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Development of Slow Release Berberine- Containing Nanoliposome for Delivery to Bone Cancer Cells Saos2

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Abstract

Background: Creating a new berberine liposome with high encapsulation efficiency and slow release formulation in the treatment of cancer is a new issue. Therefore, the aim of current study was to develop slow release berberine-containing nanoliposome for delivery to bone cancer cells Saos2.

Materials and Methods: In this experimental study, after synthesis nanoliposomal formulation, physical parameters, including size, zeta potential, and drug loading, in liposome were assessed using different techniques. Saos2 cell line was incubated in micro-plates containing Dulbecco's Modified Eagle's medium (DMEM) and FBS at 37˚C and 5% CO2. Cytotoxicity of nanoliposome was assessed using MTT assay. The release of drug from nanoliposome was assessed by dialysis method. $P< 0.05$ was assumed significant.

Results: The size of drug-free nanoliposome and drug nanoliposome (berberine-nanoliposome) was 112.1 and 114.9 nm, respectively. The zeta potential of drug-free nanoliposome and drug- nanoliposome was –16.1 and –1.9 mv, respectively. There was no significant difference between control and drug-free nanoliposome groups regarding viability ($p>0.05$). The viability of cells in different concentration of nanoliposome containing berberines in Saos2 cell line was significantly higher than that in free berberines ($p<0.05$). The release of berberine at temperature 37 º C and pH 7.4 showed that approximately 47% of the drug was released in the first 12 hours of study and then the slow release of drug was continued. IC50 value of free berberine and berberine containing nanoliposome was 137.3 and 52.2 µg / ml, respectively.

Conclusion: According to these findings, IC50 value of free berberine was 2.67 times more than berberine containing nanoliposome, indicating that nanoliposome containing berberine had more inhibition on growth of cancer cells than free berberine. In addition, the drug release was slow exposing the drug to the tumor for longer time at a lower dose and fewer injections, increasing the effect of the drug on cancer cells.

Keywords: Berberine, Bone cancer cells, Nanoliposome

Introduction

Bone cancer is a malignancy type expanding from cancerous tissue to bone and is observed in children and youths. According to findings, 75 % of patients with bone cancer are less than 25 years old (1-3). Children with cancer are at higher risk of developing bone cancer compared to any other type of cancer .Pain of bone cancer is very common and its treatment is difficult. Treatment of bone cancer usually involves administration of bisphosphonates and surgery of affected bones (4). In recent years, other treatment approaches have been provided for the treatment of bone cancer. At present, more attention has been focused on non-viral polymeric carriers, such as liposomes (5). Nanoliposome is a novel technique for encapsulation and delivery of bioactive molecules. On the other hand, nanoliposome is a bilayer polymeric vesicle which is used for loading various biological molecules. This molecule has the same structural, physical, and thermodynamic characteristic as liposome (5). In addition, nanoliposome improves the therapeutic index of established and novel drugs through changing drug absorption, decreasing metabolism, prolonging half-life, and decreasing toxicity (5, 6).

In addition, berberine hydrochloride is a component belonged to isoquinoline alkaloids and suggested for different various pharmacological functions (7). It is used for the treatment of intestinal infection, hyperlipidemia (8), diabetes (9), and arrhythmia. In addition, it inhibits the growth of sarcoma cell lines, cervical cancer cell lines, and hepatoma cell lines (H22) in mice (7). However, it has low bioavailability and solubility in the aqueous phase, which is considered as a major limitation (6-9). Studies have revealed that berberine can be loaded into liposomes, enhancing permeability and improving targeting to tumor tissue (9). Although some studies used berberine containing liposome to deliver drug to cells (7), creating a new berberine containing liposome with high encapsulation efficiency and slow release formulation in treatment of cancer is a novel issue. Therefore, since few studies have been conducted in this regard, the aim of the current study was to develop slow release nerberine-containing nanoliposome for delivery to bone cancer cells Saos2.

Materials and Methods

This experimental study was approved by Shahid Sadoughi University of Medical Sciences with number IR. SSU.MEDICINE.REC.1399.007 After synthesis nanoliposomal formulation, physical parameters, including size, zeta potential, and drug loading in liposome, were assessed using different techniques explained in the following.

Synthesis of nano-liposomal formulation and determination of physical parameters of berberine containing nano-liposome

In the first step, lipid phase containing cholesterol, phospholipid (soybean phosphatidylcholine) with molar ratio 80: 20, and hydrophobic drug were dissolved in organic solvent, and thin lipid film was prepared using a rotary. Then, phosphate buffer and hydrophilic drug were added to thin lipid film and was connected to a rotary evaporator with 150 rpm for 60 minutes at 50 º C. To decrease the size of liposome, resulting samples were sonicated. For preventing temperature raise during sonication, the balloon containing nano-liposomes was put in an ice under 60% amplitude for 30 minutes (ten seconds ON and fifteen seconds OFF). Then, the solution was passed through a filter with a diameter of 0.22 micrometers, and finally the process of separating was performed through the dialysis method for one night at a temperature of 4 º C.

In order to evaluate the release of the drug, the nanoparticles of the liposome containing the drug were placed in an environment simulated with the body, and the drug was released from the nanoliposome at certain intervals. In the next step, the specimens were assessed by Dynamic Light Scattering (DLS, USA) at room temperature.

For measuring the surface charge and zeta potential of nanoliposomes, zeta sizer (Brookhaven Instruments, USA) at 25˚C was used. In addition, morphology of nanoliposomes containing berberine was assessed by scanning electron microscope (SEM) (model EM3200, KYKY, China). Atomic force microscope (AFM) was used for taking microscopic picture of drug-free nanoliposomes and drug nanoliposome.

Determination of drug loading capacity

Standard concentrations of berberine (1 mg/ml) were prepared in isopropanol, and its absorption was read at 420 nm by spectrophotometer. Then, standard curve was obtained by using Microsoft Office Excel. In the next step, the nanoliposome containing berberine was dissolved in isopropanol with a certain dilution, and the absorption rate was read by spectrophotometer at 420 nm. Loading efficacy was calculated through Formula 1.

$$\%\ Loading\ efficiency = \frac{Total\ \ concentration\ of\ drug - \ free\ concentration\ of\ drug}{Total\ \ concentration\ of\ drug}$$

(Formula 1)

Determination of berberine release from nanoliposome

The release of berberine from nanoliposome was assessed by dialysis method at 37º C and pH 7.4 for 120 hours. In order to calculate the released drug, the dialysis medium was replaced at different times immediately with the same volume of fresh PBS. The absorbance of samples was read using spectrophotometers at 420 nm.

Assessment of cytotoxicity of nanoliposome

Human bone cell line (Saos2) was prepared from Institute Pasteur, Tehran. Cells were incubated in micro-plates containing DMEM (Dulbecco's Modified Eagle's medium) (Gibco, Germany), 10% FBS, 1% penicillin / streptomycin at 37˚C and 5% CO2. MTT assay was used to assess cytotoxicity in two time periods (48 and 72 hours after of treatment) with various concentrations of empty liposomes. After placing plates in incubator (24, 48 and 72 hours), the cells were washed with PBS, and then 20 µl of 5 mg/ml MTT diluted with PBS were added to each well. Then, plates were incubated for 4 hours to form formazan crystal. In the next step, internal solution of each well was removed and dimethyl sulfoxide (DMSO) (200 µl) added to dissolve the crystals. Then absorbance of resultant samples were measured through ELISA reader (Biotek Instruments, USA) at 570 nm and the cell viability was calculated as following.

$$\%\ Viable\ cells = \frac{Mean\ optical\ absorption\ in\ \ the\ test\ group - Average\ light\ absorbtion\ in\ culture\ medium}{Mean\ optical\ absorption\ in\ \ the\ control\ group - Average\ light\ absorbtion\ in\ culture\ medium}$$

Then, IC 50 value was obtained through graph pad software.

Statistical analysis

Data were entered to SPSS, version 19. Statistical tests, including Independent T test, were used for analysis of data. Statistically, $P<0.05$ was assumed significant.

Results

The efficiency of berberine loading in different formulation of nanoliposome is shown in Table I. As shown in Table I, berberine was successfully loaded in nanoparticles. In addition, about 85 % berberine was loaded into nonoliposome with size 114 nm. The size of drug-free nanoliposomes and drug containing nanoliposome is shown in Figures 1A and 1B. As shown in Figures 1A and 1B, the size of drug-free nanoliposomes and drug containing nanoliposomes was 112.1 and 114.9 nm, respectively. Figure 2 shows the intensity of zeta potential of drug-free nanoliposomes and drug containing nanoliposomes. As demonstrated in Figures 2A and 2B, the zeta potential of drug-free nanoliposomes and drug- containing nanoliposomes was – 16.1 and – 1.9 mv, respectively. Figure 3 shows SEM imaging of drug-free nanoliposome and drug containing nanoliposome. The SEM imaging of drug containing nanoliposome and drug free nanoliposome in Figure 3 was in accordance with size of nanoliposomes obtained with DLS. Figure 4 shows atomic force microscope (AFM) imaging of drug-free nanoliposomes and drug containing nanoliposome. The AFM imaging of drug free nanoliposomes and nanoliposomes containing berberine showed that the nanoliposomes were uniform and were not agglomerated. Figure 5 demonstrates the

release of nanoliposome containing berberine at 37º C and pH 7.4. The release of berberine at temperature of 37 º C and a pH of 7.4 showed that in the first 12 hours of study, approximately 47% of the drug was released and then the slow release of berberine containing nanoliposomes was continued (Figure 5). Figure 6 shows viability of drug free nanoliposomes in Saos2 cell line. The toxicity of nanoliposome with concentration of 1000 µg/ml on Saos2 cell line was less than 5 %, which indicated that drug free nanoliposome was not toxic. In addition, there was no significant difference between control and drug-free nanoliposome groups regarding viability ($p> 0.05$) (Figure 6).

Figure 7 shows the viability of berberine and different concentrations of nanoliposome containing berberine in Saos2 cell line. As shown in Figure 7, the viability of cells in the presence of different concentrations of nanoliposome containing berberines in Saos2 cell line was significantly lower than that in free berberines ($p<0.05$). Table II shows comparison of IC50 value(concentration that inhibits 50% cell growth) of free berberine and berberine containing nanoliposome. As shown in Table II, the half maximal inhibitory concentration of berberine containing nanoliposome was significantly lower than free berberine ($p<0.05$).

Table I : The efficiency of berberine loading in different formulation of nanoliposome

Formula	Primary Drug concentraion (mg/ml)	Cholesterol (molar ratio)	Phospholipid SPC (molar ratio)	Enteapment Efficiency (%)
1	1	30	70	70.1
2	1	20	80	84.9
3	1	10	90	72.3
4	1	5	95	38.7

Table II: Comparison of IC50 value of free berberine and berberine containing nanoliposome

Treatment type	IC50 value ($\mu g.ml^{-1}$)
Free berberine	137.3
Berberine containing nanoliposome	52.26

Table I:Frequency distribution of various subtypes of Hodgkin's lymphoma in patients

Subtype	Number	Percent
Mixed cellularity	19	55.9%
Nodular sclerosis	11	32.4%
Unclassified	1	2.9%
Lymphocyte rich	2	5.9%
Lymphocyte predominant	1	2.9%
Sum	34	100%

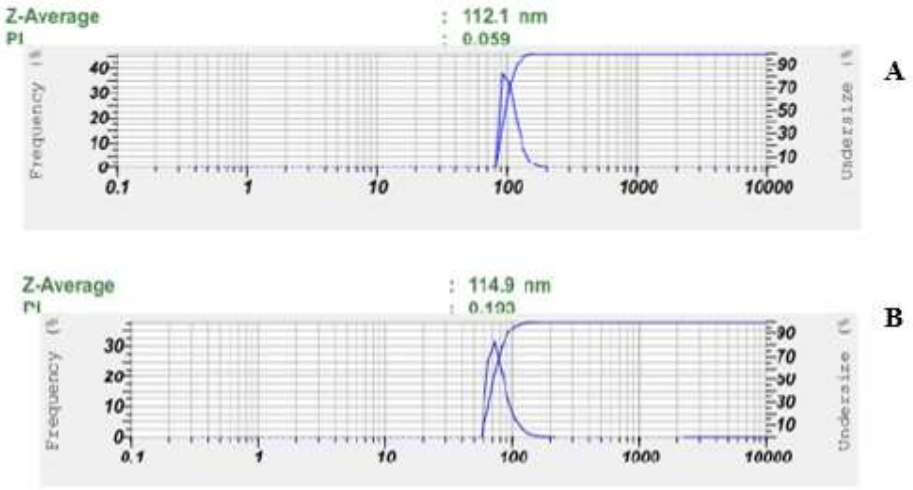

Figure 1. A: The size of drug-free nanoliposome, B : The size of drug containing nanoliposomes

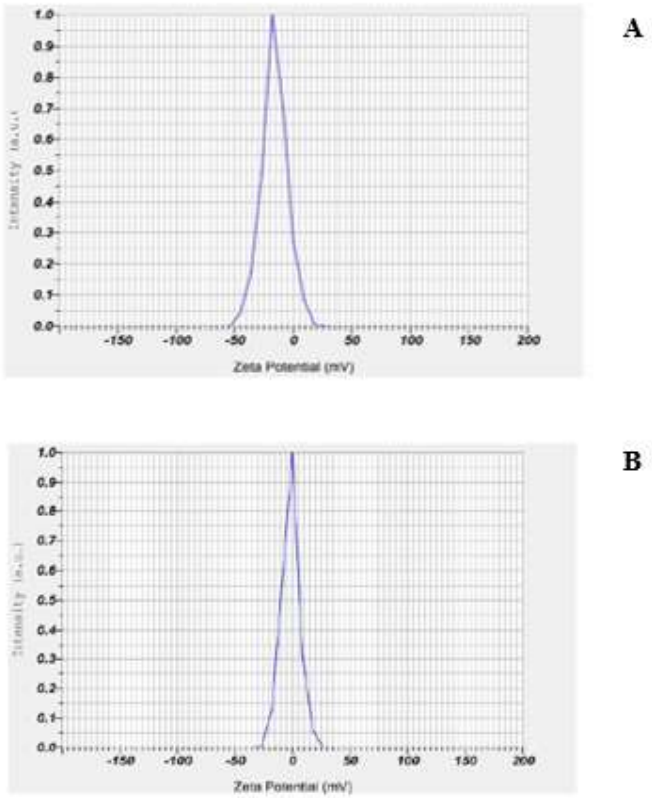

Figure 2. A: Intensity of zeta potential of drug-free nanoliposome, Figure B: Intensity of zeta potential of drug nanoliposomes

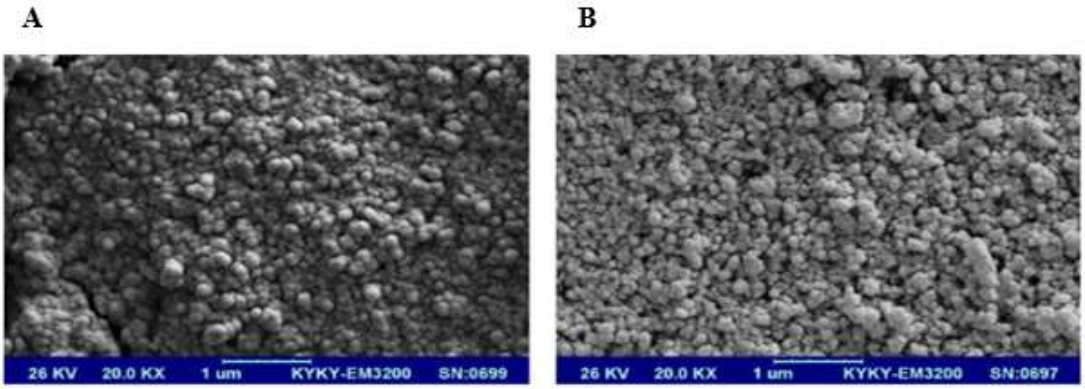

Figure 3. A: SEM imaging of drug containing nanliposome; B: SEM imaging of drug-free nanoliposome

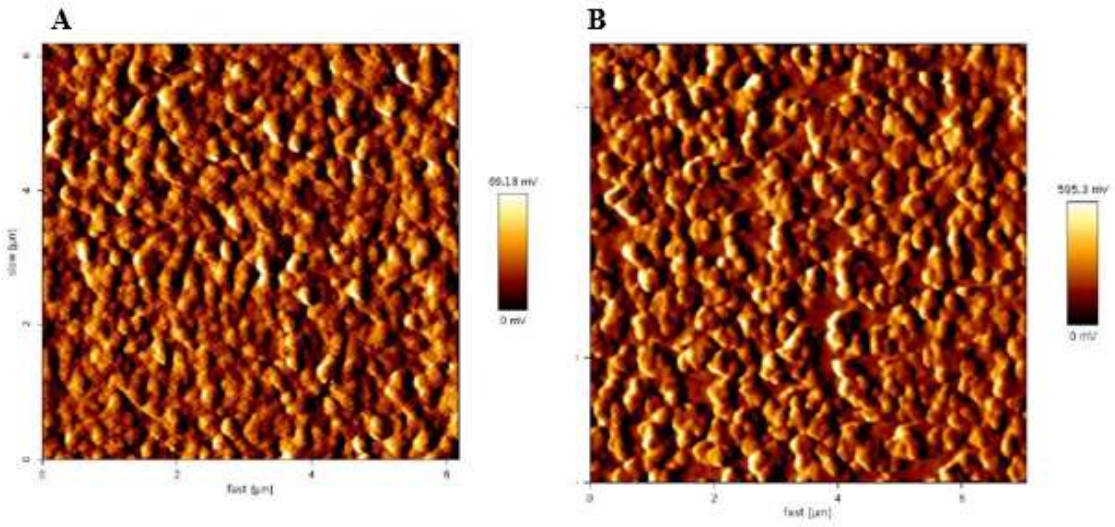

Figure 4. A: AFM imaging of drug containing nanoliposomes , B: AFM imaging of drug-free nanoliposomes

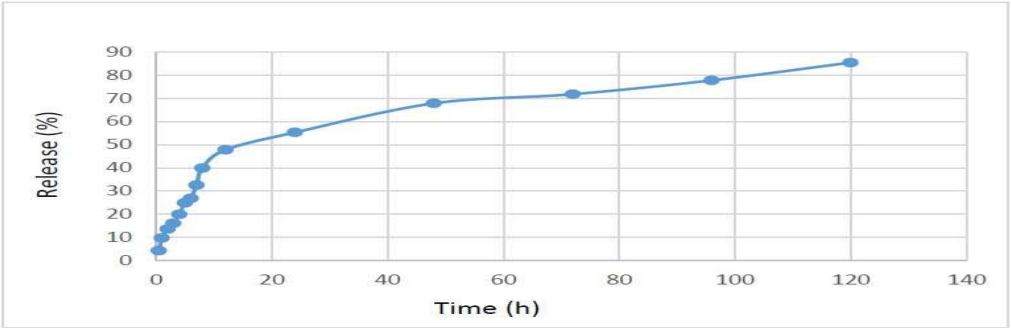

Figure 5: The release of nanoliposome containing berberine at 37º C and pH 7.4

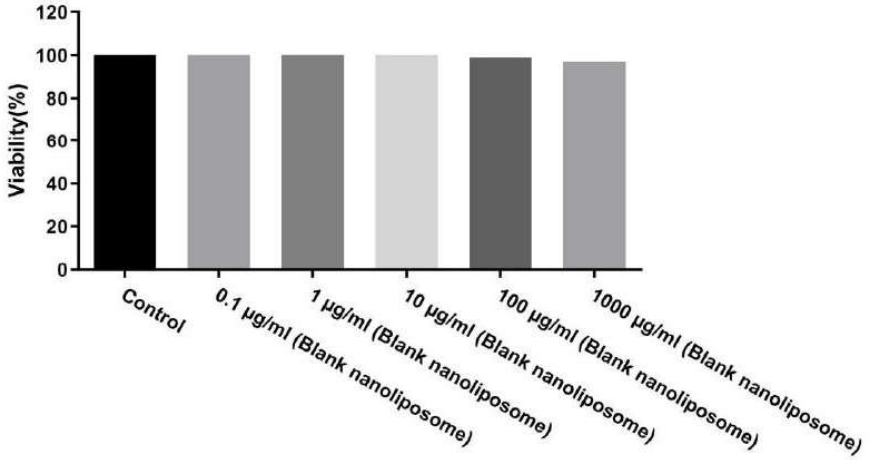

Figure 6. Viability of drug-free nanoliposomes in Saos2 cell line

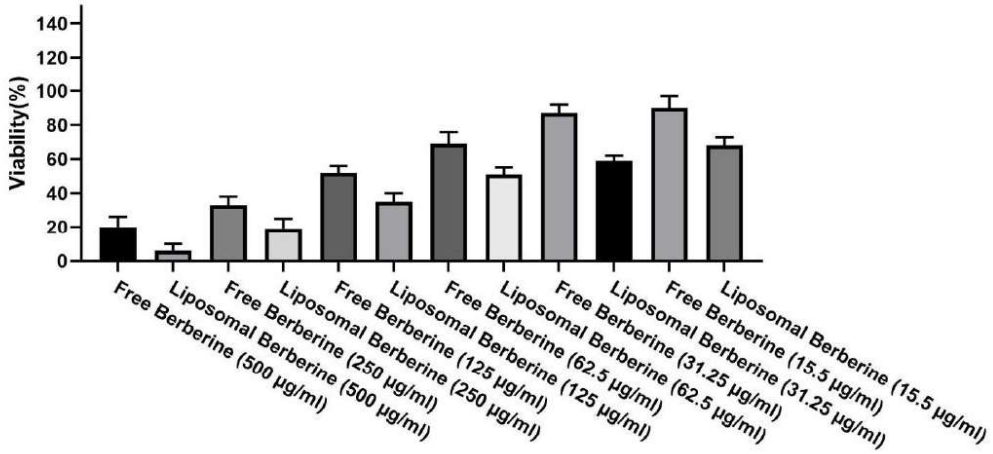

Figure 7. Viability of berberine and nanoliposome containing berberine in Saos2 cell line

Discussion

Cancer occurs when abnormal cells are divided in an uncontrolled way (10- 13). Osteosarcoma as a bone cancerous occurs less common in adolescents than children. Studies have revealed that drug delivery technologies can reduce toxic effects of the drug (5). Liposomes as a drug carrier is used in the treatment of cancer promote passive targeting for the cancer cells.

In the current study, we prepared nanoliposome containing berberine herbal drug in bone cancer cells Saos2, because liposome as a main approach in drug delivery improves the therapeutic effects and bioavailability of berberine. Our study was associated with many benefits compared to other studies. The main advantage of our study over other studies was that we used natural phospholipids) soybean phosphatidylcholine(which greatly reduced the side effects and cost of

construction compared to artificial phospholipids. Other advantage of current study was the use of minimum dosage of berberine (1 mg/ ml) and the maximum load in nanoliposome.

In addition, we used nanoliposome with size 100 nm for drug delivery to bone cancer cells Saos2. Selection of the appropriate size of nanoliposome leads to better penetration of nanoliposome to affected area, protecting them from macrophages. Malam et al., revealed that encapsulated drug with liposomes up to the size 4000 nm entered sites easily; however, there are limited by the endothelial wall of healthy tissues (14). Patel et al., also reported that delivery of biomaterials to special sites was directly influenced by size of particles (15). Mozaffari et al., also achieved similar results and reported that the delivery of these bioactive molecules to particular site of body and their release was directly influenced by particle size (6).

Nanoliposomes supply slow release of an encapsulated drug, leading to activity in the affected site and increased efficacy (15). Our findings in current study demonstrated slow release of nanoliposome containing berberine. Luo et al., evaluated the effect of liposome containing berberine hydrochloride on cancer cells and observed slow delivery of these components. They concluded that liposome containing berberine provided more stable liposomes and higher encapsulation efficiency (7). Therefore, this formula can provide a certain value for the development of liposome containing berberine.

Furthermore in our study, the viability of cells in the presence of nanoliposome containing berberine in Saos2 cell line was significantly lower than that in free berberine. Firouzabadi et al., assessed the effect of a novel liposomal nano-formulation on bone cancer and observed that encapsulated liposome compared to free drug had more toxicity, particularly in SaOs-2 cells (1). It seems that this was due to presence of positive surface charge of liposome. Cationic liposomes interact with negative charge of cell membranes, leading easy pass through membrane of cells. Other studies also assessed the toxicity of nano-liposomal artemisinin compared to standard drug on breast cancer cells and reported more cytotoxity of nano-liposomal form of artemisinin than artmisinin (16-18). Paria et al., evaluated the effect of nano-liposomal form of ginger and free ginger on MCF-7 cell line and observed that encapsulation of ginger was associated with more toxicity than ginger (19). According to these findings, it seems that nanoliposome containing drug has more toxicity than free drug; however, Lin et al., reported reduced toxicity of sulfatide-containing nanoliposomal doxorubicin compared to free doxorubicin (20).

Conclusion

According to these findings, IC50 value of free berberine was 2.67 times more than berberine containing nanoliposome, indicating that nanoliposome containing berberine had more inhibition on the growth of cancer cells than free berberine. In addition, the drug release was slow exposing the drug to the tumor for longer time at a lower dose and fewer injections, increasing the effect of the drug on cancer cells. Therefore, the use of nanoliposome containing berberine is proposed for delivery to bone cancer cells Saos2.

Conflict of interest

The authors declare no conflict of interest.

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