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Preparation and Characterization of Curcumin Loaded Dextrin Sulfate- Chitosan Nanoparticles for Promoting Curcumin Anticancer Activity

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Abstract

Curcumin as a natural medicinal agent has been proved to kill cancer cells effectively. However, its biomedical applications have been hindered owing to its poor bioavailability. Many nanoparticulate systems have been introduced to overcome this problem. Among this types polymeric-based nanoparticles which exhibit unique properties allowing their use as a efficient drug carrier. Developing a polymeric- blend nanoparticles will offer a promising nanocarrier with excellent biocompatibility, biodegradability and low immunogencity.

In this study, curcumin nano-vehicle has been made up by combining dextren sulfate and chitosan (DSCSNPs). DSCSNPs have been characterized using different techniques. Transmission electron microscopy (TEM) which revealed the spherical, smooth surface of the nano-formulation. Dynamic light scattering (DLS) for measuring DSCSNPs hydrodynamic- diameter. Zeta potential measurements showed nanoparticles high stability. Fourier transform infrared spectroscopy (FTIR) confirmed successful combination between the two polymers and curcumin loading on naoparticles surface. Curcumin release profile out of DSCSNPs showed high drug release in tumor acidic microenvironment. In vitro cytotoxicity measurements demonstrated that curcumin loaded polymeric nanoparticles (DSCSNPs-Cur) have high therapeutic efficacy against colon (HCT-116) and breast (MCF-7) cancer cells compared with free curcumin. DSCSNPs as a combined biopolymers is an excellent candidate for improving curcumin bioavailability allowing its use as anticancer agent.

Keywords

Curcumin; Dextren sulfate chitosan nanoparticles; physicochemical characterization; drug release profile; in vitro cytotoxicity

1. Introduction

Curcumin is a nutraceutical bioactive compound that exhibit an activity against different cancer types including skin, oral cavity, mammary glands, digestive system organs (esophagus, colon, liver,...etc), [1-3]. Curcumin showed an inhibitory effect on cancer proliferation through its inclusion in many signaling pathways as well as molecular targets, [4]. Curcumin molecular targets involve the modulation of transcription and growth factors, apoptosis and cell proliferation genes and cytokines, [1,4]. Alongside with curcumin antiproliferation activity, curcumin can strongly induce apoptosis, arrest cell cycle, inhibit cancer invasion and angiogenesis, [4,5].

In the last decades many studies have reported the effectiveness of curcumin against different types of cancer cell lines, [6-10].

Despite of its potential activity against cancers, curcumin bioavailability is low owing to its: hydrophobic nature [11], lack of tissue absorption, rapid metabolism and systemic clearance [12,13]. Such reduced bioavailability of curcumin hinder its therapeutic efficacy [14].

Numerous nanoformulations have been developed and extensively used to overcome the problems of curcumin poor solubility, metabolic stability, prolonging circulation time, thus, enhancing curcumin bioavailability [15,16].

Among various nanoparticles types, polymeric based nanoparticles that have drawn an attention due to their unique properties. Polymeric nanoparticles are characterized by their biocompatibility and biodegradability specially natural fabricated ones. Polysaccharides are considered as a natural polymeric nanocarriers due to their elegant physical and biological properties [17]. Chitosan nanoparticles have been studied as curcumin nanocarrier, showing an enhancement in curcumin cellular uptake as well as bioavailability [17-21].

Incorporating and mixing different polymeric types have been introduced to improve the properties of polymeric based nanocarrier. Hybrid nanocarrier made from dextran sulphate and chitosan has gained an attention owing to exhibiting new properties over using each polymer individually.

The current study aims to prepare dextran sulphate- chitosan nanoparticles (DSCSNPs) as a promising nanocarrier that characterized by its high biocompatability and biodegradability, in addition to excellent stability in different pHs. DSCSNPs as a nanocarrier for curcumin is expected to greatly improve its bioavailability and enhance its therapeutic efficacy against cancer.

2. Materials and Methods

2.1 Materials

Curcumin (95% total curcuminoid content, 85% Curcumin) from turmeric rhizome, gold (III) chloride trihydrate ($HAuCl_4$. $3H_2O$, ≥99.9% trace metals basis), MTT Assay Kit (3-4, 5-Dimethylthiazol-2-yl 2, 5-diphenyltetrazolium bromide), medium molecular weight chitosan (degree of deacetylation, DD >75%) , ammonia hydroxide (NH_4OH, 28%), sodium salt of dextran sulphate (Mol wt >500 kDa), and tripolyphosphate (TPP) were purchased from Sigma-Aldrich (Germany). Solution of 2 M sodium hydroxide (NaOH), 2- ethoxyethanol ($C_2H_5OCH_2CH_2OH$) and absolute ethanol were purchased from Merck. Deionized (DI) water was used throughout the study.

2.2 Methods

2.2.1 Preparation of dextran sulphate-chitosan nanoparticles (DSCSNPs):

Chitosan solution (0.1 %) was prepared in acetic acid (1%). Dextran sulphate solution (0.1 %) was prepared using DI water. Upon mixing the two solutions with a ratio of 3:2 (V/V) under vigorous stirring for 15 min, DSCSNPs were formed instantaneously. The prepared DSCSNPs were separated from the suspension using a centrifuge for 50 min at 13,000 rpm. DSCSNPs washed twice in DI and re-suspended in 10 mM phosphate buffer solution (PBS).

2.2.2 Preparation of dextran sulphate - chitosan nanoparticles loaded curcumin (DSCSNPs-Cur):

Curcumin was dissolved in ethanol (2 mg/ 2 ml). Curcumin was loaded into the DSCSNPs by adding the curcumin solution to DSCSNPs solution.

To facilitate the evaporation of ethanol, DSCSNPs-Cur was stirred for 30 min in a warm water bath (40 ^{o}C). Then, curcumin loaded DSCSNPs was collected using centrifuge for 50 min at13,000 rpm. The pellets were

washed twice and re-dispersed in 10 mM PBS. The supernatant was carefully collected for encapsulation efficiency calculations. Free curcumin content in the supernatant was measured using spectrofluorometer at an excitation λ 420 nm and emission λ 550 nm and calculated from the calibration curve in order to determine the loading efficiency of curcumin in DSCSNPs.

2.2.3 Physicochemical characterization:

The morphology of dextran sulphate - chitosan nanoparticles (DSCSNPs-Cur) was characterized by Transmission electron microscope (TEM). An aqueous solution of uranyl acetate (1% w/v) was used as a staining agent; kept on the grid and placed on filter paper (to remove the excess solution) for 2 min at room temperature.

Dynamic light scattering was used to measure the size distribution of DSCSNPs-Cur. Furthermore, zeta potential of DSCSNPs were measured in DI water.

Also, FTIR spectra of dextran sulphate, chitosan, curcumin, DSCSNPs and DSCSNPs-Cur were recorded for lyophilized samples.

2.2.4 Loading efficiency and in vitro release profile of curcumin:

Curcumin loading efficiency:

After preparation of DSCSNPs-Cur, the collected free curcumin concentration in the supernatant was calculated at an excitation λ 420 nm and emission λ 550 nm using a spectrofluorometer (Shimadzu, RF5301pc, Japan). Then, the loading efficiency was calculated using the following formula:

Loading efficiency (%) =

$$\frac{Initial\ amount\ of\ Cur. - Supernatent\ free\ amount\ of\ Cur.}{Initial\ amount\ of\ Cur.}$$

Cumulative curcumin release profile:

In vitro release profile of curcumin release from DSCSNPs-Cur were measured the technique of diffusion dialysis bag [22].

A dialysis bags with a molecular-weight cut-off of 12,000 Da were used for the release experiment. Before starting the experiment the dialysis bags were kept in the release media overnight. Phosphate buffered saline (PBS) solutions with two different pHs: 7.4 and 5.5, simulating normal blood/tissues and tumor microenvironment, respectively were used as curcumin release media to. Curcumin loaded NPs (1 ml) was centrifuged and dispersed into the release media (1 ml), then put into the dialysis bags. The two dialysis bags (well sealed) were placed separately into two bottles containing the release media (50 ml). The two bottles were placed in water bathed shaker (100 rpm) at 37 $^{\circ}$C under light-sealed condition. In vitro curcumin release study was done for 7 days. At certain time points, curcumin concentration was quantified, by withdrawing an amount from the release media (3 ml), using spectrofluorometer. The withdrawn amount was returned to the original release media.

The concentrations of the released curcumin were calculated from the calibration curves at an excitation λ of 420 nm and emission λ of 550 nm.

The cumulative release (CR %) was quantified as follows:

$$CR(\%) = \frac{\text{Amount of curcumin released}}{\text{Total amount of curcumin}} \times 100$$

All experiments were carried out in triplicate and the standard deviation (SD) was calculated using origin 8.0 software.

2.2.5 In vitro cytotoxicity by MTT assay:

The anticancer activity of DSCSNPs-Cur and free curcumin (with different concentrations) against both colon (HCT-116) and breast (MCF-7) were analyzed by MTT assay and imaged under inverted microscopy.

3. Results and Discussion

DSCSNPs-Cur, will be used as nano-carrier for curcumin to enhance its solubility, stability and cellular uptake which in turn increase its therapeutic potential.

The combination of dextran sulfate (DS) and chitosan formed a promising nanohybrid system of poly-anion with a negatively charged sulfate groups. The prepared DSCSNPs exhibit high biocompatibility and biodegradability allowing its use in pharmaceutical products as an effective capsule for controlled drug release. Since DSCSNPs have good stability, so they do not need any stabilizing agent during its preparation. DSCSNPs have been developed to control drug release in delivering curcumin to tumor site [23].

3.1 Dextran sulphate-chitosan nanoparticles loaded curcumin (DSCSNPs-Cur**):**

3.1.1 Physicochemical characterization of DSCSNPs-Cur:

3.1.1 (a) Transmission electron microscope:

Nanoparticles size was affected by the of chitosan and dextran sulfate molecular weights greatly affect the size of the prepared formulation [24,25].

The surface morphology and approximate size of the prepared DSCSNPs was studied by TEM. Figure 3.1 a&b clearly showed the spherical morphology and the soomth surface of the prepared DSCSNPs with particale size of about 100 nm.

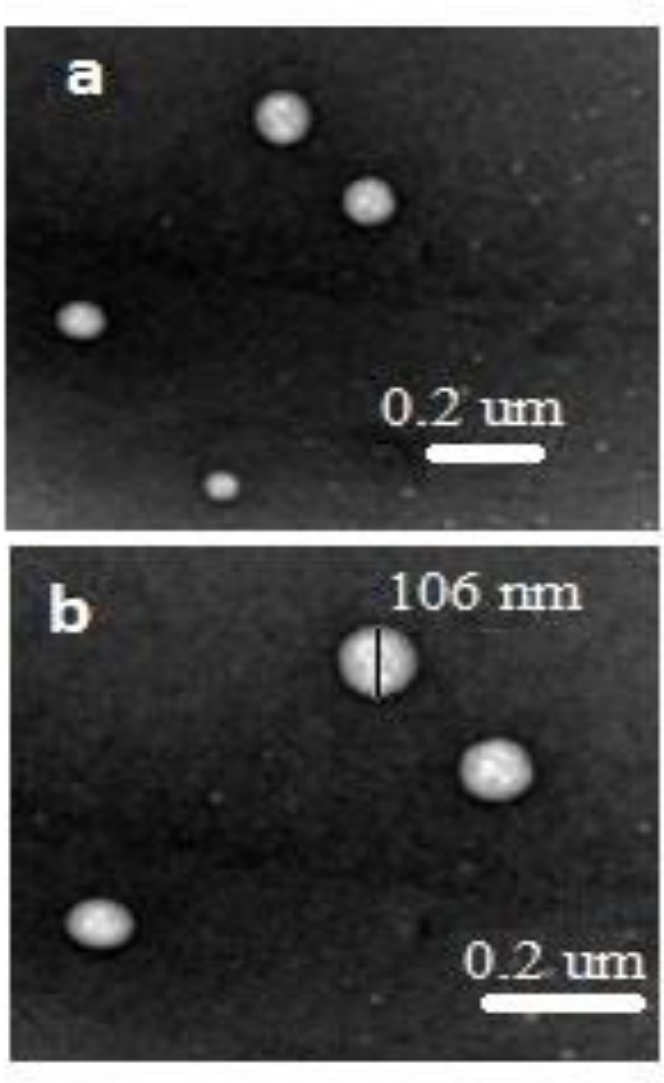

Figure (3.1 a&b) Transmission electron microscopy images of dextran sulphate - chitosan nanoparticles (DSCSNPs) stained with uranyl acetate.

3.3.1 (b) Zeta potential measurement:

The formation of dextran sulphate – chitosan nanoparticles (DS-ChNPs) is based on the electrostatic interaction between dextran sulphate and chitosan. Varying the ratio between the concentrations of the two-polymers can modulate the surface charge of nanoparticle[26]. The prepared DS-ChNPs have good stability and do not require any stabilizers.

Stability of both DS-ChNPs and DS-ChNPs@Cur were investigated by measuring zeta potential. The average zeta potential values for DS-ChNPs and DS-ChNPs@Cur were -26 ± 2.8 and -27.7± 2.92 respectively. This indicated the stability and monodispersity of the prepared formulation.The negative surface charge of curcumin loaded NPs (DS-ChNPs@Cur) is attributed to dextran sulphate high charge density.

Positively charged nanoparticles as well as hydrophobic surfaces are more susceptible to reticuloendothelial system (RES) clearance than the negatively charged ones. Since the prepared DS-ChNPs is negatively charged and fabricated from hydrophilic material so it can easily avert the clearance by RES as well as avoiding opsonization [27].

3.3.1 (c) Fourier transform infrared (FTIR) spectroscopy:

FTIR spectrum was used to study the interaction between the two polymers (dextran sulphate and chitosan) and curcumin.

The co-acervation reaction between the negatively charged dextran sulphate and the positively charged chitosan resulting in the immediate formation of DSCSNPs. This reaction based mainly on the amino-sulphate groups interaction.

FTIR spectrum of chitosan showed peak at 1653 cm^{-1} that is corresponding to the bending vibration of amide I . In the DSCSNPs spectrum, this wave number is shifted to 1639 cm^{-1} upon the formation of DSCSNPs. The sulphate group of dextran sulphate showed a broad vibrational band in the region of 1200–1280 cm^{-1} Figure (3.2). The interaction between the two groups was indicated by the change in peaks position in these regions. For sulphate stretching vibrations, the peak was shifted from 1275 to 1257 cm^{-1}. In the spectrum of dextran sulphate–chitosan nanoparticles loaded curcumin DSCSNPs-Cur Figure (3.2), the peak at 1510 cm^{-1} demonstrated –NH deformation. The peak of curcumin keto group was shifted from 1037 cm^{-1} to 1077 cm^{-1} after the formation of DSCSNPs-Cur [23].

Thus, FTIR data confirmed the formation of DSCSNPs which in turn induce curcumin loading. Moreover, this type of interaction control the release rate of curcumin out from the nanocarriers.

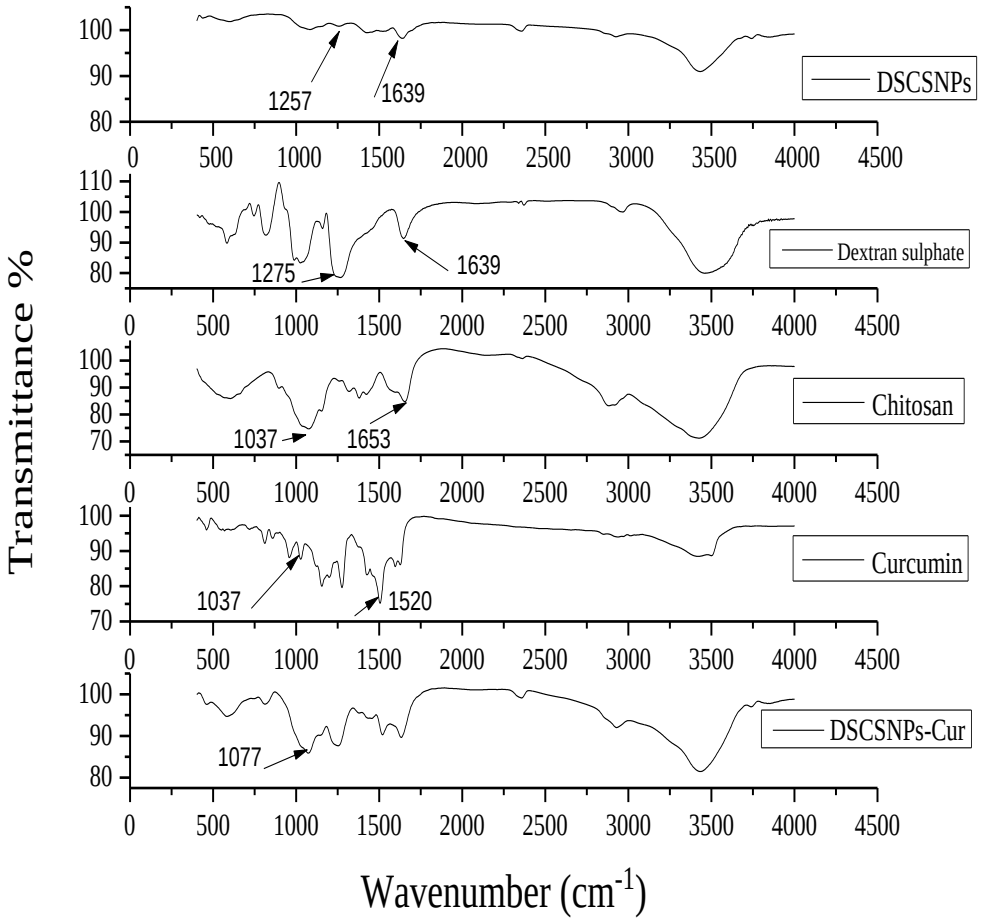

Figure (3.2) FTIR spectrum of dextran sulphate, chitosan, curcumin, dextran sulphate-chitosan nanoparticles (DSCSNPs), and curcumin loaded dextran sulphate-chitosan nanoparticles (DSCSNPs-Cur).

3.3.1 (d) Curcumin loading efficiency and in vitro release profile measurements:

The loading efficiency of curcumin on DSCSNPs was found to be ≈75%.

Curcumin release out of the nanoparticles was carried out at different pHs (pH 5.5 and 7.4) as shown in Figure (3.3).

Curcumin release profile showed a surge release in the first 2 hr followed by curcumin steep release over a period of one week. 62% of curcumin was released throughout this time period at pH 5.5, while it reached about 38 % of released curcumin at the end of the week at pH 7.4. In acidic environment, the high release rate of curcumin is attributed to the protonation of chitosan amine group [28]. The adsorbed curcumin on DSCSNPs surface and that entrapped near the nanocarrier surface might be the reason for the initial surge release in the first 2 hr. Furthermore, as polymers dissolution rate at the surface is high, curcumin release will be also high.

Since most of the tumors have poor blood vasculatures, cell metabolic end products will be accumulated in tumor microenvironment that generate acidic pH around tumor cells. Thus, the swelled DSCSNPs in tumor site would release its curcumin content more rapidly due to protonation of chitosan.

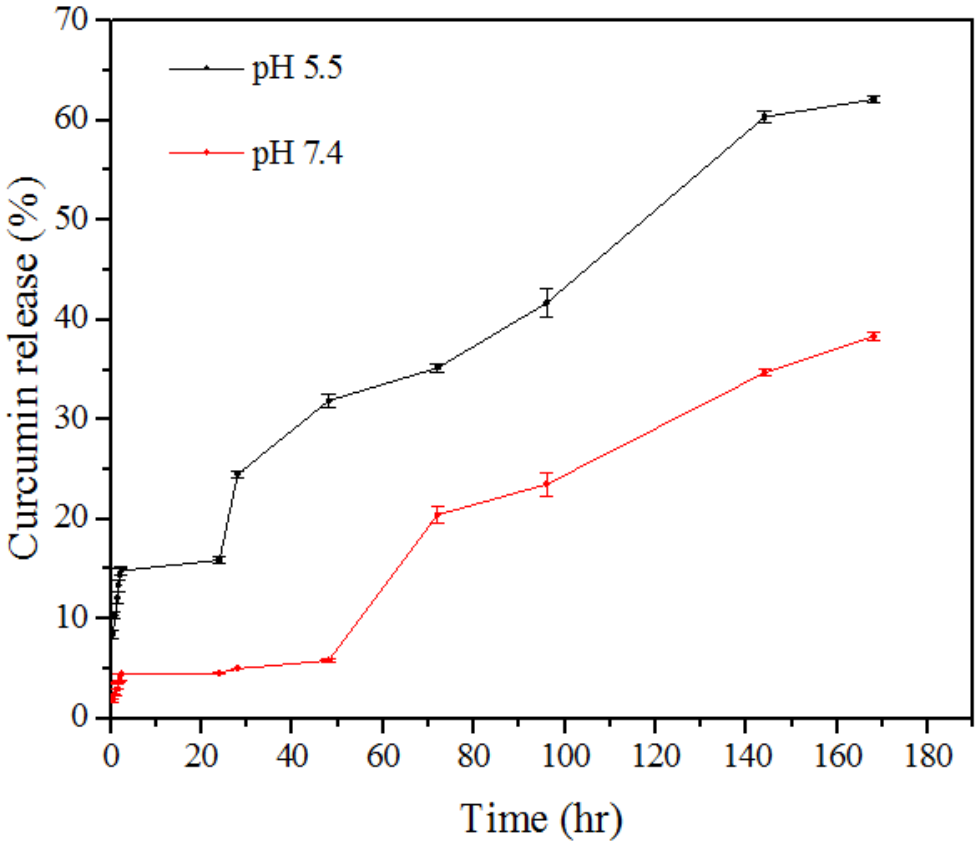

Figure (3.3) Drug release profile of DSCSNPs-Cur at pH 7.4 and 5.5 over a time period of one week.

3.3.2 In vitro anticancer activity of DSCSNPs-Cur:

3.3.2 (a) In vitro cytotoxicity by MTT assay:

The anticancer activity of DSCSNPs-Cur and free curcumin (different concentrations) against both colon (HCT-116) and breast (MCF-7) was measured by MTT assay Figure (3.4 a & b).

MTT results showed that DSCSNPs-Cur were more toxic against both cancer cell lines compared to cells treated with free curcumin. DSCSNPs-Cur treated cells showed cell viability of approximately 13 %, 24 hr post incubation (at higher curcumin concentration ~ 17 µg/ml). While in case of free curcumin, cell viability was ~ 50 % at the same curcumin concentration. For the lowest curcumin concentration (~ 6 µg /ml), both HCT-116 and MCF-7 cells treated with DSCSNPs-Cur showed cell viability of 50% compared to 73 % and 93% in HCT-116 and MCF-7 treated with free curcumin. The IC_{50} of DSCSNPs-Cur was found to be around 6.3 µg, while that of free curcumin was about 16 µg among all cancer cell lines. Previous studies demonstrated that bare DS-CSNPs showed almost no toxicity against cancer and normal cells [28]confirming the anticancer activity curcumin. Consequently, DSCSNPs could be used as an ideal carrier to deliver hydrophobic drugs, like curcumin, to cancer cells.

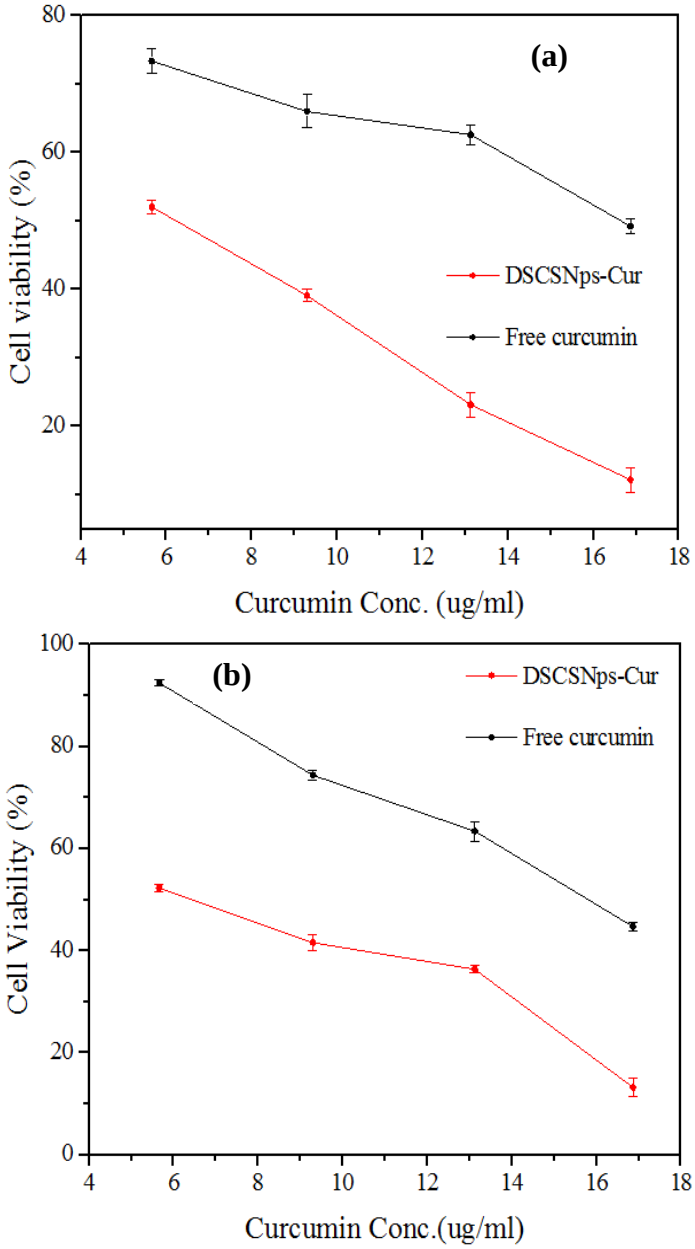

Figure (3.4) Cytotoxicity of DSCSNPs-Cur and free curcumin against (a) HCT-116 and (b) MCF-7 cancer cell lines, 24 hr post incubation.

3.3.2 (b) Microscopic examination for cancer cells:

The morphology of HCT-116 and MCF-7 cancer cells either treated with DSCSNPs-Cur and free curcumin or untreated (control) were examined using inverted microscopy. Figure (3.5) revealed the toxicity of the prepared DSCSNPs-Cur and free curcumin on both cancer cell lines morphology compared to untreated cells (control). Control HCT-116 and MCF-7 cancer cells possess a normal spindle shape Figure (3.5 a & d). Upon treatment with DSCSNPs-Cur, cells of HCT-116 and MCF-7 had lost their contact and normal spindle shape and appeared spherical indicating apoptosis induction, Figure (3.5 c & f) respectively. The observed dark aggregates in DSCSNPs-Cur treated cells might be attributed to the accumulation of the nanoparticles inside the cells and the release of curcumin in the cell cytoplasm leading to condensed cytoplasm (apoptotic features). In contrary, free curcumin treated HCT-116 and MCF-7 cells appeared intact with their normal spindle shape with no observed dark aggregates Figure (3.5 b&e). These results revealed that polymeric curcumin nanoparticles (dextran and chitosan) not only enhance the solubility of curcumin and its cellular uptake but also increase its anticancer effect due to the efficient internalization into cancer cells. Hence, the polymeric DSCSNPs-Cur can be a useful tool for transferring hydrophobic drug into cells.

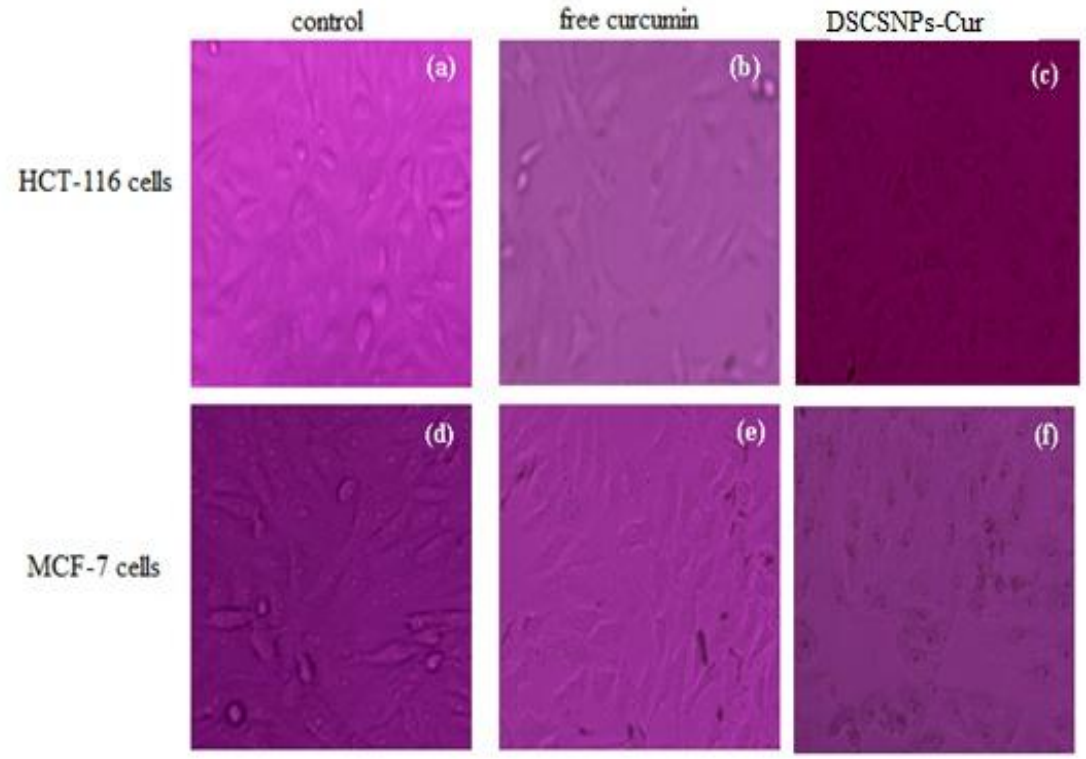

Figure (3.5) Inverted microscopy images of: (a) HCT-116 untreated cells (control), (b) HCT-116 cells incubated with free curcumin, (c) HCT-116 cells incubated with DSCSNPs-Cur, (d) MCF-7 untreated cells (control), (e) MCF-7 cells incubated with free curcumin, (f) MCF-7 cells incubated with DSCSNPs-Cur.

Conflict of interest

Authors have no conflict of interest.

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