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Research Article

Anticancer Activity of Curcumin Loaded Beta Carotene Nanoparticles in Cancer Cell Lines

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Abstract

Anticancer properties of curcumin are studied by many groups in the past and they suggest that the activity is poor because of the poor solubility and bioavailability of curcumin. Research also suggested that the nanoparticles can enhance the bioavailability of curcumin and thus enhance the anticancer activity. However, all the studies were done with nanoparticle matrices like poly lactide co glycolic acid, polycaprolactone, or other popularly known polymers. Solid lipid nanoparticles were also prepared to enhance the bioavailability. In our study, we focused on hybrid nanoparticles that are in between solid lipid nanoparticles and polymeric nanoparticles. We used beta-carotene as a matrix material to encapsulate curcumin. Further, we studied the anticancer activity in IMR 32 cells (Neuro Blastoma cells), MCF 7 cells (Breast cancer cells), HEK 293 cells (Human embryonic kidney cells). The curcumin-loaded beta-carotene nanoparticles showed potent activity compared to curcumin alone in all the cell lines. Thus, it can be concluded that the curcumin-loaded beta-carotene nanoparticles are a good option for studying the benefits of curcumin in cancer.

Keywords: beta carotene; cell culture studies; curcumin; nanoparticles

Introduction

Curcumin, a natural compound found in turmeric, has been the subject of scientific research for its potential health benefits, including its effects on cancer. While more studies are needed to fully understand its mechanisms and efficacy, some potential benefits of curcumin in cancer are anti-inflammatory, antioxidant, anticancer properties, angiogenesis inhibition, etc. Studies suggest it has strong anti-inflammatory properties, which can help reduce inflammation in the body [1]. Chronic inflammation is linked to the development and progression of cancer, and curcumin may play a role in inhibiting pro-inflammatory pathways. Curcumin is a potent antioxidant, meaning it can neutralize free radicals and reduce oxidative stress. Free radicals can damage cells and DNA, leading to cancer development, and the antioxidant properties of curcumin may help prevent or slow down cancer progression. Anti-cancer activity: Curcumin has demonstrated anti-cancer effects in preclinical studies, primarily through its ability to inhibit the growth and spread of cancer cells and induce apoptosis (programmed cell death) in cancer cells [3,4]. It may also interfere with various molecular signaling pathways involved in cancer development and progression. Angiogenesis inhibition: Curcumin has been shown to inhibit angiogenesis, the process by which new blood vessels form to supply tumors with nutrients and oxygen. By limiting angiogenesis, curcumin may hinder tumor growth and metastasis. Enhancement of chemotherapy and radiation therapy: Some studies suggest that curcumin can sensitize cancer cells to chemotherapy and radiation, making cancer treatments more effective. It may also protect normal cells from the harmful effects of these treatments. Prevention and treatment of cancer-related side effects: Curcumin's anti-inflammatory properties may help alleviate some cancer-related symptoms, such as pain, fatigue, and loss of appetite. It may also mitigate treatment side effects and improve the overall quality of life for cancer patients [5-8].

While the potential benefits of curcumin in cancer are promising, it's essential to note that research is still ongoing, and curcumin is not a replacement for conventional cancer treatments. It should be used as a complementary approach alongside standard medical therapies. Additionally, curcumin's bioavailability is limited, meaning it may not be efficiently absorbed by the body [9]. Researchers are exploring different formulations and delivery methods to improve their effectiveness. We have explored the nanoparticulate approach to the improvement in bioavailability and thus its anticancer property.

Materials and methods

Curcumin and beta carotene were purchased from Yucca Enterprises, India, and Hefei TNJ Chemical Industries Co., Ltd, China, respectively. Ethyl acetate, EDTA, sucrose, mannitol, dextrose, trehalose, and polyvinyl alcohol were procured from Sigma-Aldrich. Purified reverse osmosis water was used for all the experiments.

Analytical method to estimate curcumin by UV-Visible spectrophotometer

To determine the wavelength at which curcumin demonstrates its highest level of absorption (λmax), a stock solution of 100 µg/mL was prepared by diluting 10 mg of curcumin in 100 mL of methanol. This solution was then analyzed using a spectrophotometer within the wavelength range of 800 to 400 nm, with methanol serving as the blank. The absorption curve revealed that the unique absorption maxima of the drug occurred at approximately 421 nm.

To create the calibration curve, a series of serial dilutions were performed starting from the stock solution of 100 µg/mL. The standards used for these dilutions ranged from 1 to 6 µg/mL.

Preparation of curcumin-loaded beta-carotene nanoparticles

The emulsion-diffusion-evaporation method was adapted to produce nanoparticles (NPs) of beta carotene. Beta carotene was dissolved in ethyl acetate due to its highly favorable solubility in this solvent, which is crucial for emulsification-based NP development. To prepare the NPs, the required amount of beta carotene was first dissolved in dichloromethane or chloroform and then emulsified in a stabilizer solution containing poly(vinyl alcohol), a commonly used nanoparticle preparation agent. Subsequently, this emulsion was diluted with water to facilitate the diffusion of the organic solvent, leading to the formation of a nanosuspension [10].

In brief, a mixture of 2.5 mL of dichloromethane or chloroform was stirred at 1200 rpm for 30 minutes until curcumin, beta-carotene, and the polymer fully dissolved (as shown in Table 3). This organic phase was then slowly added drop-by-drop into an aqueous phase containing a stabilizer to form an emulsion. To minimize droplet size, the emulsion was homogenized using a Polytron PT4000 (Polytron Kinematica, Switzerland) after one hour of agitation. Afterward, a significant volume of water was added to the emulsion to dilute it, resulting in the diffusion of the organic solvent and nanoprecipitation. Curcumin loading of 10 mg and 20 mg were used for every 50 mg of beta-carotene, as indicated in Table 3.

Freeze drying of nanoparticles

For freeze-drying, an automated system (VirTis, AdVantage, USA) previously customized for curcumin nanoparticles (NPs) was utilized. The freeze-drying process maintained a condenser temperature of 60°C and a pressure of 200 Torr throughout each stage. The nanoparticle suspension (2 ml) was introduced into glass vials with a capacity of 5 ml. The freeze-drying technique was optimized to achieve NPs with excellent dispersibility. To obtain a free-flowing and easily dispersible powder, different cryoprotectants and their concentrations were evaluated. Specifically, mannitol, sucrose, and trehalose were used as cryoprotectants at a 5% concentration relative to the NPs. After freeze-drying, the samples were reconstituted in demineralized water, and a series of tests were conducted to assess their size, drug content, surface charge, and morphology.

Characterization of nanoparticles

Zetasizer nanoZS (Malvern, UK) and the Malvern PCS software version 6.20 were used to perform Photon Correlation Spectroscopy (PCS) measurements of particle sizeand polydispersity index. Zetasizer nanoZS was used to measure the zeta potential inelectrophoretic light scattering (ELS) mode. The device was kept at 25ºC while the data was being collected. The stated values were the average of three different measurements.

Loading capacity and percentage yield

The weight of the freshly created formulation equal to one unit dose was taken and diluted with chloroform up to 10 mL. The resulting suspension was vortexed for one hour and then centrifuged at 6,000 rpm for 45 minutes (5804R, Eppendorf, Germany). A 0.2-m filter was used to separate and filter the supernatant. The filtrate was diluted with chloroform before being subjected to a UV spectrophotometer analysis at 421nm (Systronics 2203 Smart, India). The analysis was conducted using a UV calibration curve that was created at the same wavelength. The formulations made without curcumin were handled similarly and served as a control for the measurements. The experiment was performed 3 times using various preparations. The formula was used to determine encapsulation effectiveness.

$$\text{Encapsulation Efficiency} = \frac{weight\ of\ curcumin\ in\ nanoparticlesn}{weight\ of\ curcumin\ added}$$

In vitro cell culture studies

In vitro, cytotoxicity assays are commonly used for the evaluation of biomaterials / medical devices. “Cytotoxicity can be determined by evaluation of cell morphology, cell damage, and cell growth or by measurement of cellular activity. 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide” (MTT) assay is one of the method strongly recommended byISO 10993-5 [11].

IMR 32 cells (Neuro Blastoma cells), MCF 7 cells (Breast cancer cells), HEK 293 cells (Human embryonic kidney cells), were all kept as monolayer cultures in a 25 sq. cm flask using DMEM media that was augmented with 2% FCS and some antibiotics such as kanamycin, Penicillin, and Streptomycin. The cells were cultivated in an incubator containing 5% CO2 and maintained at 37ºC until they reached a confluence of 70%. The cells were then washed with 1x PBS, and 200 µL of 0.2% (Trypsin-EDTA) solution was added. The mixture was then allowed to incubate at room temperature for 5 minutes. The cells were then suspended in 5 ml of complete media, and 100 µL was subsequently transferred to 96 well ELISA plates. 48 hours were allotted for the growth of the cells (Table 1).

After removing the old medium from the ELISA plates containing the IMR 32 cells, MCF 7cells, and HEK 293 cells and adding 100 µL of the fresh medium, the plates were examined under a microscope for signs of contamination. Cells were treated with seven distinct doses of undiluted test materials in triplicate, each time. Place the plate in an incubator with a humidity of 5% and 37 ºC for a whole day. The plates were frequently removed for microscopic analysis to look for visible indicators of toxicity in response to the test ingredients and the controls. These visual signs include a change in the size or appearance of cellular components or a disturbance in their configuration. After a treatment of 24 hours,20 µL of MTT solution (10 mg per mL of stock) was added to each well, and the plates were placed in an incubator at 37ºC for four hours. The medium was discarded, and the formazan crystal was dissolved in an isopropanol solution that included 0.04N HCl and observed an OD of 570 nm.

All the samples were dissolved in 100 % DMSO. Concentrations of curcumin or beta carotene or nanoparticles ranging from 12.5 µg to 150

µg were utilized. These amounts correspond to 25, 50, 75, 100, and 125 respectively have been used for the MTT assay. The study was conducted in a single assay with a triplicate well.

$$\% \text{ of cell viability } = \frac{(\text{OD of Experiment})}{(\text{OD of control}) \text{ x}100}$$

Formulation	1	2	3	4	5	6	7
Curcumin (µg)	12.5	25	50	75	100	125	150
Beta-carotene (µg)	12.5	25	50	75	100	125	150
Nanoparticles (µg)	12.5	25	50	75	100	125	150
Control (DMSO) (µl)	10	10	10	10	10	10	10

Table 1: MTT assay concentrations

Results and discussion

Analytical method

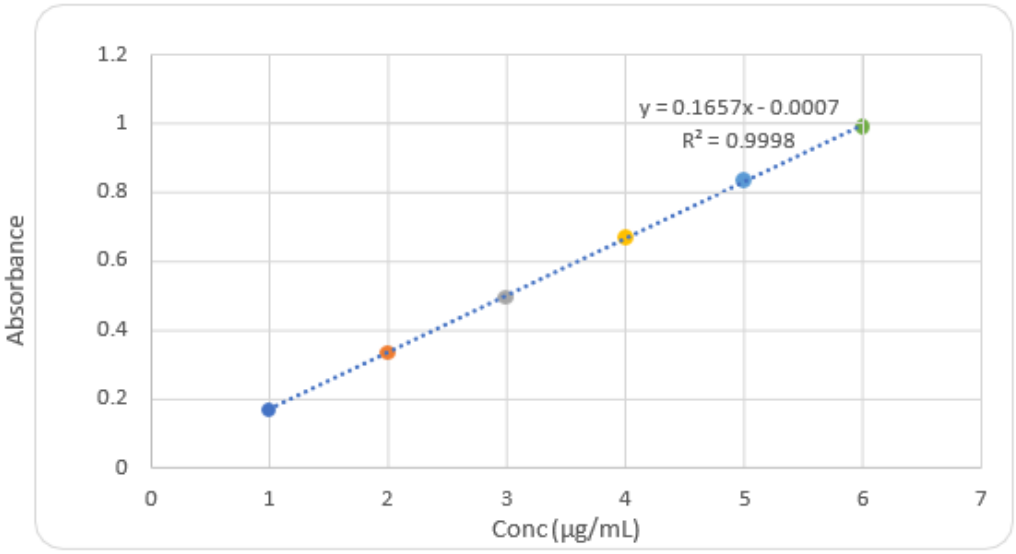

Figure 1: UV Calibration curve of curcumin

To determine the wavelength at which curcumin exhibits the highest level of absorption, known as λ max, a stock solution of 100 µg/ml was made by diluting 10 mg of the substance in 100 ml of methanol. Using a spectrophotometer, this solution was analyzed within the wavelength range of 800 to 400 nm against methanol, which served as a blank. The drug's unique absorption maxima were found to occur around 421 nm along the absorption curve. The calibration curve of curcumin was plotted in methanol at 421nm and a straight line was obtained. The equation obtained was y= 0.165x-0.0007 and the value of R^2 was 0.999. The absorbance values are shown in Table 5 and the standard graph is shown in Figure 1.

Development of blank and curcumin-loaded beta carotene nanoparticles

All the developed nanoparticles showed particle sizes less than 200 nm (Table 2). Blanknanoparticles showed lower sizes than the drug-loaded nanoparticles. Nanoparticles showed almost similar size to the blank nanoparticles suggesting the compact nature of the beta carotene nanoparticles. The zeta potential is negative in all cases and the entrapment efficiency of curcumin resulted to be over 90%.

Freeze drying of developed nanoparticles

The freeze-dried nanoparticles retained properties similar to the initial nanoparticles with trehalose. The size seems to be slightly increased and the zeta potential decreased slightly after the freeze-drying process (Table 2) (Figure 1). The particles seem to be spherical and the size distribution seemed to be good in all cases. In particles prepared with beta carotene, there is a small peak of particles indicating some of the particles are very small in comparison to the other particles. This could be due to the lipid agglomeration that might have happened during the preparation process but not the freeze-drying process. The freeze-dried particles retained almost all the characteristics of the initial nanoparticles and are easily redispersible. For all the remaining studies freeze-dried nanoparticles were used. Before using them, they were kept at -80 degrees.

Formulation	Particle size		Zeta potential	
	Before	After	Before	After
Blank	100 ± 3.2	110 ± 5.2	-21.3 ± 2.8	-17.2 ± 1.7
Nanoparticles	116.2±5.7	126.5 ± 6.9	-28.5±3.4	-18.9 ± 2.5

Table 2: Characteristics of nanoparticles

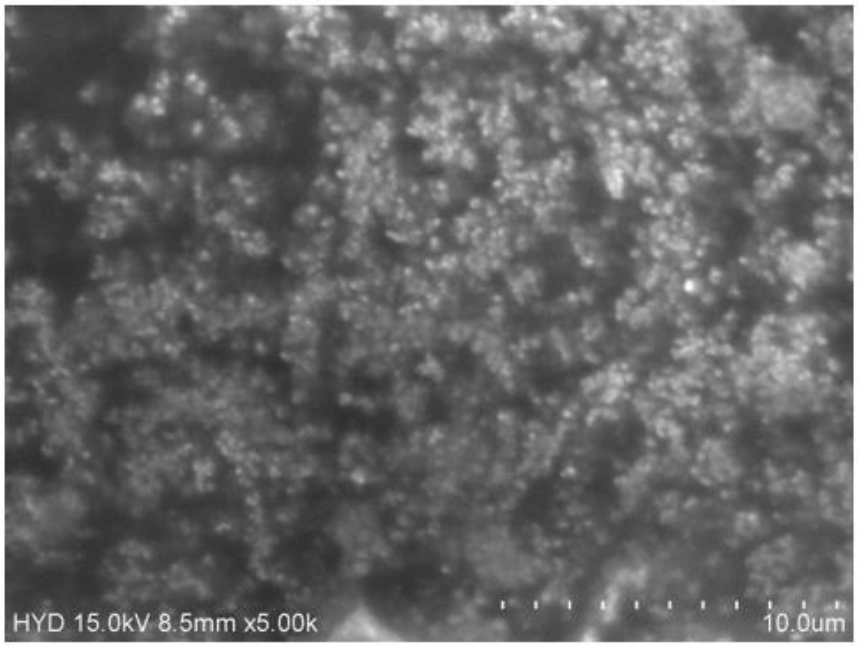

Figure 2: SEM image of nanoparticles after freeze-drying

In vitro cell culture studies

Group	12.5 µg	25 µg	50 µg	75 µg	100 µg	125 µg	150 µg
curcumin-IMR CELLS	94%	83.33%	74.92%	**53%**	36.92%	24.66%	17.16%
beta carotene-IMR CELLS	94.48%	83.35%	72.39%	62.30%	**50.86%**	34.27%	20.04%
nanoparticles-IMR CELLS	84.39%	74.74%	**51.27%**	41.40%	35.67%	23.96%	11.60%
curcuminMCF-7 CELLS	94.71%	86.58%	67.88%	54.83%	**41.60%**	25.87%	16.78%
beta carotene-MCF-7 CELLS	92.73%	83.91%	71.57%	64.08%	**49.67%**	28.42%	13.76%
nanoparticles MCF-7 CELLS	85.42%	73.07%	48.53%	35.66%	24.54%	18.93%	11.15%
curcumin-HEK 293 CELLS	95.41%	88.89%	79.99%	68.38%	58.76%	**49.79%**	32.67%
beta carotene HEK 293 CELLS	96.03%	88.09%	76.33%	68.98%	58.62%	**49.60%**	35.21%
nanoparticles HEK 293 CELLS	96.20%	88.94%	79.33%	66.33%	56.97%	**49.89%**	34.00%

Table 3: Cell Viability Percentage in IMR32, MCF-7, HEK293 Cells

Cytotoxicity assay results show 53 % cell viability was observed in 75 µg curcumin-treated cells. 50.86 % cell viability was observed in 100 µg Beta carotene treated cells and 51.27% cell viability was observed in 50µg nanoparticles treated cells. Based on the MTT assay in IMR 32 cells the IC-50 value is 75 µg for curcumin, Beta Carotene 100 µg, and 50 µg for nanoparticles (Table 3).

Cytotoxicity assay results show 54.83 % cell viability was observed in 75 µg curcumin-treated cells. 59.67 % cell viability was observed in 100 µg Beta carotene treated cells. 45.53% cell viability was observed in 50µg nanoparticle-treated cells. Based on the MTT assay results on MCF 7 cells the IC-50 value for curcumin is 75 µg, Beta Carotene 100 µg for, and 50µg for nanoparticles (Table 3).

Cytotoxicity assay results showed around 50% cell viability was observed in 125 µg curcumin, 125 µg Beta carotene, and 125 µg nanoparticles treated cells. Based on the MTT assay results on MCF 7 cells the IC-50 value for curcumin is 125 µg, Beta Carotene and nanoparticles (Table 3).

From the in vitro cell culture studies, it can be understood that the nanoparticles are more potent than the curcumin suspension. In two of the studied three cell lines the nanoparticles showed enhanced activity probably due to the sustained release of the drug for prolonged periods or protecting the drug from degradation or location-specific drug delivery to tumor cell lines. The curcumin suspension in other cell lime showed a similar effect concerning the nanoparticles. The reason could be hard to predict. One possibility is the permeability of the cells for the nanoparticles could be lower than the two other cell lines studied. In any case, nanoparticles proved to be efficient delivery agents in vitro to treat cancer cell lines. Literature reports also suggest that curcumin in advanced drug delivery systems is better in eliciting potent anticancer activity. [3,4,12]

Conclusion

The use of nanoparticles as drug delivery systems has gained significant attention in cancer research. In our study, curcumin-loaded beta-carotene nanoparticles were successfully prepared and characterized. We found that these nanoparticles were less than 200nm and possess negative zeta potential. The curcumin nanoparticles exhibited cytotoxicity against cancer cell lines, indicating their potential to enhance the anticancer activity of the loaded curcumin. The ability of nanoparticles to improve drug delivery and increase the effectiveness of therapeutic agents is attributed to their small size, which allows them to penetrate tumor tissues more efficiently. Beta carotene, a natural compound found in various fruits and vegetables, has been studied for its potential as a carrier for drug

delivery systems. Its favorable characteristics, such as biocompatibility and stability, contribute to its effectiveness in improving the bioavailability of curcumin and the reason for the higher potency of nanoparticles in cytotoxicity to cancer cells.

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