RESEARCH ARTICLE

Nanoniosomes Loaded with Catechin for Anticancer Activity on Hepatoma HepG2 Cells

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ABSTRACT
This study focuses on formulation and assessment of catechin-loaded nanoniosomes and their cytotoxicity against HepG2 hepatoma cells. Catechin, a natural antioxidant with potential anticancer properties, has limited clinical use due to its poor bioavailability and stability. Using thin-film hydration approach, nanoniosomes were produced and characterised for encapsulation efficiency, particle size, and zeta potential in order to address these obstacles. cytotoxic activity of catechin-loaded nanoniosomes was assessed on HepG2 cells using MTT assay, and results were compared to free catechin and the chemotherapeutic drug cisplatin. The nanoniosomes demonstrated improved encapsulation efficiency and sustained release properties. Furthermore, catechin-loaded nanoniosomes exhibited significantly enhanced cytotoxicity against HepG2 cells compared to free catechin, and cisplatin. This study highlights the potential of nanoniosomal systems in improving the therapeutic efficacy of catechin for hepatocellular carcinoma treatment.
Keyword: Catechin, HepG2, nanoniosomes, anticancer, cytotoxicity, MTT
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INTRODUCTION

The stability, biocompatibility, and capacity to encapsulate hydrophilic and lipophilic chemicals of nanoniosomes, a new nanocarrier system, are making them an attractive candidate for medication delivery. Cholesterol and non-ionic surfactants come together to generate these vesicles, which are perfect for cancer treatment since they can transport drugs to specific areas[1]. Catechin, a polyphenolic compound found in green tea, have shown promising anticancer effects, especially against HCC, most communal form of liver cancer[2]. Despite catechin's well-deserved reputation as an antioxidant, anti-inflammatory, and anticancer agent, its fast metabolism, low bioavailability, and poor absorption severely restrict its therapeutic utility. Encapsulating catechin in nanoniosomes may enhances its stability, bioavailability, and cellular uptake, significantly improving its therapeutic potential against cancer cells[3]. Catechin is flavonoidcreate to inhibit lipid peroxidation in cell culture, enhanced cytotoxicity, reduced cell proliferation, and increased apoptosis[4]. Use of nanoniosomes as a delivery system for catechin may offers a promising approach for treating HCC by combining the natural anticancer properties of catechin with the targeted, efficient delivery capabilities of nanotechnology.Our objective in this study was to enhance the stability, bioavailability, and sustained release of catechins from niosomal vesicles. To that end, we designed and tested nanoniosomes bearing catechins on HepG2 cells. The niosomes was prepared using thin-film hydration method and subjected to a comprehensive characterization process, including assessments of PDI, zeta potential, particle size, FTIR, encapsulation efficiency, and *in vitro* drug release.Additionally, release kinetics of catechins from the niosomes will be analysed to elucidate underlying release mechanisms, providing insights into probable of niosomes as an actual delivery system for catechins.

MATERIALS AND METHOD

Span 60, Cholesterol was obtained from Loba Chemie Pvt. Ltd. Mumbai India. Catechin was purchased from Yucca Enterprises Mumbai. Chloroform, potassium dihydrogen phosphate, disodium hydrogen phosphate, dialysis bag was obtained from New Neeta Chemical Pune India. MTT reagent and dimethyl sulfoxide were obtained from Himedia Thane India. HepG2cell line was obtained from the NCCS, Pune.

Formulation of niosomes loaded with Catechin

Thin film hydration approach was used to formulatenanoniosomes loaded with catechin. A round-bottom flask was used to dissolve surfactant (Span 60) and cholesterol in chloroform. A thin film was produced by evaporating this solution using a Rota-evaporator. The 10 mL of pH 7.4 phosphate buffer was subsequently used to hydrate the film. containing catechin. Whole mixture was again subjected to Rota-evaporator for 2 hours to form the niosomes. formed niosomal dispersion was then put in bath sonicator for 5 min. Different batches were formulated as per table 1. The formed niosomes was then centrifuged. Supernatant was discarded and pellet was then re-suspended in water and subjected to lyophilization[5]. The

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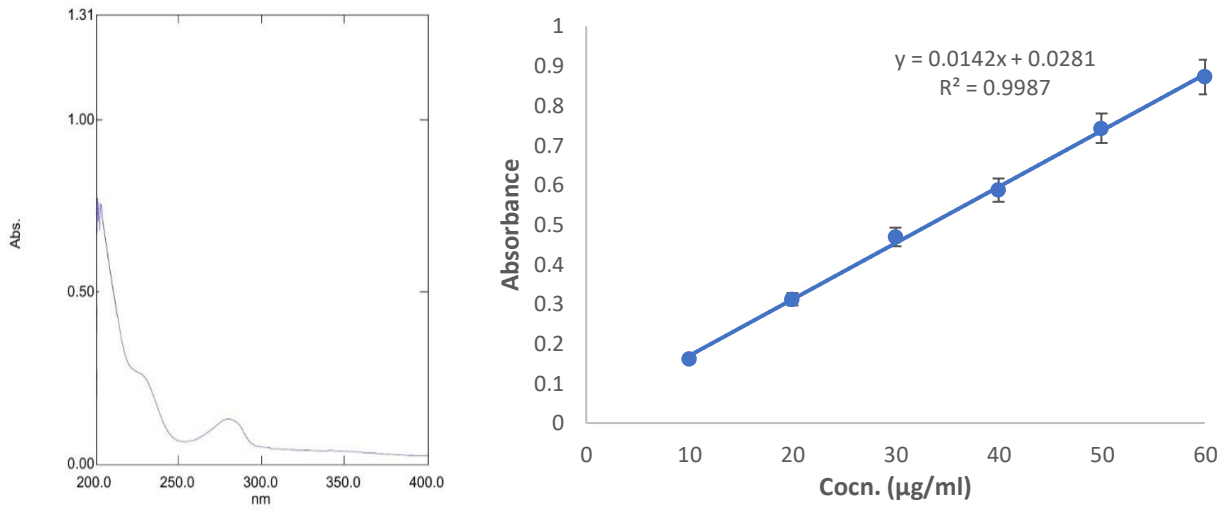

Figure 1: UV absorption spectrum for Catechin and linearity graph

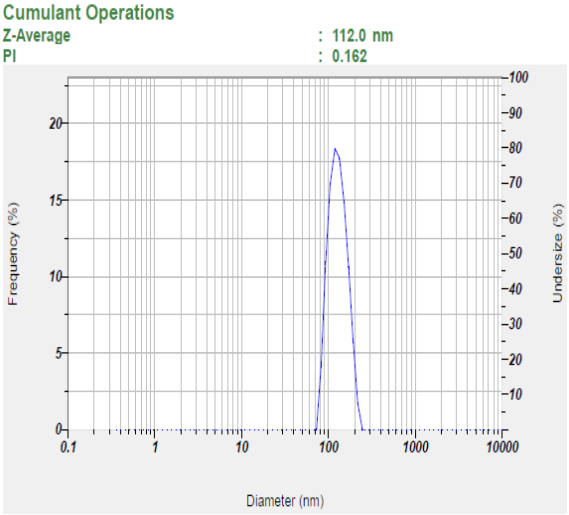

Figure 2: Average particle size and PDI of optimized Catechin niosomes

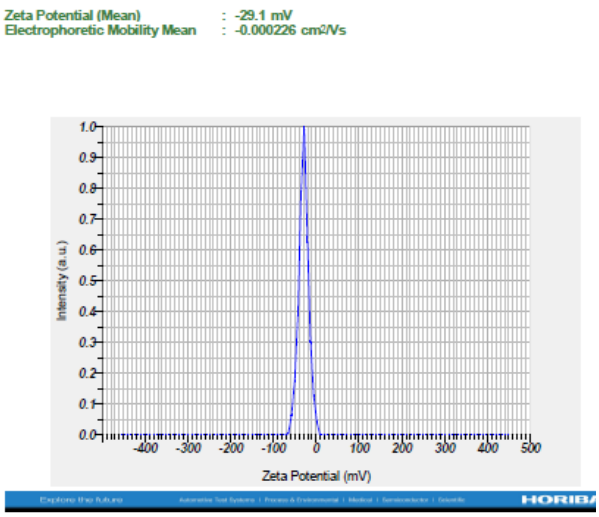

Figure 3: Zeta potential of optimized Catechin niosomes

formed niosomes was then evaluated. The batches are shown in Table 1.

Characterization

Melting Point

A small quantity of catechin was loaded into a capillary tube and gently tapped on the bench to position it at the bottom. The tube was placed in a device that measures melting points. The temperature was recorded as the catechin started to melt and continued until it was completely liquid.

Analytical method development

It was made easier to work at the laboratory level by developing a UV spectroscopic method for determining catechin. As part of standard laboratory procedures, UV method allows for determination of drugs. For purpose of analysing catechin, a straightforward, accurate, precise, and less time-consuming UV method was created and verified[6].

Calibration & linearity curve

Calibration curve was plotted for resolve of wavelength of maximum absorption using UV spectrophotometer for catechin. A linearity curve was developed in phosphate buffer having pH 7.4.

Saturation solubility

Table 1: Formulation table of Catechin niosomes

Ingredients	(+) Catechin (mg)	Span 60 (mg)	Cholesterol (mg)
F5	1	3	2
F4	1	2	3
F3	1	2	2
F2	1	1	2
F1	1	2	1

An additionalquantity of catechin was dissolved in 10 ml suitable solvent in which saturation solubility have to determine. This saturated solution is placed on mechanical shaker for 48 hrs. After the equilibrium is achieved collect supernatant and filter through Whatman filter paper. Filtrate were examined for using UV at 279 nmafterward suitable dilution. Calculate amount of catechin concentration using slope equation[7].

Practical yield

Practical yield of freeze dried catechin loaded niosomes was calculated by using following formula[8].

Table 2: Formulation and optimization results of Catechin loaded niosomes

S. No.	Formulation Code	Catechin: Span 60: Cholesterol (% w/w/w)	Average particle size (nm)	PDI	Zeta potential (mV)	Entrapment efficiency (%)
1.	F1	1:2:1	99.8± 9.83	0.380 ± 0.097	-20.0± 1.45	43.2 ± 1.74
2.	F2	1:1:2	137.2 ± 10.24	0.078 ± 0.11	-24.8 ± 1.97	62.37 ± 2.87
3.	F3	1:2:2	141.1± 7.68	0.098 ± 0.104	-26.10± 1.74	79.18± 1.29
4.	F4	1:2:3	112 ± 8.14	0.162 ± 0.08	-34.1 ± 1.87	88.44 ± 2.16
5.	F5	1:3:2	145.5± 8.28	0.118 ± 0.093	-21.57 ± 1.52	89.25 ± 2.28

Table 3: *In vitro* kinetic release data from Catechin niosomes

Kinetic model	r^2
Zero order kinetic	0.9758
First order kinetics	0.965
Higuchi	0.9081
Korsmeyer Peppas	0.3016

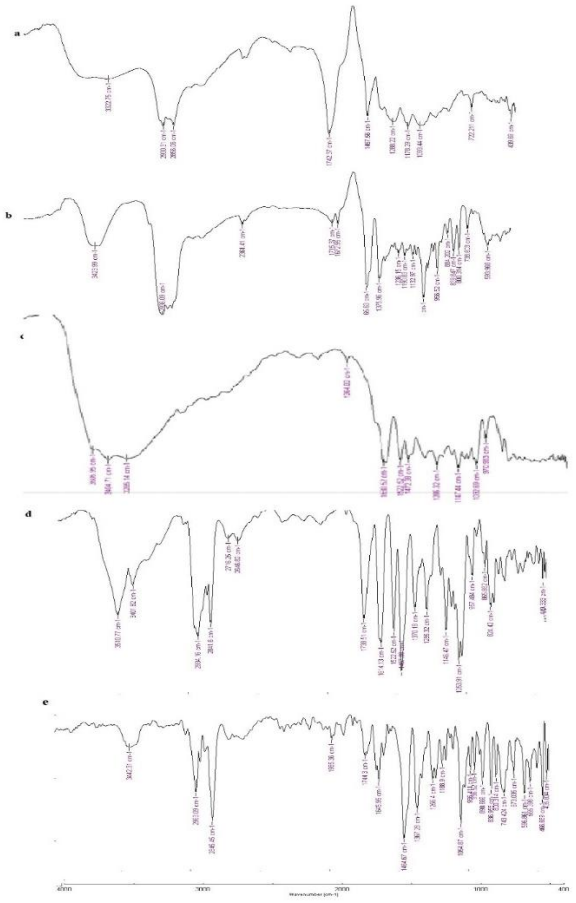

Figure 4: FTIR of a) Span60 b) Cholesterol c) Pure Catechin d) Physical mixture e) niosome formulation

% Practical Yield = Actual weight of catechin niosomes/Total theoreticalweight X 100

Percentage drug content

Take 100 mg of freeze-dried catechin loaded nanoniosomes and add into 100 ml volumetric flask and add enough methanol. The suspension was put in bath sonicator for 15 mins. Then the final volume was made with methanol. One millilitre of the filtrate was transferred to a ten millilitre volumetric flask after the suspension was filtered with Whatman filter paper. For analysis of filtered sample, a UV spectrophotometer set at 279 nm was utilised. This formula was used to determine % of drug content in niosomes that were produced[8].

% Drug Content = Actual drug content/Theoretical drug content X 100

Average particle diameter (size), PDI, and zeta-potential

Niosomal formulations were analyzed for mean particle diameter, PDI, and ζ-potential using a Nano-ZS Zetasizer. Measurements were conducted by evaluating the autocorrelation function at a 90° angle and a 25°C temperature. The instrument provided the average particle size along with standard error based on the fitted data. Respectively experimentation was executed in triplicate to confirm accuracy[9].

Entrapment efficiency

Entrapment efficiency was determined using a centrifugation technique. A specified amount of catechin loaded niosomal suspension was transferred into a centrifuge tube. Centrifuge the sample at high rpm for 25 min. After centrifugation, carefully remove supernatant. Examined the transparent supernatant solution using UV spectrophotometry at 279 nm to find out the free catechin[10].

FTIR

FTIR was used to obtain a spectrum of individual apparatuses of niosomesi.e catechin, span 60, cholesterol, physical mixture of niosomes component and optimized catechin loaded niosomes. A hydraulic press was used to obtain spectra in the 400–4000 nm range using the KBr disc method. The structural characteristics of produced niosomes and drug-excipient compatibility were investigated using FTIR analysis[11].

Scanning electron microscope (SEM)

With the use of a SEM, we examined the formulation's surface morphology. A dry film was formed by pouring formulation onto a circular aluminium plate and formerly drying it in a vacuum oven. Resulting film was then examined using a scanning electron microscope[12].

In- vitro drug release

A pre-activated dialysis bag comprising 2 mg of catechin and an equivalent quantity of niosomal preparation was immersed in a beaker having 100 mL of PBS. Maintaining a temperature of 37°C, buffer was constantly mixed at 150 rpm. The solution was removed in 1 mL increments and replaced with freshly made PBS at exact intervals. Using a UV technique spectrometer, we were able to ascertain the catechin concentration in the samples. It was determined what percentage of catechin was released from the niosome formulation. The release kinetics were analyzed using a

variety of mathematical models, such as zero, first-order, Higuchi, and Korsmeyer-Peppas models[13,14].

***In vitro* cytotoxicity study (MTT assay)**

Preparation of Cells

Hepatoma HepG2 cancer cell was purchased from NCCS Pune. A medium called Modified Eagle Medium (MEM) was used to keep HepG2 liver cancer cells. This medium also contained Non-Essential Amino Acids (NEAA) and 10% foetal bovine serum (v/v). Every two to three days, the cells were grown in a humidified chamber with 5% CO2 and 37°C.

MTT Assay Procedure

Cells were seeded into 96-well plates at a density of 100,000 cells/well in 100 µL of medium. For 24 hours, the plates were incubated at 37°C with 5% CO_2. They kept an eye on the cells for half a confluence monolayer after the incubation time was up. We removed the cells from the culture media and treated them with pure catechin, a nanoniosomal formulation of catechin, and cisplatin at seven different concentrations. As a control, cells were cultured in cell culture media without any test item and subjected to the same conditions. We incubated plates at 37°C by 5% CO_2 for 24 hours[15]. After 24hrs, cells were observed under inverted microscope for changes in morphology or death if any. After observation, culture medium was removed and 100ul fresh medium added with 10ul mg/ml MTT reagent in individually well[16]. Plated were incubated for 4hrs at 37°C in 5% CO_2 incubator.100ul solubilization (DMSO) added into each well and allowed to stand for 1hr at 37°C in 5% CO_2. The absorbance at 570 nm was measured using a microplate reader once formazan purple crystals had dissolved entirely[17]. A graph showing test substance concentrations vs percentage of cell inhibition was used to estimate IC50 values[18].

RESULTS AND DISCUSSION

Melting Point

Catechin melting point was be 172-174°C.

Calibration curve

calibration curve of catechin was constructed to determine the UV absorptionwavelength. For Catechin it was found to be 279 nm.Linearity curve was constructed in phosphate buffer (pH 7.4) at concentration rang of 10 to 60 µg/mL and it shows linear relationship with $R^2 = 0.9987$ (Figure 1).

Saturation solubility

Saturation solubility of catechin was determined in different solvents and it was found as 0.48 ± 0.112 mg/ml in water, 54.1 ± 0.740 mg/ml in ethanol, 34.7 ± 0.281 mg/ml in chloroform, 15.68± 0.014 mg/ml in PBS pH 7.4 at RT

Analytical method development

The UV analytical method development for catechin demonstrated satisfactory precision and accuracy. Intraday and interday precision showed %RSD values within the acceptable range (less than 2%), confirming consistent results both within a single day and across multiple days. Robustness testing, involving small changes in wavelength indicated minimal variation in results, confirming the method's reliability under varied conditions. Similarly, ruggedness testing across different analysts produced consistent outcomes, highlighting the method's reproducibility. accuracy of method was validated with recovery study. It indicating that the method accurately quantifies catechin with excellent recovery within the desired range.

Practical yield

Percentage practical yield of freeze dried catechin niosomes was determine and it was in the range of 57.36 ± 0.74 to 93.96 ± 0.13.

Percentage drug content

Percentage drug content of freeze dried catechin niosomes was calculated. For various batches from F1 to F5 and it was found in the range of 43.06 ± 0.58 to 89.96 ± 0.24.

Average particle size and PDI

Average particle size was in range of 99.8 ± 9.83 to 145.5 ± 8.28 nm (Table 2). The optimised catechin niosomes' average particle size and PDI are displayed in Figure 2. The statistics on particle size clearly show that formulation's increase in span 60:cholesterol ratio increase the niosomal vesicle size, which gradually enlarges. The mean particle size targeted can be produced by manipulation of the combined effects of cholesterol and surfactant concentration. Smaller particle size is often desirable for niosomes as they can enhance the bioavailability of encapsulating drug by facilitating better penetration through biological barriers and improving cellular uptake. Smaller particles are also associated with a larger surface area-to-volume ratio, which can enhance the interaction of niosomes with target cells and tissues[19,20].

The PDI values of different formulation were found between 0.078 ± 0.11 and 0.162 ± 0.08 (Table 2). A lower PDI is also indicative of a homogenous niosomal population, which is important for achieving reliable and reproducible therapeutic outcomes. In contrast, a high PDI value indicates a broad size distribution, which can lead to inconsistent drug delivery and reduced formulation stability[21].

Zeta potential

All formulation batches of catechin loaded niosomes exhibited negative zeta potential value from -20.0 ± 1.45 to -34.1 ± 1.87 mV (Table 2). Figure 3 shows zeta potential of optimized catechin niosomes[22]. Negative zeta potential value might be due to free hydroxyl group present in cholesterol, non-ionic surfactant molecules and addition of negatively charge inducing molecule i.e. DCP. The higher negative charge might be reducing the tendency of niosome aggregation which result in increase in stability ofniosomes during storage condition[23,24].

Entrapment Efficiency

Several factors influence efficacy of entrapment (EE), including vesicle size, surfactant type, and cholesterol concentration. Table 2 shows that range of % EE for several formulations was between 43.2 ± 1.74 and 89.25 ± 2.28. Formulation F4 shows maximum entrapment efficiency. The presence of cholesterol and a non-ionic surfactant in niosomes makes the vesicles more stable and reduces the likelihood of leakage. Cholesterol stabilizes the bilayer, prevent the leakage of vesicle.A linear correlation is present between the cholesterol content and EE up to certain level cholesterol concentration above that level there is decrease in EE because there may be competition between the

cholesterol and drug to be placed between the bilayer of niosomes and hence less amount of drug may be entrapped in niosomal vesicles[25]. Based on zeta potential,particle size, and entrapment efficiency F4 batch was considered as optimized batch.

Fourier Transformation Infrared Spectroscopy (FTIR)
The FTIR spectra **(Figure 4)** of catechin, excipients i.e span 60 and physical mixture,cholesterol,and catechin niosomes were recorded as shown in figure 4. stretching vibration of the O-H bond of catechin was attributed to the absorption

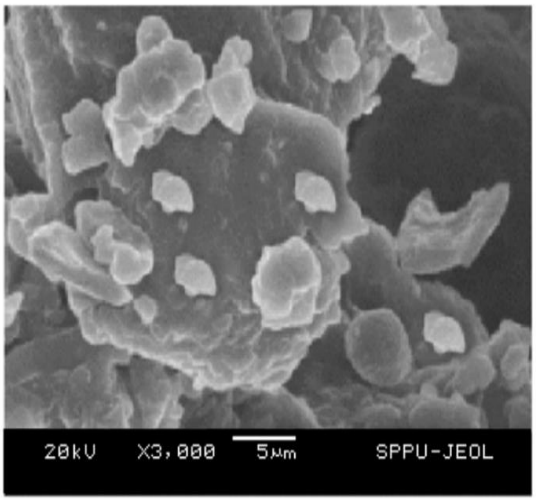

Figure 5: Morphology of Catechin niosomes

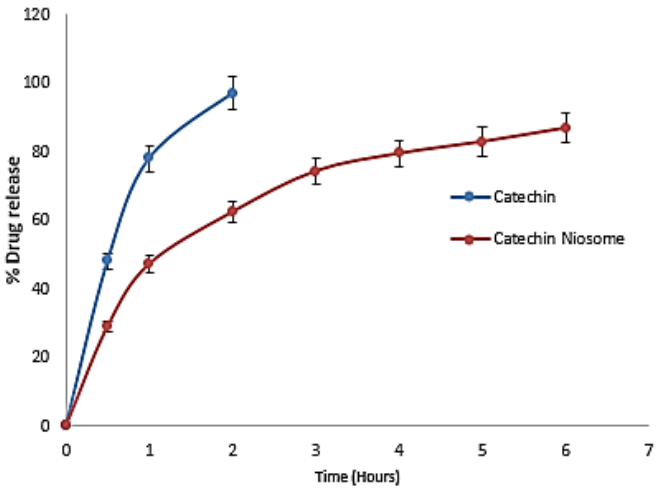

Figure 6: *In vitro* release of Catechin from catechin solution and Catechin loaded niosomes

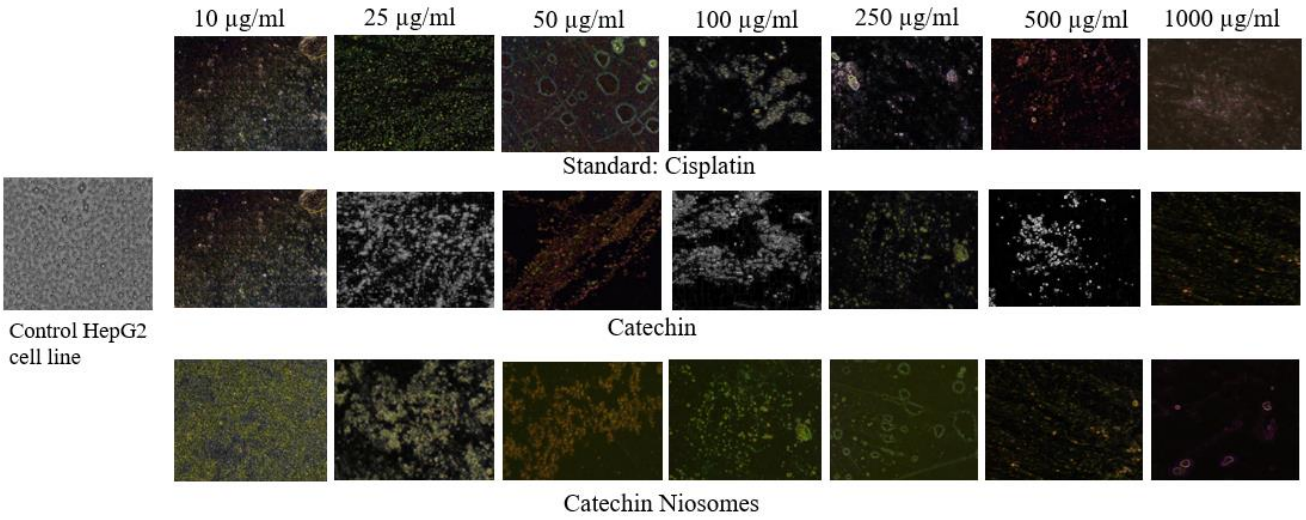

Figure 7: Microscopic changes in HepG2 cell at different concentration of cisplatin, free catechin and catechin niosomes

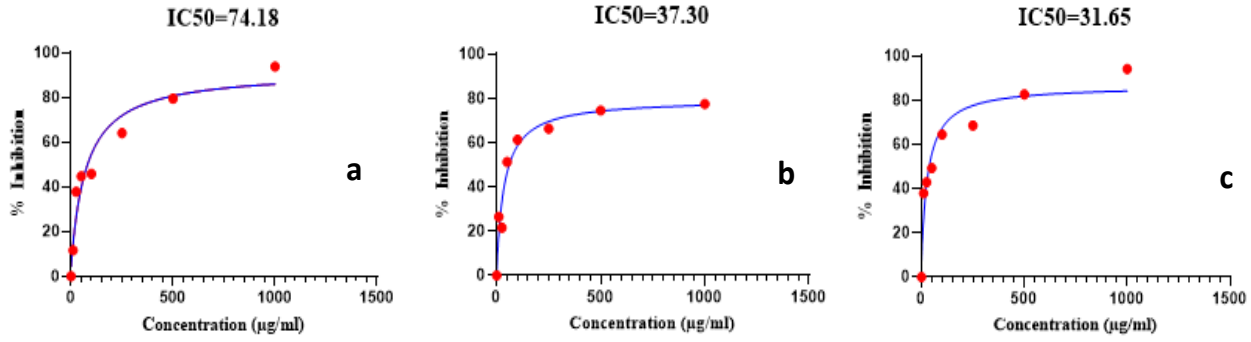

Figure 8: % Inhibition as a function of concentration of a) cisplatin b) Catechin c) Catechin niosomes

band extending from 3500 to 3200 cm^{-1} in the spectra. The C=C group of the benzene ring in catechin was determined to be the source of absorption peak at 1630.52 cm^{-1}. It was catechin C-O stretching vibration that caused absorption peaks at 1285.32 cm^{-1} and 1147.84 cm^{-1}. Cholesterol shows characteristics -OH peak at 3423.99 cm-1. Span 60 shows acetic anhydride peak at 1742.37 cm^{-1} and -OH peak at 3322.75 cm^{-1}. All peaks of catechin, span 60 and cholesterol were reappeared in FTIR of physical mixture. Hence, it was confirmed that all excipients and drug was confirmed that all excipients and drug was compatible with each other. The absence of new peaks confirms that there were no chemical interactions among catechin and other excipients. In FTIR spectrum of catechin niosomes there was shift in wavenumber from physical mixture related to OH stretch from 3401cm-1 to 3547cm^{-1}, CH stretch and aromatic from 2934cm^{-1} to 2973 cm^{-1}, C=O ester from 1738cm^{-1} to 1742cm^{-1}, C = C stretch in alkenes from 1614cm^{-1} to 1655cm^{-1} and C = C aromatics from 1467cm^{-1} to 1473cm^{-1}. This proved that final niosome structure included catechin and niosome vesicles. Since from above interpretation it can be conclude that catechin is encapsulated inside niosome vesicles[26].

SEM

From the SEM image of catechin loaded niosomes, it can be concluded that vesicles were well identified and presented in a nearly spherical shape as shown in figure 5.

***In vitro* drug release**

The *in vitro* drug release from catechin was measured using Dialysis bag method in phosphate buffer pH 7.4. Release of catechin from catechin solution and catechin niosomes was found to be 97.42 ± 0.58 in 2 hrs and 86.89 ± 0.65% in 6 hrs respectively (Figure 6). Catechin niosomes shows a sustained release compare to pure catechin it might be due to encapsulation of catechin in niosomal vesicle. The gradual diffusion of catechin from the niosomal vesicles, might be influenced by the vesicular composition and the encapsulation efficiency of the niosomes[27,28]. **Release Kinetic Behaviour**

In order to assess data on the release *in vitro*, several kinetic models were employed. kinetic models of zero and first-order, Higuchi, and Korsmeyer-Peppas were used to study the release of drugs from niosomes[29]. So that we can figure out how the niosomal formulation releases its medication[30]. As can be seen from result in Table 3 vitro release of catechin from niosomes was best explained by zero order kinetic model for selected formulation owing to highest value[31,32] of r^2.

Cell cytotoxicity study (MTT assay)

Various concentrations of free catechin, catechin loaded niosomes, and conventional cisplatin were tested on HepG2 cells during 24 hours using the MTT assay[33,34]. The concentrations ranged from 10 to 1000 µg/ml. The results demonstrate that compared to free catechin and cisplatin, the cell cytotoxicity activity of niosomes loaded with catechin is substantially higher[35,36]. The half-life of catechin niosomes was 31.65 µg/mL, whereas for free catechin cisplatin it rose to 37.30 µg/mL and 74.18 µg/mL, respectively. Niosomes laden with catechin have improved cellular absorption and increased *In vitro* anticancer activity, which in turn increases their cytotoxicity[37,38]. These conclusions are in agreement with optical microscopy studies that examined cell proliferation on samples treated with cisplatin, free catechin, and niosomes laden with catechin[39,40] (Figure 7).

Catechin niosomes having a lower IC_{50} values than free catechin and standard cisplatin suggests that catechin niosomes are a promising delivery system for cancer therapy, potentially offering improved efficacy and reduced toxicity compare to free catechin and conventional chemotherapeutics like cisplatin[41](Figure 8).

CONCLUSION

Catechin as an efficient anticancer agent loaded into niosomes and its physicochemical characteristics were evaluated. Morphology of resulting niosomal formulation was spherical. Entrapment efficiency of catechin was 88.44% and its release behaviours from niosomes showed a sustained release compare to free catechin.MTT assay provide that catechin loaded niosomes has a significant anticancer effect on HepG2 cancer cell line as compared to free catechin and cisplatin. Thus, nanoniosomes loaded with catechin show promise as a vehicle for the regulated, prolonged, and effective administration of catechin.

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