undesired systemic distribution. Overall, the successful synthesis of DAS-NSPs represents a promising approach to improving the pharmacological properties of DAS, enhancing its therapeutic efficacy and patient compliance [1, 8]. The synthesized DAS-NSPs exhibited a homogeneous nanosuspension with particle sizes ranging from 148.38 ± 2.64 nm and a PdI of 0.221 ± 0.043, indicating a uniform distribution. The zeta potential of the improved formulation was -22.54 ± 2.83 mV, suggesting effective steric stabilization. The yield percentage was 65.84 ± 3.41%, and the EE was 72.62 ± 3.31%. FT-IR analysis revealed characteristic peaks for both DAS and HPβCD, with the drug's peaks being masked in the NSP spectrum, indicating potential interactions between the drug and the NSPs. DSC and XRD studies confirmed that the drug underwent solid-state complexation within the NSPs, as evidenced by the absence of the drug's melting peak in the formulation. Drug release studies showed a significant improvement in sustained release with the NSPs, as 95.42 ± 7.16% of the drug was released over 60 hours, compared to 92.62 ± 8.18% for the simple inclusion complex and 30.66 ± 6.24% for the plain drug. Stability testing over three months indicated no significant changes in the appearance, encapsulation efficiency, or particle size of the NSPs, although a slight decrease in zeta potential was observed [10, 12]. In this study, the cytotoxic effects of DAS pure drug and DAS-NSPwere systematically evaluated on A498 and MCF-7 cell lines through an MTT assay. The results of the cytotoxicity studies presented in Tables 1-5 and Figures 1-7 offer insightful observations regarding the comparative efficacy of the pure drug and its NSP formulation, underscoring the influence of

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formulation strategies on drug delivery and therapeutic outcomes [13]. DAS, a potent tyrosine kinase inhibitor, exhibited significant dose-dependent cytotoxicity across both A498 and MCF-7 cell lines. In A498 cells (Table 1, Figure 1), the IC50 value of DAS pure drug was recorded as 40.23 µg/ml, demonstrating a substantial reduction in cell viability as the drug concentration increased. The drug's cytotoxic effect was clearly evident, with a 21.45 ± 2.67% cell viability observed at the highest concentration (100 µg/ml), which was consistent with its known therapeutic action in inhibiting tumor cell proliferation.

Table 1: Cytotoxicity of Dasatinib Pure drug on A498 cell line

Dasatinib Pure drug	% Mean Viability	IC50 value (µg/ml)
Blank	-	40.23
Vehicle Control	100.00±0.00	
5.00	97.87±2.98	
10.00	79.53±2.46	
20.00	52.47±2.48	
30.00	39.65±2.78	
50.00	27.56±2.46	
100.00	21.45±2.67	

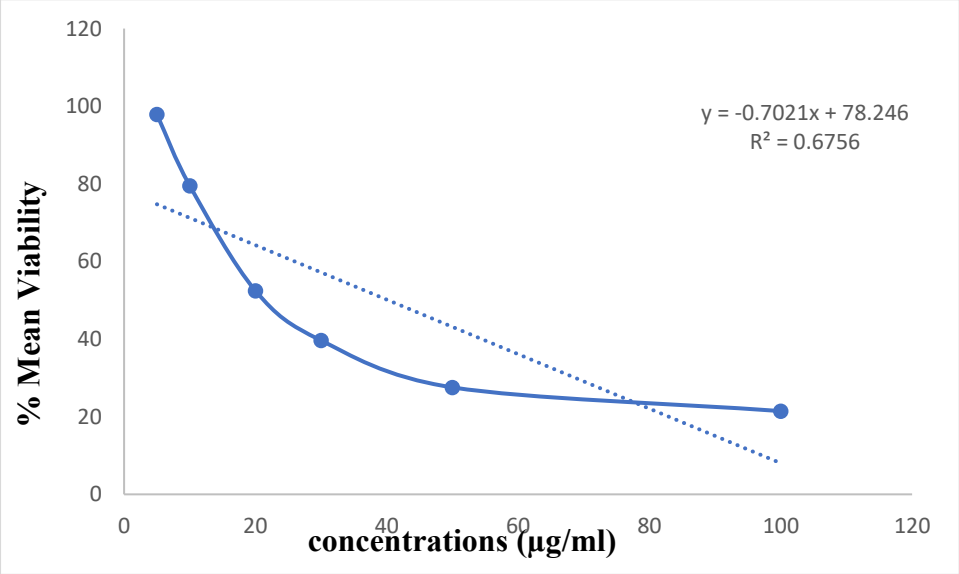

Figure 1: Cytotoxicity of Dasatinib Pure drug on A498 cell line

In contrast, the DAS-NSP exhibited a comparatively lower cytotoxicity profile, with higher IC50 values recorded for both A498 and MCF-7 cell lines. Specifically, the IC50 value for DAS-NSP on A498 cells was found to be 73.155 µg/ml (Table 2, Figure 2), which is significantly higher than the pure drug. This suggests that the formulation of DAS-NSP reduces its immediate cytotoxicity. At 100 µg/ml, the cell viability was 35.45 ± 2.44%, indicating that the NSPs still retained significant cytotoxic

potential but required higher concentrations for comparable effects.

Table 2: Cytotoxicity of Dasatinib nano sponges on A498 cell line

Dasatinib nano sponges (µg/ml)	% Mean Viability	IC50 value (µg/ml)
Blank	-	73.155
Vehicle Control	100.00±0.00	
5.00	96.57±2.57	
10.00	93.85±2.64	
20.00	87.89±2.34	
30.00	76.58±2.57	
50.00	58.56±2.47	
100.00	35.45±2.44	

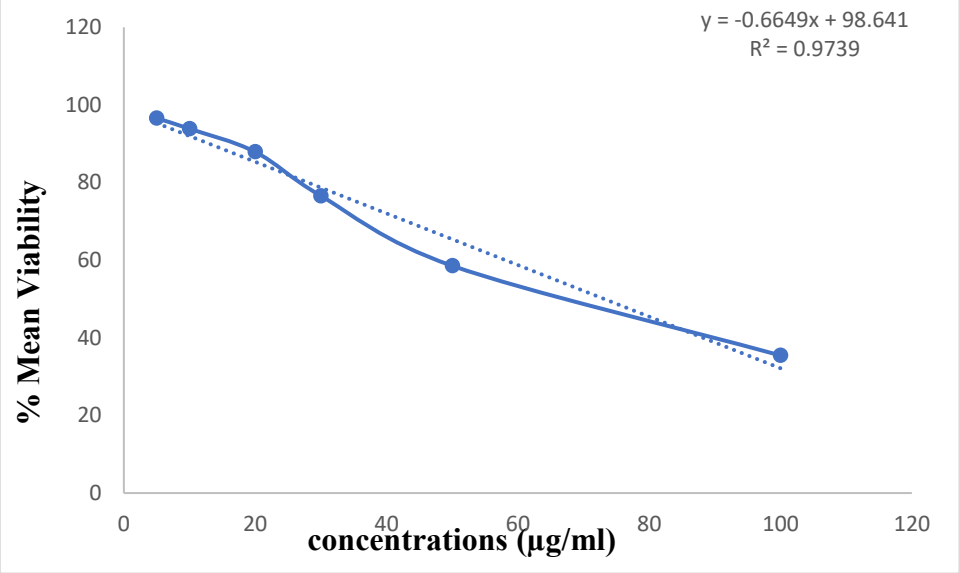

Figure 2: Cytotoxicity of Dasatinib nano sponges on A498 cell line

Similarly, on MCF-7 cells, the IC50 value for DAS-NSP was 53.34 µg/ml (Table 3, Figure 3), also higher than the pure drug's IC50, and the viability at 100 µg/ml was reduced to 17.76 ± 1.48%.

Similarly, when applied to MCF-7 cells (Table 3, Figure 3), DAS pure drug showed a comparable dose-response relationship, with an IC50 value of 32.54 µg/ml, confirming its cytotoxic potential against breast cancer cells. The decrease in cell viability reached 25.76 ± 1.87% at the highest dose, reflecting its potential efficacy in cancer therapy [18]. These results align with the pharmacological profile of DAS, which is a potent anticancer agent, particularly effective in treating cancers like renal cell carcinoma (A498 cells) and breast cancer (MCF-7 cells) due to its inhibitory action on several tyrosine kinases involved in cellular proliferation and survival. The marked reduction in cell viability as the concentration of DAS increased confirms its potent antitumor activity across both cell lines [19, 20].

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Table 3: Cytotoxicity of Dasatinib nano sponges on MCF-7 cell line

Dasatinib nano sponges (µg/ml)	% Mean Viability	IC50 value (µg/ml)
Blank	-	53.34
Vehicle Control	100.00±0.00	
5.00	94.28±2.59	
10.00	92.57±2.87	
20.00	82.76±2.59	
30.00	67.57±2.49	
50.00	35.59±2.48	
100.00	17.76±1.48	

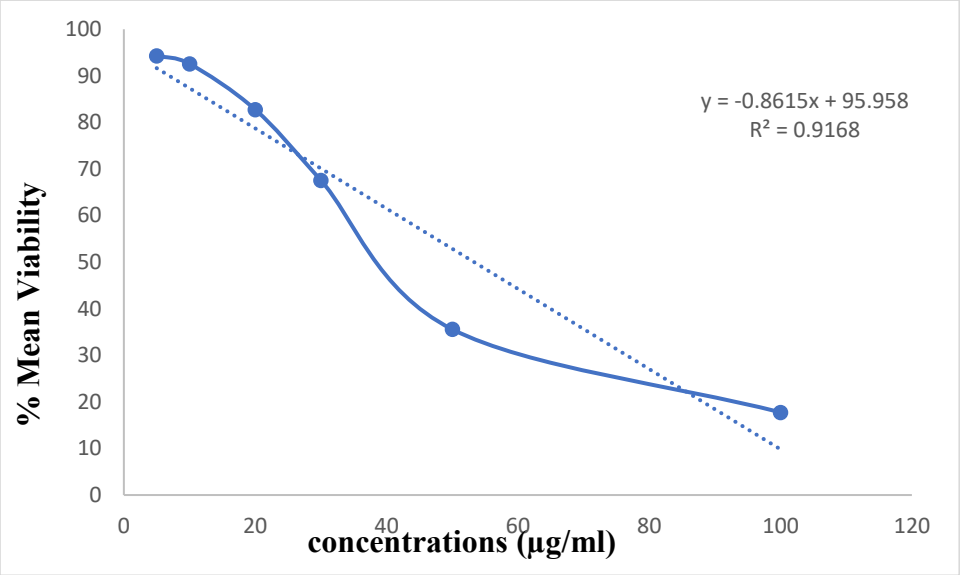

Figure 3: Cytotoxicity of Dasatinib nano sponges on MCF-7 cell line

This lower cytotoxicity observed with the NSPs could be attributed to the encapsulation of DAS-NSP, which likely alters the drug's release dynamics [21]. NP typically release drugs in a controlled manner, which may reduce the peak drug concentrations within cells and lead to a slower, more sustained therapeutic effect. This sustained release can potentially allow for prolonged therapeutic activity without causing the rapid cytotoxic effects seen with the free drug [22]. Furthermore, the observed differences in IC50 values between the free drug and the NSPs suggest that the NSPs may offer a more favorable pharmacokinetic profile. The formulation may enhance the solubility and stability of DAS, thereby allowing for better drug delivery and sustained therapeutic effects with reduced side effects. The NSPs' ability to release DAS over an extended period could be an advantage, particularly for patients requiring prolonged therapeutic intervention [23]. The IC50 values for both formulations in A498 and MCF-7 cell lines further support the idea that the NSPs reduce the immediate cytotoxicity of the pure drug. As shown in Table 4, the IC50 values of DAS-NSP were consistently higher than those of the pure drug in both cell lines, with the pure drug achieving its cytotoxic effects at lower concentrations. This could reflect the influence of the NSPs' encapsulation mechanism, which modulates the release of the drug, thus necessitating higher concentrations to achieve comparable effects [24].

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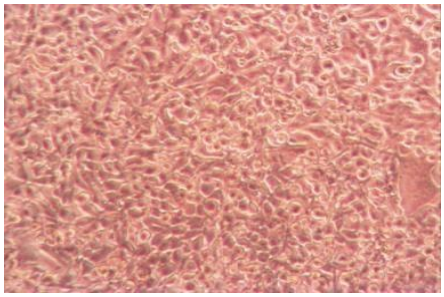
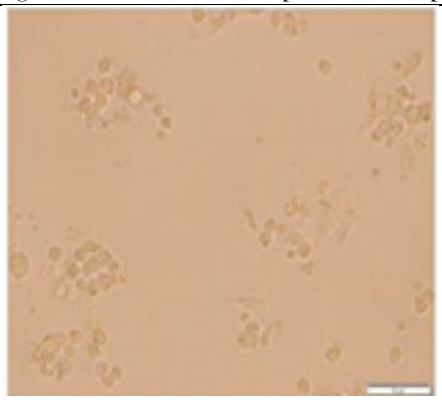
Table 4: IC50 Values of both nanobubbles and Pure drug

Compound	IC50 value (µg/ml)	
	A 498	MCF-7
Dasatinib nano sponges	73.155	52.15
Dasatinib Pure drug	40.23	32.54

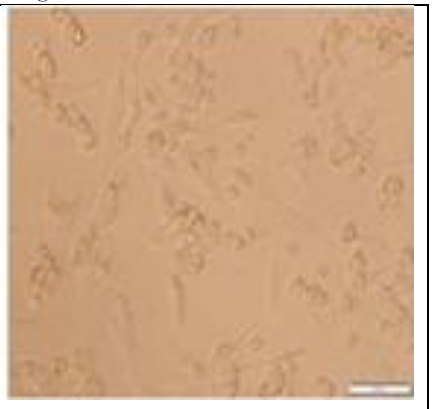
In addition to the viability measurements, Figures 4 illustrate the morphological changes observed in both A498 and MCF-7 cells after treatment with DAS-NSP. The NSPs induced cell shrinkage and membrane blebbing, characteristic of apoptosis, at higher concentrations. These morphological alterations corroborate the cytotoxicity data, confirming that the NSPseffectively exert their therapeutic effects over time, though at a slower rate compared to the pure drug.



vehicle control

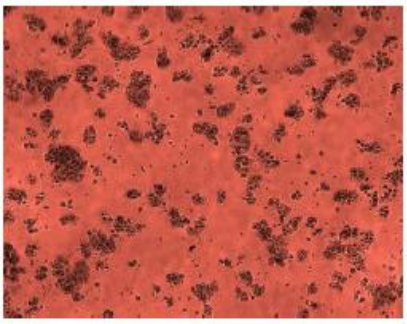


Dasatinib nano sponges against MCF-7 cellline



Dasatinib nano sponges against A498 cellline

Dasatinib Pure drugon MCF-7 cell line



Dasatinib Pure drug on A498 cell line

Figure 4: Morphological changes of MCF-7, A498 cancer cells when treated with Dasatinib nano sponges.

The results of this study underscore the potential of DAS-NSP as an alternative drug delivery system. By encapsulating DAS-NSP, the drug's bioavailability and therapeutic efficacy could be enhanced, while simultaneously reducing the rapid onset of cytotoxicity observed with the pure drug. NSPs offer the advantage of controlled drug release, which could improve the therapeutic window by minimizing off-target toxicity and prolonging the drug's effectiveness at tumor sites. This is particularly important for

cancer therapies, where achieving sustained drug levels over extended periods without excessive side effects is crucial for patient compliance and treatment success [25]. The difference in IC50 values between the pure drug and the NSPs also suggests that NSPsmay provide a strategy to optimize the pharmacokinetic and pharmacodynamic profiles of DAS, ensuring that the drug reaches therapeutic levels in the tumor while minimizing systemic toxicity. Moreover, the ability of NSPs to enhance drug stability and solubility could be especially beneficial for hydrophobic drugs like DAS, which face challenges in traditional oral or injectable formulations [22]. MTT assay is a colorimetric assay based on assessing the cell metabolic activity. MCF-7, A498 cell line was used to see the cytotoxic potential of a test drug for initial screening of apoptosis or necrosis. The biochemical mechanism behind the MTT assay involves NAD (P) H-dependent cellular oxidoreductase enzyme that converts the yellow tetrazolium MTT [3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] into insoluble (E,Z)-5-(4,5-dimethylthiazol-2-yl)-1,3-diphenylformazan (formazan). In this study, the SMEDDS formulation containing the above-mentioned safe substances showed almost no cytotoxicity in a safety test using the MTT assay. We confirmed that the cell viability (%) of the pretreated media with a fabricated formulation was over 97% (<3% cell death at all concentrations). This result indicates that the prepared microemulsion has almost no cytotoxic effects on the MCF-7, A498 cell line.

4. CONCLUSION

In conclusion, the findings from this study highlight the comparative effectiveness of DAS-NSP versus the pure drug in terms of cytotoxicity across A498 and MCF-7 cell lines. While the pure drug exhibited higher cytotoxicity with lower IC50 values, the NSPs demonstrated a controlled release profile that could potentially offer sustained therapeutic effects. These results suggest that DAS-NSP could serve as an effective alternative drug delivery system, with the potential for enhanced drug stability, bioavailability, and controlled release, ultimately leading to more effective cancer treatment regimens with reduced toxicity.

CONFILT OF INTEREST

None

FUNDING

None

AUTHOR CONTRIBUTION

AS contributed to the study design and collaborated with data collecting and analysis, while SC, supported in paper writing, assuring a collaborative and balanced effort throughout the research process. Both Authors reviewed and approved the final draft.

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