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Formulation and Optimization of Catechin-Loaded Niosomes for Improved Pharmacokinetic Performance

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Abstract

Background

This study reports the design, characterization and biological evaluation of catechin-loaded niosomes to enhance the anticancer efficacy and pharmacokinetic profile of catechin. Catechin is a green tea derived polyphenolic with established antioxidant, anti-inflammatory and anticancer activity, but its clinical application is limited by poor solubility and bioavailability. To address these constraints, nanoniosomal carriers were fabricated.

Method

Catechin-loaded nanoniosomes were prepared by the thin-film hydration method and evaluated for particle size, zeta potential, encapsulation efficiency, morphology, in vitro drug release, and stability. The optimized formulation was further assessed by MTT assay against HepG2 cells and evaluated for pharmacokinetic performance in rats following oral administration.

Result

The prepared niosomes showed a particle size of 112 ± 8.14nm with stable zeta potential of 34.1± 1.87m. , SEM revealed spherical vesicles with smooth surface. It showed an excellent physical stability at 4 °C. In vitro Cytotoxic against HepG2 hepatocellular carcinoma cells was accessed by MTT assay, benchmarking catechin loaded nanoniosomes against to free catechin and standard cisplatin. Catechin loaded nanoniosomes exhibited significantly enhanced cytotoxicity against HepG2 cells compared to free catechin and cisplatin with IC_{50} value 31.65µg/ml, 37.30µg/ml and 74.18µg/ml respectively. Following oral administration in animal model, pharmacokinetic analysis showed marked improvements in C_{max}, AUC, $t_{1/2}$, T_{max}, elimination rate constant, apparent volume of distribution, systemic clearance and MRT collectively indicating enhanced bioavailability and prolonged systemic circulation of catechin when delivered via niosomes.

Conclusion

The findings indicate that catechin-loaded niosomes may improve the pharmacokinetic performance and sustained delivery of catechin, supporting their potential for hepatocellular carcinoma therapy. Further preclinical studies are needed to confirm their therapeutic efficacy and safety.

Keywords: Hepatocellular carcinoma, Nanotechnology, Pharmacokinetics, Bioavailability, HepG2 cells, Polyphenol

1. Introduction

Hepatocellular carcinoma (HCC) is a major primary liver malignancy associated with high mortality and limited therapeutic outcomes[1]. The limitations of conventional anticancer therapy, including systemic toxicity, poor selectivity and therapeutic resistance have stimulated interest in naturally occurring bioactive compounds as potential therapeutic candidates[2,3]. Catechin, a naturally occurring flavan-3-ol polyphenol found abundantly in green tea and medicinal plants, possesses antioxidant, anti-inflammatory, hepatoprotective and anticancer

activities. Its anticancer effects are associated with modulation of oxidative stress, cellular signaling, proliferation and apoptosis[4].

Despite its promising pharmacological activity, the therapeutic application of catechin is restricted by poor aqueous solubility, limited absorption and low systemic bioavailability[4]. These limitations can reduce its plasma exposure and consequently compromise therapeutic efficacy. Nanotechnology based delivery systems have therefore attracted considerable interest for enhancing the solubility, stability, absorption and bioavailability of poorly water soluble natural compounds [5].

Niosomes are vesicular nanocarriers composed mainly of non-ionic surfactants and cholesterol. They offer advantages such as good physicochemical stability, high drug-encapsulation capacity, controlled drug release and potential enhancement of bioavailability. Recent studies have highlighted the potential of niosomes for delivery of anticancer drugs and natural bioactive compounds by improving drug stability, cellular uptake and systemic exposure[6]. Thus, niosomal encapsulation may provide a promising approach for overcoming the biopharmaceutical limitations of catechin.

Therefore, the present study was undertaken to formulate and evaluate catechin niosomes for their physicochemical characteristics, encapsulation efficiency, morphology, in vitro drug release and stability. The optimized formulation was further investigated for anticancer activity against HepG2 cells and compared with free catechin. In addition, an oral pharmacokinetic study was performed to determine whether niosomal delivery could improve the systemic exposure and pharmacokinetic behavior of catechin.

1. Material and Method

Cholestero, Span 60, Chloroform, disodium hydrogen phosphate , potassium dihydrogen phosphate, dialysis bag were procured from New Neeta Chemical Pune India. Catechin was procured from Yucca Enterprises Mumbai. Dimethyl sulfoxide and MTT reagent were obtained from Himedia Thane, India. The NCCS in Pune provided the HepG2 cell line.

2. Formulation of Catechin Niosomes

In a round bottom flask containing chloroform add sorbitan monostearate (Span 60) and cholesterol in order to create catechin-loaded niosomes. To create a thin layer, this solution was subsequently evaporated using a Rota-evaporator. After that, 10 mL of pH 7.4 phosphate buffer containing catechin was used to hydrate this film. To create the niosomes, the entire mixture was once more run thru a Rota-evaporator for two hours. After that, the niosomal dispersion was placed in a bath sonicator for five minutes. After that, the niosomes were centrifuged. After discarding the supernatant, the pellet was washed with water to get rid of any extra solvent.

The formed noisome was then re-suspended in 5 mL Millipore water and then 1% Mannitol was added in it. This suspension was then allowed to freeze overnight in -20^{O}C refrigerator. The frozen niosomes were then subjected to lyophilize to get powder. The formed niosomes was then evaluated for further characterization[7].

3. Characterization

3.1. Calibration & Linearity Curve

Using a UV spectrophotometer a calibration curve for catechin was built to determine the wavelength of maximum absorption. In phosphate buffer with a pH of 7.4, a linearity curve covering a concentration range of 10 to 70 µg/ml was created.

3.2. Percentage Practical Yeld

Percentage yield of lyophilized niosomal powder was calculated by using following formula[8].

$$\% \text{ Practical Yield} = \frac{\text{Actual weight of OA niosomes}}{\text{Total theoretical weight}} \text{ x } 100$$

3.3 Percentage Drug Content

Methanol was added to a 100 mL volumetric flask containing 10 g of freeze dried catechin niosomes. After 15 minutes of sonication on a bath sonicator, the dispersion's volume was increased to 100 milliliters using methanol. After filtering the dispersion thru Whatman filter paper (0.22 µ), 1 ml of the filtrate was added to a 10 ml volumetric flask and PBS 7.4 was used to make up the volume. Use a UV spectrophotometer to examine the diluted sample[8].

$$\% \text{ Drug Content} = \frac{\text{Actual amount of drug content}}{\text{Theoretical amount of drug content}} \text{ x } 100$$

To determine the drug content from catechin niosomes UV analysis was done at 279 nm.

3.4 Particle Size and Polydispersity Index (PDI)

Catechin niosomal suspension was diluted with deionized water to achieve a suitable concentration. Diluted sample was loaded into a cuvette and analyzed using the Nano-ZS Zetasizer at 25°C. The average particle size and PDI were measured to evaluate the size distribution and homogeneity of the niosomes. Each experiment was performed in triplicate[9].

3.5 Zeta Potential

The catechin niosomal suspension was diluted with deionized water to an appropriate concentration. The diluted niosomal dispersion was placed in a electrophoretic cell and analyzed using the Nano-ZS Zetasizer at 25°C. To ascertain the niosomes' stability and surface charge, the zeta potential was evaluated[9]. Each experiment was performed in triplicate.

3.6 Entrapment Efficiency

Encapsulation efficiency of catechin-loaded niosomes was determined by separating free drug through centrifugation. The niosomal dispersion was centrifuged at 18,000 rpm for 25 min, and the resulting supernatant liquid was carefully collected and filtered through Whatman filter paper. The filtrate was appropriately diluted using pH 7.4 phosphate buffer and the concentration of free catechin was quantified by UV spectrophotometry at 279 nm [7].

$$\% \text{ Entrapment efficiency} = \frac{\text{Conc.of total drug} - \text{Conc.of free drug}}{\text{Conc.of total drug}} \text{ x } 100$$

3.7 Scanning Electron Microscopy (SEM)

The surface characteristics of catechin-loaded niosomes were evaluated by SEM. To determine the surface features of prepared catechin niosomes, a small amount of niosomal powder was placed on a conductive substrate, such as a metal stub, and allowed to dry. After that a thin film of conductive platinum was applied on the niosomal powder to stop it from charging under the electron beam. The prepared sample was placed in the SEM (FEI, Quantum 200E Instrument) chamber, where a focused electron beam scans the surface to generate high-resolution images. These images are analyzed to determine particle size, shape and surface morphology of the catechin niosomes[10].

3.8 *In Vitro* Drug Release

A pre-treated dialysis bag containing 2 mg of catechin and an equivalent amount of the niosomal formulation was immersed separately in a flask containing 100 mL of phosphate-buffered saline. The buffer was kept at 37°C while being constantly agitated at 150 rpm. At specified intervals, 1 mL of the solution was taken out and refilled with freshly prepared PBS. The amount of the active component in the samples was assessed using UV method at 279 nm. The percent active component release from the niosome formulation was computed[9].

3.9 Stability Testing

Stability testing of niosomes is crucial to evaluate their capacity to retain structural integrity, drug loading and therapeutic efficacy over time. To ensure product reliability, short-term stability studies were conducted for three months following ICH guidelines. Optimized catechin niosomal formulations were stored in sealed glass vials at 4°C and at room temperature. Observations were made over 0, 30 and 90 days. At each interval, the formulations were assessed for physical appearance, particle size, PDI, zeta potential and entrapment efficiency. This evaluation helped determine the influence of storage conditions on the formulation[11].

3.10 *In Vitro* Cytotoxicity Study (MTT assay)

HepG2 hepatocellular carcinoma cells were cultured in Modified Eagle Medium (MEM) supplemented with Non-Essential Amino Acids (NEAA) and 10% (v/v) fetal bovine serum and maintained at 37°C in a humidified 5% CO_2 atmosphere, with the culture medium replaced every 2–3 days.

Cells were seeded into 96-well plates at a density of 100,000 cells/well in 100 µL of culture medium and incubated for 24 h under the same conditions until approximately 50% confluence was achieved. The medium was then removed and cells were exposed to seven different concentrations of cisplatin (standard), pure catechin and

niosomal formulations. Untreated cells maintained in culture medium served as the control. Following 24 h of treatment, cellular morphology and viability were examined using an inverted microscope. The treatment medium was subsequently removed and 100 µL of fresh medium containing 10 µL of MTT reagent (5 mg/mL) was added to each well. The plates were incubated for 4 h at 37°C under 5% CO_2. Thereafter 100 µL of DMSO was added to each well to dissolve the formed formazan crystals, followed by incubation for 1 h. Absorbance was measured at 570 nm using a microplate reader. The IC_{50} values were determined by plotting the concentration of each test formulation against the percentage of cell inhibition using GhraphPad prism[12].

$$\text{Percent Cell Survival (\%)} = \frac{\text{Absorbance of test}}{\text{Absorbance of control)}} \text{ x } 100$$

3.11 Pharmacokinetic Study

Study design: The pharmacokinetic study was performed following approval from the Institutional Animal Ethical Committee (IAEC) under Project Proposal No. SC/IAEC/2024/042. The study aimed to compare the pharmacokinetic profile of pure catechin with that of catechin-loaded niosomes. Female Albino Wistar rats weighing 200 ± 20 g were selected and placed under standard laboratory conditions and provided unrestricted access to food and water. The animals were fasted overnight before drug administration and randomly allocated into two groups, each comprising six rats. A single oral dose equivalent to 35 mg/kg body weight of catechin was administered to both groups [18]. Group I received pure catechin solution, while Group II received the catechin-loaded niosomal formulation. Blood samples (approximately 0.5 mL) were collected from the retro-orbital plexus into heparinized tubes at predetermined time points of 0, 0.5, 1, 2, 4, 8, 12 and 48 h post-dose. Mild ketamine anesthesia was administered during blood collection. [13].

Extraction of drug from plasma: The collected blood samples were centrifuged at 10,000 rpm for 10 min at 4°C using a microcentrifuge to separate the plasma. For protein precipitation mix 100 µl plasma and 280 µl methanol into the Eppendorf tube. The mixture was vigorously vortexed for 2 min and subsequently centrifuged at 10,000 × g for 10 min to facilitate complete settling of the precipitated material. The upper clear solution was separated and 20 µl sample were injected to HPLC column to determine drug present in blood sample after each time point. The same procedure was followed for all sample[14].

Chromatographic Conditions for Detection of Catechin: Chromatographic separation was carried out on a Phenyl Hexyl column (2.7 µm, 100 × 4.6 mm) with a 20 µL injection volume and a flow rate of 0.8 mL/min. The mobile phase comprised 0.6% formic acid in water and acetonitrile (92:8, v/v). Catechin was detected at 279 nm using a photodiode array (PDA) detector[15].

Preparation of Standard Solution: Standard stock solution was prepared by dissolving 10 mg of catechin in methanol with adequate shaking and final volume is make upto 10 mL of methanol. The stock solution was further diluted with the mobile phase comprising 0.6% formic acid in water and acetonitrile (92:8, v/v) to obtain working standard solutions of 25, 10, 5, 2, 1, and 0.5 µg/mL[15].

Sample Preparation and Analysis: For analysis, transfer 100 µl drug free plasma into o the Eppendorf tube and add 280 µl working standard solution. This solution was mix well and vortex for 2 min and then centrifuge at 10000 g for 10 min until the precipitate was settled completely. Supernatant clear solution was separated and 20 µl sample were injected to HPLC column for analysis. The same procedure was followed for all sample and plot a linearity graph concentration v/s peak area[15].

Screening Parameters: After analyzing HPLC data, pharmacokinetic parameters were screened by plotting the drug concentration against time to create a plasma drug concentration-time profile. Pharmacokinetic parameters, including half-life ($t_{1/2}$), time to reach maximum plasma concentration (T_{max}), maximum plasma concentration (C_{max}), area under the plasma concentration–time curve (AUC), elimination rate constant, volume of distribution, apparent systemic clearance and mean residence time (MRT) were calculated using the PKSolver software based on the plasma concentration–time data obtained for pure catechin and the catechin-loaded niosomal formulation. These pharmacokinetic parameters were used to evaluate and compare the absorption, distribution, metabolism, and elimination characteristics of pure catechin and noisome formulation.

Statistical Analysis: Pharmacokinetic parameters were statistically analyzed using GraphPad Prism version 8.42. Data were presented as mean ± standard deviation and differences between groups were evaluated using the Student's t-test. Statistical significance was defined as $P < 0.05$ with significance levels of *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$ relative to the pure catechin group.

4 Result and Discussion

4.1 Calibration & Linearity Curve

The UV calibration curve of catechin was established to determine its maximum absorption wavelength, as shown in Figure 1. Catechin exhibited a λmax of 279 nm. Linearity was evaluated in phosphate buffer (pH 7.4) over the concentration range of 10–70 µg/mL. A strong linear correlation between catechin concentration and absorbance was observed, yielding a R^2 of 0.9987.

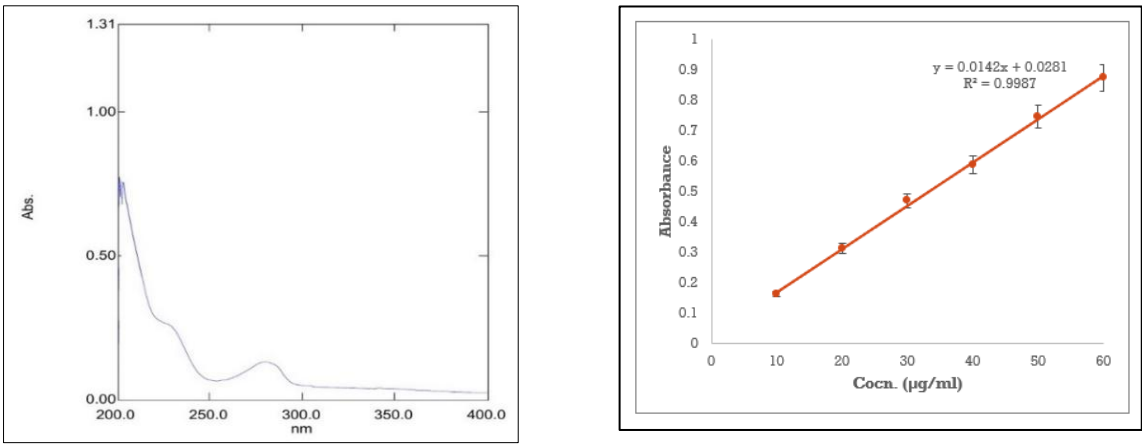


Figure 1: Calibration and linearity graph of catechin by UV method

4.2 Percentage Drug Content

The freeze-dried catechin-loaded niosomes exhibited a drug content of 88.86 ± 0.39%, demonstrating satisfactory drug incorporation within the formulation.

4.3 Particle Size and PDI

The average particle size and PDI of catechin loaded niosomal formulation was measured by using Nano-ZS Zetasizer. The mean particle size and PDI was found tobe 112 ± 8.14 and 0.162 ± 0.08 respectively (Figure 2). A smaller particle size is considered advantageous for niosomal formulations as it can improve the bioavailability of the encapsulated drug by facilitating its passage across biological barriers and promoting cellular uptake. Nanosized particles possess a higher surface area to volume ratio, which may enhance the interaction of niosomes with target cells and tissues, thereby improving drug delivery efficiency[16]. Lower PDI value indicates monodisperse sample with homogenous population of particle and is an acceptable nanocarrier for drug delivery system. PDI Values should be between 0.05 – 0.7. PDI of 0.3 and below is acceptable in vesicular drug delivery system[16,17].

4.4 Zeta Potential

The catechin-loaded niosomes exhibited a negative zeta potential of −34.1 ± 1.87 mV (Figure 3). This high negative surface charge is likely to minimize vesicle aggregation through electrostatic repulsion, thereby enhancing the physical stability of the niosomes during storage [9]. The negative zeta potential may be attributed to the presence of free hydroxyl groups in cholesterol, the non-ionic surfactant molecules, and the incorporation of dicetyl phosphate (DCP) as a negatively charged stabilizing agent.

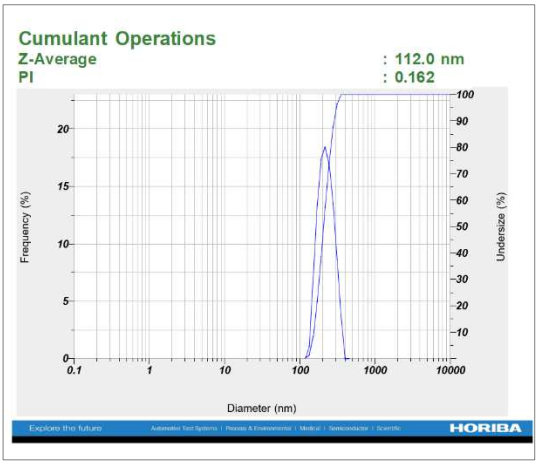


Figure 2: Particle size & PDI of catechin niosomes

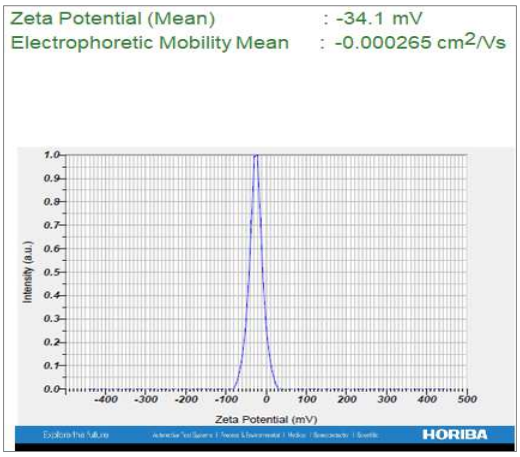


Figure 3: Zeta potential of catechin niosomes

4.5 Entrapment Efficiency

Factors influencing the effectiveness of entrapment include vesicle size, type of surfactant and concentration of cholesterol[18]. The % EE was found to be 88.44 ± 2.16. This high entrapment efficiency is attributed to the blend of non-ionic surface-active agent and cholesterol, which leads to the formation of extra stable and more impermeable niosome vesicles.

4.5 Scanning Electron Microscopy

SEM analysis of the catechin-loaded niosomes revealed clearly defined vesicles with a predominantly spherical morphology, as illustrated in Figure 4.

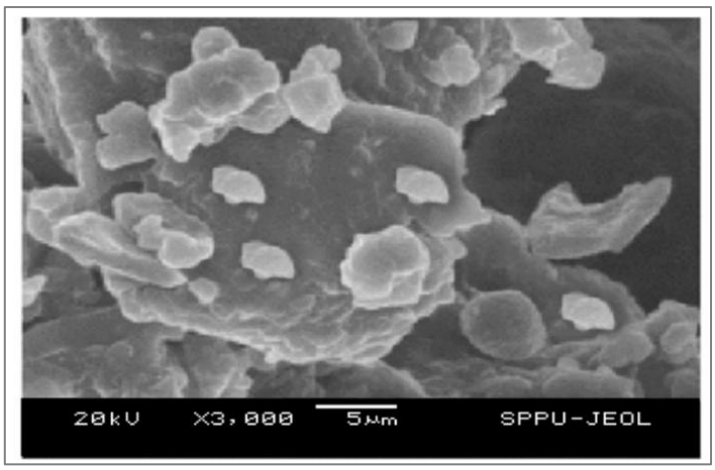


Figure 4: Morphology of Catechin niosomes

4.6 *In Vitro* Drug Release

The in vitro release profile of catechin was evaluated using the dialysis bag diffusion method with phosphate buffer (pH 7.4) serving as the release medium. Results revealed that catechin released from the catechin solution reached 97.42 ± 0.58% within 2 hours, whereas catechin released from the niosomes was 86.89 ± 0.65% over 6 hours (Figure 5). These findings indicate that catechin niosomes exhibit a sustained release profile compared to the rapid release observed with pure catechin. The sustained release profile may be attributed due to the entrapment of catechin within the niosomal vesicles, which regulates and prolongs its diffusion into the release medium.

The sustained release of catechin from the niosomal formulation may be attributed to the combined influence of several factors, including the composition of the vesicular bilayer and the efficiency of catechin encapsulation.

The bilayer's rigidity and stability, enhanced by the presence of span 60 and cholesterol, likely restrict the immediate diffusion of catechin, resulting in a more prolonged release. Additionally, the vesicular structure provides a barrier that slows down drug release, ensuring a steady diffusion over an extended period[19-20].

This sustained release profile is advantageous for therapeutic applications as it may improve bioavailability, and reduce dosing frequency. Moreover, the prolonged release ensures a more consistent drug concentration at the target site, potentially increasing efficacy while minimizing side effects associated with rapid drug release. Thus, catechin-loaded niosomes represent a promising delivery system for achieving controlled release of drug.

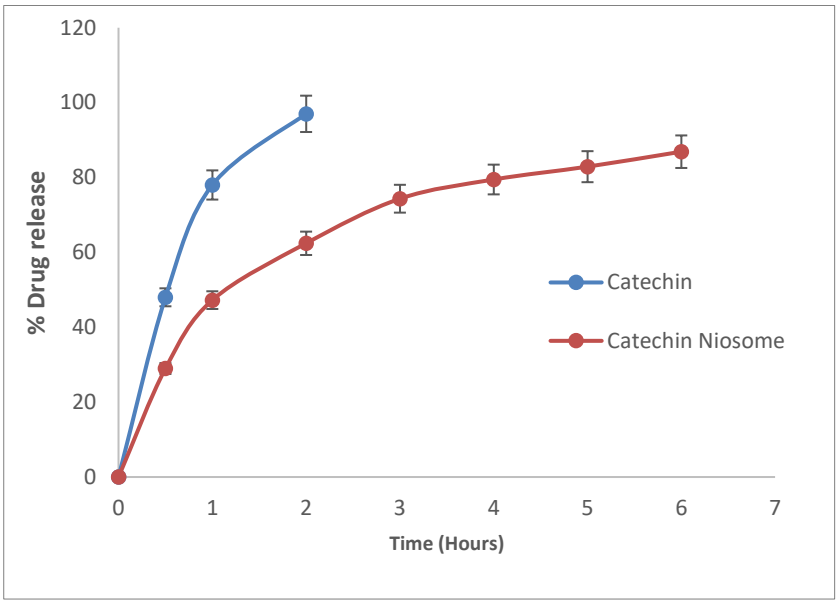


Figure 5: Invitro drug release

4.8 Stability Testing

The stability of catechin-loaded niosomes was assessed over a 90 days under refrigerated (4°C) and room-temperature (25°C) storage conditions. The result is showing in table 1. At 4°C, the niosomes retained a consistent light brown appearance with negligible changes in average particle size, PDI, zeta potential and entrapment efficiency indicating good stability. In contrast, room temperature storage led to the formation of dispersible aggregates with days, an increase in particle size from 112 nm to 191.4 nm and PDI from 0.162 to 0.343, a decline in zeta potential from -34.1 mV to -28.9 mV and a marked reduction in drug entrapment efficiency from 88.44 % to 68.17 %.

Table 1: Summary of stability testing of catechin niosomes at 4°c & RT

Parameters	(4° C)			RT		
	0 Day	30 Days	90 Days	0 Day	30 Days	90 Days
Physical appearance	Light brown solution	Light brown solution	Light brown solution	Light brown solution	Sight discoloration of solution	Sight discoloration of solution with redispersible flocculation
Particle size	112 ± 8.14	121.5 ± 9.37	137.8 ± 10. 56	112 ± 8.14	151.9 ± 8.78	191.4 ± 9.89
PDI	0.162 ± 0.08	0.167 ± 0.11	0.169 ± 0.78	0.162 ± 0.08	0.174 ± 0.19	0.343 ± 0.27
Zeta potential	-34.1 ± 1.87	-33.8 ± 2.15	-32.5 ± 1.89	-34.1 ± 1.87	-31 ± 2.20	-28.96 ± 3.28
% EE	88.44 ± 2.16	87.63 ± 1.90	86.35 ± 2.01	88.44 ± 2.16	79.48 ± 2.14	68.17 ± 1.34

These changes are attributed to temperature induced destabilization mechanisms such as increased cholesterol fluidity, structural rearrangement in the bilayer and possible degradation of surfactant components, which collectively promoted aggregation and drug leakage. Overall, the results highlight that low-temperature storage (4°C) is essential for maintaining the physical and chemical integrity of catechin niosomes, making it the preferred condition for long-term preservation of the formulation's stability and performance.

4.9 In Vitro Cytotoxicity Study (MTT assay)

The cytotoxic effects of cisplatin (standard), free catechin and catechin-loaded niosomes were evaluated in HepG2 cells using the MTT assay across concentrations of 10–1000 µg/mL. The percentage of cell survival decreased in a dose-dependent manner for all treatments, while cell inhibition (figure 6) increased with higher concentrations. At all tested concentrations, catechin niosomes showed significantly greater cell inhibition compared to free catechin, indicating the enhanced cytotoxic effect of the niosomal formulation.

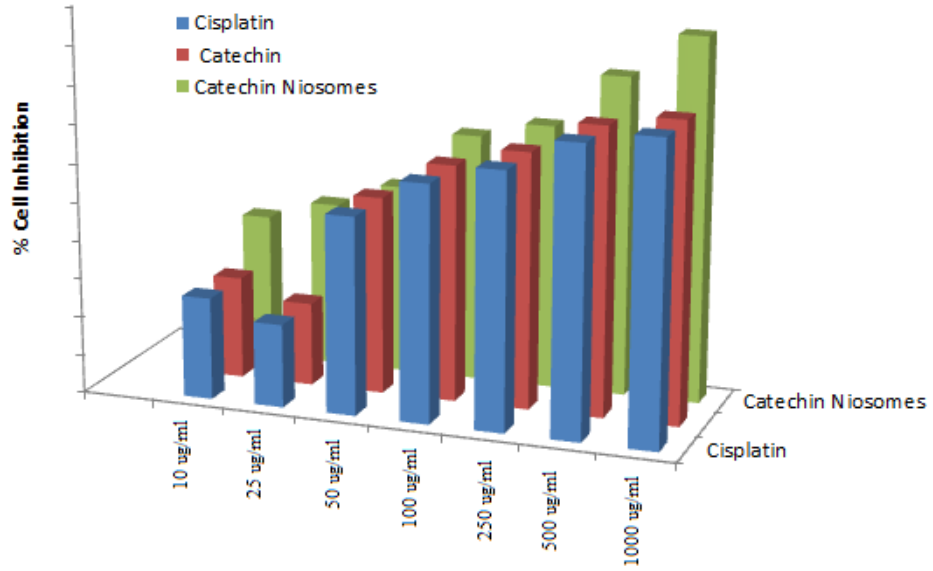


Figure 6: % cell inhibition as a function of catechin concentration

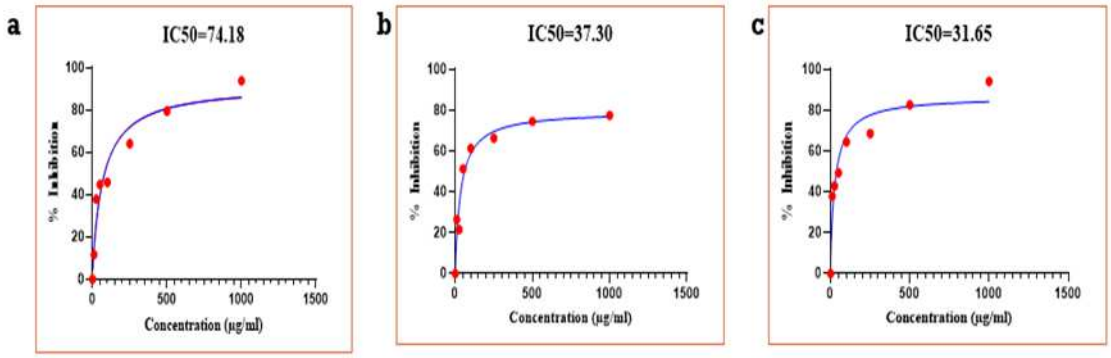


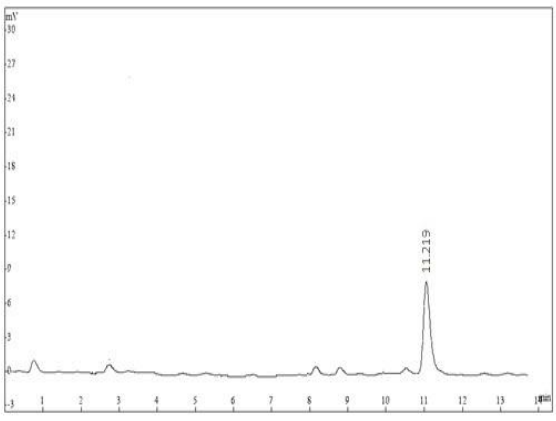
Figure 7: IC_{50} value of a) cisplatin b) catechin c) catechin niosomes

The IC_{50} value (figure 7) for cisplatin was 74.18 µg/mL, reflecting its well-known anticancer activity. Free catechin exhibited an IC_{50} value of 37.30 µg/mL, demonstrating moderate cytotoxicity against HepG2 cells. In contrast, catechin-loaded niosomes showed a significantly lower IC_{50} value of 31.65 µg/mL, indicating a stronger cytotoxic effect. The lower IC_{50} value for catechin-loaded niosomes can be attributed to the enhanced bioavailability, improved cellular uptake and sustained release of catechin. Niosomes, as drug delivery systems, facilitate the efficient encapsulation and release of catechin, which not only protects it from degradation but also ensures better penetration into the cells[21]. Additionally, the niosomal formulation provides a controlled release profile, allowing for prolonged exposure of the cells to catechin, which enhances its cytotoxic effects. The improved cytotoxicity of catechin-loaded niosomes, as evidenced by the lower IC_{50} value. In conclusion, the MTT assay results indicate that catechin-loaded niosomes are more effective in inhibiting HepG2 cell proliferation compared to free catechin and cisplatin, with a lower IC_{50} value. This enhanced cytotoxicity can be attributed to the controlled release and improved cellular uptake of catechin in the niosomal formulation[21,22].

4.10 Pharmacokinetic Study

A bioanalytical HPLC method was developed to quantify catechin in plasma following oral administration. Prior to the experiment, a calibration curve was established, with the acceptance criteria requiring a correlation value greater than 0.99. The developed method showed excellent linearity over the concentration range of 0.5–25 µg/mL (Figure 8), with a high $R^2 = 0.9939$ value.

Plasma catechin levels in the collected blood samples were determined by HPLC analysis. The plasma concentration–time profiles obtained after oral administration of pure catechin solution and catechin-loaded niosomal suspension are shown in Figure 9. Pharmacokinetic parameters were calculated using PKSolver software based on a one-compartment model. The evaluated parameters included T_{max}, C_{max}, AUC, MRT, $t_{1/2}$, elimination rate constant, mean residence time, apparent volume of distribution and apparent systemic clearance with the corresponding values presented in Table 2. All data were expressed as mean ± standard deviation (SD).



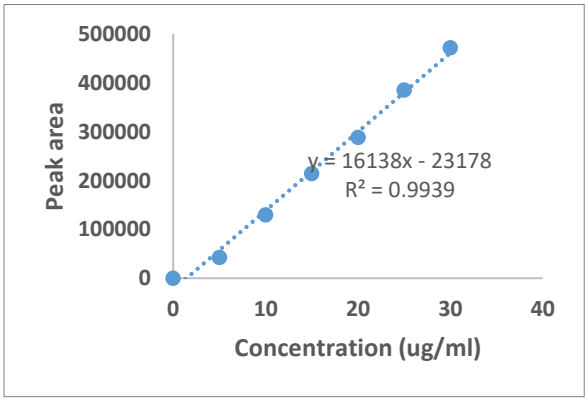


Figure 8: HPLC linearity curve of catechin

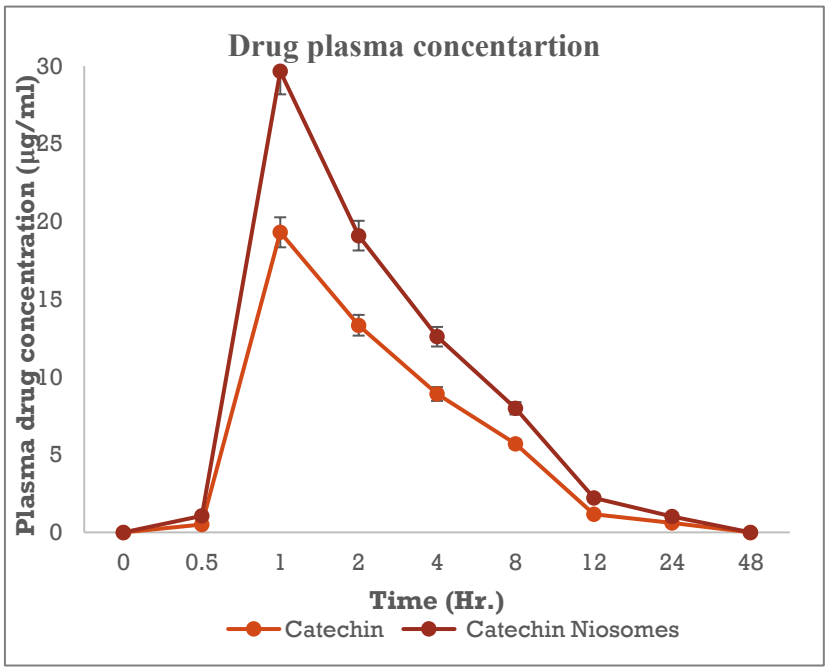


Figure 9: Plasma concentration of catechin after oral administration of catechin and catechin niosomes

The plasma drug concentration (C_{max}) verses time graph shows an elevation in plasma concentration of catechin from niosomes compared to free catechin as shown in figure 9. C_{max} of the catechin was increased from 19.303 ± 0.5427 µg/ml to 29.660 ± 0.341 µg/ml with the use of niosomes. There was increase in C_{max} because nanosized niosomes were efficiently taken up by RES system, leading to enhanced permeability and sustained release and hence statistical significance of catechin niosomes was considered at ****P<0.0001 as compared to pure catechin. There was no statistical difference in T_{max} (Table 2) following oral administration of catechin and catechin niosomes suggests that the rate of absorption remains similar for both formulations. This indicates that niosomes may enhance bioavailability without altering the time taken to reach peak concentration.

As shown in Table 2, the elimination rate constant significantly decreased (****P<0.0001) while the half-life ($t_{1/2}$) increased for catechin loaded niosomes compared to pure catechin (***P<0.001), likely due to the lipophilic and nanosized nature of niosomes that enhance drug stability and prolong circulation. The AUC also showed a marked increase from 122.177 ± 1.574 to 553.498 ± 5.516 µg·h/mL (***P<0.001), indicating improved bioavailability and systemic exposure. No statistical difference in the apparent volume of distribution (table 2) between catechin niosomes and the free catechin suggesting similar tissue distribution. Additionally, catechin niosomes demonstrated a significantly longer mean residence time ****P<0.0001) and reduced systemic clearance (***P<0.001) suggesting sustained release and better pharmacokinetic performance over free catechin.

Table 2: Pharmacokinetic parameter of catechin and catechin niosomes after oral administration

Sr. No	Parameter	Unit	Catechin	Catechin Niosomes
1	**Elimination rate constant**	1/h	0.311 ± 0.0082	0.131 ± 0.004
2	**$t_{1/2}$**	H	5.238 ± 0.259	9.739 ± 0.465
3	**T_{max}**	H	1.000 ± 0	1.000 ± 0
4	**C_{max}**	µg/ml	19.303 ± 0.5427	29.660 ± 0.341
5	**AUC**	µg/ml*h	122.177 ± 1.574	553.498 ± 5.516

6	**Mean Residence time**	h	3.630 ± 0.301	11.306 ± 0.305
7	**Apparent Volume of distribution**	(mg)/(µg/ml)	2.105 ± 0.135	2.0583 ± 0.059
8	**Apparent systemic clearance**	(mg)/(µg/ml)/h	0.898 ± 0.364	0.2300.039

5 Conclusion

The present study successfully demonstrates that catechin niosomes significantly enhance the pharmacokinetic performance and anticancer potential of catechin, offering a promising strategy for hepatic cancer therapy. The developed niosomal formulation exhibited high entrapment efficiency of 88.44 ± 2.16%, nanosized particles of 112 ± 8.14 nm, low PDI value of 0.162 ± 0.08, optimum negative zeta potential value -34.1±1.87 mV. The catechin loaded niosomes showed an excellent physical stability at 4 °C. SEM analyses confirmed vesicular integrity of catechin in niosomal vesicles. In vitro drug release studies revealed that catechin released from the catechin solution reached 97.42 ± 0.58% within 2 hours, whereas catechin released from the niosomes was 86.89 ± 0.65% over 6 hours. These findings indicate that catechin niosomes exhibit a sustained release profile compared to the rapid release observed with pure catechin. Cell cytotoxicity assays showed superior anticancer efficacy of catechin-loaded niosomes compared to free catechin and cisplatin, as evidenced by the lowest IC_{50} value 31.65 µg/ml. The formulation was studied for oral pharmacokinetic in rat and bioavailability was determined using the pharmacokinetic parameters and quantification of drug in plasma. The maximum plasma concentration achieved was 19.303 ± 0.5427 µg/ml as compare to pure catechin 29.660 ± 0.341 µg/ml. The mean residence time of the drug was also increase from 3.630 ± 0.301 hrs to 11.306 ± 0.305 hrs. The other key pharmacokinetic metrics such as AUC, $t_{1/2}$, elimination rate constant, apparent volume of distribution, systemic clearance, MRT were significantly improved, reflecting enhanced bioavailability and prolonged systemic circulation. Therefore, it is recommended that catechin niosomes is secure and useful as nanocarrier which can enhance the catechin bioavailability.

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7 Author Contributions

Sonali Devne contributed to the conceptualization, methodology, formulation development, experimental investigation, data analysis and preparation of the original manuscript. Jyoti Jawale contributed to experimental work, data collection and analysis. Vikash Shende contributed to the pharmacokinetic investigation, data interpretation and manuscript review. Pramod Ingale gives critical revision of the manuscript. All authors read and approved the final manuscript.

8 Ethics Declaration

The animal study was approved by the Institutional Animal Ethics Committee (IAEC) under Project Proposal No. SC/IAEC/2024/042 and conducted according to applicable ethical guidelines.

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The research did not receive any funds from any source.

10 Conflict of interests

The authors declared no conflict of interest.

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