Nanomed. J. 9(3): 205-215, Summer 2022

RESEARCH PAPER

Synthesis, physicochemical characterization and pharmaceutical function of niosomal nanoparticles-encapsulated bioactive compound for osteosarcoma treatment

Mohamad Taebpour [1], Milad Akhlaghi [2], Shayesteh Shahriary [3], Saeedeh Hajihosseini [4], Fatemeh Haghiralsadat [1, 5]*, Fatemeh Oroojalian [6, 7]*, Davood Tofighi [8]

[1]Depatment of Medical Biotechnology, Faculty of Medicine, Shahid Sadoughi University of Medical sciences, Yazd, Iran
[2]Department of Clinical Biochemistry, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[3]Department of Medical Biotechnology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[4]Department of Mechanical Engineering, University of Tehran, Tehran, Iran
[5]Department of Advanced Medical Sciences and Technologies, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[6]Department of Advanced Technologies, School of Medicine, North Khorasan University of Medical Sciences, Bojnurd, Iran
[7]Natural Products and Medicinal Plants Research Center, North Khorasan University of Medical Sciences, Bojnurd, Iran
[8]Department of Psychology, University of New Mexico, Albuquerque, New Mexico, USA

ABSTRACT

Objective(s): The purpose of this study is to synthesizing and characterizing niosomes containing curcumin in order to delivery to bone cancer cells. Nano-carriers were synthesized using thin film method and curcumin was loaded into them by active hydration method.

Materials and Methods: The optimal formula was selected based on the encapsulation efficiency and release profile. Then the physicochemical properties of nanoparticles such as size and zeta potential, morphology and system-drug interaction were evaluated by using DLS, SEM, AFM and FTIR methods. Finally, the toxicity of nanosystems on bone cancer cell line MG-63 as resistant cells to treatment was examined by MTT assay.

Results: Niosomes containing curcumin with size of 90.8 nm, PDI of 0.236, zeta potential of -8.9 and encapsulation rate of 73.5 ± 1.8 have slow-release profile. The maximum release rate of the drug for this nano-carrier in healthy and cancerous within 72 hr was 60.12% and 64.35% respectively. IR and morphological investigations showed no chemical interaction between curcumin and nanocarrier and the particles are spherical in shape. The results of the MTT assay also showed that by encapsulating curcumin, its effect on bone cancer cells increased and the resistance of MG-63 cells to treatment decreased.

Conclusion: The results of this study showed that niosomes containing curcumin with appropriate physicochemical properties can improve the treatment process in bone cancer cells and also reduce the resistance of this cell to the drug and could be proposed as a new therapeutic strategy to help the treatment of osteosarcoma.

Keywords: *Bioactive compound, Curcumin, MG-63 cells, Niosomal nanoparticles, Osteosarcoma*

How to cite this article
Taebpour M, Akhlaghi M, Shahriary Sh, Hajihosseini S, Haghiralsadat F, Oroojalian F, Tofighi D. Synthesis, physicochemical characterization and pharmaceutical function of niosomal nanoparticles-encapsulated bioactive compound for osteosarcoma treatment. Nanomed J. 2022; 9(3): 205-215. DOI: 10.22038/NMJ.2022.63770.1665

INTRODUCTION

Osteosarcoma (OS) represents one of the most common primary malignancy of bone in children and adolescents (1). The incidence rate of OS is approximately 3.1 cases per million in United State, as an annual average of 900 new cases being diagnosed with osteosarcoma in America (2). OS is the third most common cancer in adolescents, with only lymphomas and brain tumors being more prevalent and is accounts for 3 to 5% of all cancers diagnosed in children (3). Although the primary tumor in patients with OS is often removed surgically, this malignancy is highly prone to metastasis, especially pulmonary metastasis (4).

* *Corresponding authors: Emails: fhaghirosadat@gmail.com; f.oroojalian@nkums.ac.ir; f.oroojalian@gmail.com.*
Note. This manuscript was submitted on February 15, 2022; approved on April 30, 2022

OS in the adolescent is due to intense linear bone growth. Tumors often develop in the long bones, especially in the most rapid growing areas (arms, legs, knees, and shoulders) (5). Abnormalities in signaling pathways of TP53, Rb, RecQ are risk factors in the pathogenesis of osteosarcoma so children with genetic syndromes such as Li-Fraumeni, hereditary retinoblastoma, Rothmund - Thomson, Bloom or Werner syndrome are more endangered for OS (1). Prior 1970, surgical tumor resection was the first and most important treatment for this malignancy but later, the advent of chemotherapy led to significant advances in the treatment of this malignancy (6). Adriamycin (7), Cisplatin (DDP), Methotrexate (MTX), Cyclophosphamide (CTX) and Epirubicin (EPI) are the main chemotherapy agents used to treat OS (8). However, resistance to chemotherapy, as well as its adverse side effects (9), highlights the need for a new therapeutic strategy to improve the treatment process in patients with OS.

Nowadays, many studies have proven that herbs could be useful in treatment of many diseases such as diabetes (10), atherosclerosis (11), neurological disorders(12), depression (11) and cancer (13). The use of medicinal plants is increasing sharply worldwide due to its reasonable price, availability and low side effects (14). Also, the natural origin of plants makes them better adapted to living organisms, including the human body, compared to chemical drugs (15). Curcumin [1,7-bis (4-hydroxy-3-methoxyphenyl) -1,6-heptadiene-3,5-dione] or diferuloylmethane is the main natural polyphenol in rhizome of Curcuma longa (turmeric). Curcumin is a bright yellow to orange crystalline compound that is used as an edible colorant (16). The use of turmeric as an herbal plant in traditional medicine of Asian countries has been prevalent from the past to the present (17). It is the principal curcuminoid of turmeric (Curcuma longa), a member of the ginger family, Zingiberaceae. It is sold as an herbal supplement, cosmetics ingredient, food flavoring, and food coloring. Chemically, curcumin is a diarylheptanoid, belonging to the group of curcuminoids, which are phenolic pigments responsible for the yellow color of turmeric. Curcumin has antioxidant. Today, proven that most of the therapeutic features of turmeric is because of presence of curcumin (18), anti-diabetic (18), anti-inflammatory (19), antimicrobial (20) and anti-tumor (21) properties and also improves the function of the gastrointestinal tract, liver, heart, eyes, nervous system, respiratory system, kidneys and reproductive system (22, 23). As mentioned, the use of herbal plants is increasing rapidly due to low side effects, but their use in conventional ways faces many challenges such as low impact on target tissue, impact on non-target tissue, low solubility and oxidation of some compounds in their composition (24) which this clarify the essential need for new strategies for their prescription, such as the use of nano-drug carriers like liposome and niosome, which solve these problems and improve their effectiveness. The nanostructure could change the specifications and the behavior of the natural drugs inside the body. It can maintain natural drugs from degeneracy and in delivering them to their target tissue. Also, lengthening of blood circulation time, augmentation of drug accumulation in the pathological tissues and reduction toxicity can organize the application of the nanostructure for many pharmaceutical uses (25-28).

Niosomes are formed from the self-assembly of non-ionic amphiphiles in aqueous media resulting in closed bilayer structures which first reported in the 1970 by researchers in the cosmetic industry. In many cases, cholesterol and its derivatives are used to make niosomes. Easy design, biocompatibility, flexibility and slow drug release are among advantages of using niosomes. Niosomes are capable of loading hydrophilic and hydrophobic compounds due to their unique structure. Hydrophilic drugs were trapped within the aqueous nucleus of niosomes and hydrophobic drugs were trapped between the membrane bilayers of niosomes. The chemical stability of the niosome compared to the liposome as an important drug nanocarrier is one of its advantages. Unlike liposomes, niosomes have a longer shelf life and are more resistant to oxidation and degradation. (7, 29-31).

The aim of this study is to synthesizing and characterizing niosomes containing curcumin in order to delivery to bone cancer cells and evaluating its effect as a new treatment strategy for using in the treatment of osteosarcoma so that new windows may be opened for the treatment of this disease.

MATERIALS AND METHODS

In this study, the curcumin, SPAN 60 and cholesterol prepared from (Sigma, USA) along with the solvents such as chloroform, methanol and isopropanol purchased from (Merck, Germany).

Determine the maximum absorption wavelength (λ_{max}) and plot standard curves

Spectrophotometry was used to determine the maximum absorption wavelength of curcumin. In this method, at first stock solution of curcumin with a concentration of 1 mg/mL in PBS (Sigma, USA) and isopropyl (Merck, Germany) was prepared. Then, by using stock solution, different concentrations of curcumin solution (500, 250, 125, 62.5, 30, 15 and 7.5 mg/mL) were prepared by using dilution methods in PBS and isopropyl solvents. After that, the absorption spectrum was read by a spectrophotometer (Epoch, USA) in the range of 200 to 800 nm for all concentrations. The wavelength at which the highest amount of adsorption occurred at all concentrations was considered as the maximum absorption wavelength. Then, by using the absorption spectrum obtained from different concentrations at the maximum absorption wavelength, the standard curve of curcumin was plotted in the PBS and isopropyl buffers and the normalized equation of line in PBS and isopropyl was calculated by using the standard curve. The experiments were repeated three times at this stage.

Preparation of nano-niosome formulation by thin-film hydration method

To synthesize and optimize nanoniosome containing curcumin and to achieve the best formulation concerning the drug entrapment and release rate, the following procedures were carried out:

a) The assessment of various molar ratios of SPAN60: cholesterol. b) Functionality tests: the assessment of nanoniosomes release pattern and determination of cytotoxicity of both curcumin-containing niosomes and free form of niosome.

Briefly, 0.003 g of curcumin and different mole ratios of cholesterol and span60 according to Table 1, were be solved in the chloroform which then evaporated under vacuum condition at 45˚C using a rotary evaporator (Heidolph, Germany). Dried film was hydrated with 5.5 ml of sterilized distilled water while rotating for 30 min at 55˚C to perform spherical vesicles. Niosome were thereafter reduced using both microtip probe sonicator (E–Chrome Tech Co, Taiwan) for 10 min and bath ultrasonic (Elmasonic S, Germany) at 45˚C for 1 hr. The vesicles were homogenized, and their size was further reduced using 0.22 mm filtration (32).

Evaluation of curcumin accumulation in the nano-niosome

At first, first free curcumin was removed using a dialysis bag at 4 ° C in PBS buffer. Then, to evaluate the encapsulation rate of curcumin in nano-niosome, the prepared niosomes were mixed with isopropanol with ratios of one to nine, one to nineteen and one to thirty-nine (the membrane of the liposomes is broken down using isopropanol, which causes the spilled out of the drug from the liposome.) and at a maximum absorption wavelength curcumin (420 nm), its UV absorption was determined and the concentration of the encapsulated drug was obtained according to the standard curve and Equation 1.

$$Percentage\ of\ \ loaded\ curcumin = \frac{\text{entrapted drug in nanoniosomes}}{\text{initial drug}} \times 100$$

Investigating the drug release pattern from niosomal formulation

To examine the drug release pattern from nanoniosomes, a certain amount of the formulation was put into a dialysis bag and placed in a 10 mL PBS buffer and entire system was put in water. Sampling from water at various times (0.5, 1, 2, 3, 4, 5, 6,7, 8, 24 and 48 hr) and at two various temperatures and pH conditions (37˚C, pH=7.4 and 45˚C, pH=5.4) was carried out. The absorbance of the samples at maximum absorption wavelength was evaluated by spectrophotometer and the release pattern of curcumin from nano-niosome was assayed.

Determination of size, polydispersity index, and the zeta potential of nano-niosomes

Dynamic light scattering (DLS) (Brookhaven Instruments Corp) was applied to figure out the mean diameter of nano-niosomes containing curcumin. Nanoniosomes were measured at an angle of 90° and laser radiation with a wavelength of 657 nm at 25°C. The samples were also measured five times and each time with a duration of 30 sec. To

Table 1. Formulas made with different molar ratios of span60 and cholesterol

Formulations	SPAN 60 (%)	SPAN 80 (%)	Cholesterol (%)
Formulation 0 (F0)	80	0	20
Formulation 1 (F1*)	75	0	25
Formulation 2 (F2)	70	0	30

figure out the size of particle, 600 µL of sample, with concentration of 0.5 to 1.0 µg/mL was used. Zeta potential was also figured out by using a Zeta Sizer apparatus (Brookhaven Instruments Corp) to measure the surface charge of nano-noisomes at 25°C.

Investigation the morphology of synthesized nano-niosomes

Microscopic observation was used to study the morphology and shape and structure of the synthesized nano-niosome. The Scanning Electron Microscope (SEM) (modelKYKY-EM3200-30 kV, KYKY Technology Development Ltd., Beijing, China) and Atomic Force Microscopy (AFM) were applied to determine the morphology of nano-niosomes containing curcumin. In order to analyze the sample by SEM, first a few drops of the sample with a concentration of 0.1 mg / ml was poured on the mesh copper grid 400. Then, in order to evaporate the solvent, the grid was put in an evacuated desiccator and after drying, sample coated with a thin layer of gold. AFM was used to evaluate the morphology of the synthesized nano-niosomes in shape, uniformity and roughness. Atomic force microscopy (AFM) is used to examine samples with dimensions in the range of nanometer and also to examine their surface topography. This microscope is equipped with a sharp silicone needle with a diameter of less than 10 nm and a length of 2 microns, which is located on a lever and because of the force between the sample and the needle the lever bends and the reflection of laser light on the detector is shifted.

Analysis of synthesized niosomes containing curcumin using FTIR infrared spectroscopy

Infrared spectroscopy (FTIR) technique was used to investigate the interaction between the synthesized niosomes and curcumin. In this method, we used free from of curcumin and blank synthesized niosome, and the FTIR spectra of these samples were obtained separately. For this purpose, first 1 mg of each sample in a ratio of 1 to 100 added to potassium bromide (KBr) and then, the samples were located in a hydraulic press to form the pellets. Each sample was analyzed by FT-IR spectrum instrument (Brucker, Germany) at a wavelength of 400-4000 cm^{-1} and its functional groups were identified.

Cellular uptake

Imaging was performed by fluorescent microscopy (Olympus, Japan) to examine the cellular uptake of nanosystems by cancer cells. Cancerous cells were cultured in 6-well plates (5×10^6 cells per well) containing DMEM culture medium with %10 bovine fetal serum (FBS). The culture medium was then changed and the cells were treated with a new culture medium containing diluted curcumin nanoparticles (10 mg/mL). After 3 hr of incubation and three times rinsing with PBS buffer, the cells were fixed with paraformaldehyde and DPAI was added to stain the cell nucleus and imaging was done.

Cell lines and culture conditions

The cells used in this study were MG-63 bone cancer cells that are more resistant to chemotherapy than other types of bone cancer cells. They were purchased from Pasteur Institute in Tehran, Iran and cultured according to cells culture protocol ATCC (*American Type Culture Collection)*. Cells were cultured in DMEM medium enriched with 10% bovine fetal serum (FBS) at 37 °c and 95% humidity.

Cytotoxicity assay (MTT Method)

MTT method was used to assess the cytotoxicity of the nano-system in this study. The MTT assay is a colorimetric test. The basis of this method is the reduction of yellow crystals tetrazolium (3-[4,5-dimethylthiazol-2-yl] -2,5-diphenyl-tetrazolium bromide (MTT)) by mitochondrial reductases enzymes of living cells. For this purpose, MG-63 cells were cultured in 96 plates for 48 hr. After reaching the required number of cells (10^4), the cells were treated with different concentrations (5, 10, 20, 40, 80 and 160 µg/mL) of blank niosomes, free form of curcumin and niosomes containing curcumin for 48 hr. Then 20 µl of MTT salt with a concentration of 0.5 µl was added to each well and incubated for 4 hr. After that, the supernatant was removed and 150 µL of DMSO was added to each well to remove Formazon crystals, and samples incubated for another 30 min. Finally, the absorbance of each well was measured at wavelength of 570 nm by EPOCH microplate spectrophotometer (synergy HTX, Bio Tek, USA) and cells viability was calculated.

RESULTS

Evaluation of the absorption spectrum of curcumin at range of 200 to 800 nm showed that the maximum absorption at different concentrations of curcumin is at wavelength of 420 nm (Fig. 1A).

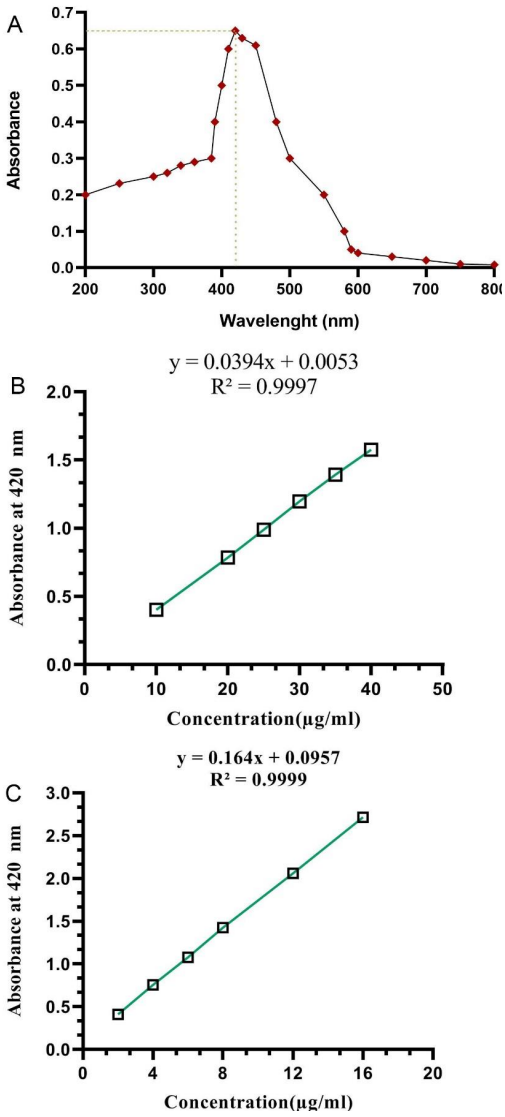

Fig. 1. A) Absorption spectrum of curcumin at wavelengths of 200 to 800 nm. The maximum absorption value is at 420 nm. B) The standard curve of curcumin in PBS buffer is derived from the evaluation of the absorption spectra in different concentrations of curcumin at its maximal absorption wavelength. C) The standard curve of curcumin in isopropyl buffer is derived from the evaluation of the absorption spectra in different concentrations of curcumin at its maximal absorption wavelength

Then, using this wavelength, standard curves of curcumin were plotted in PBS and isopropanol buffers. The standard curve of curcumin in PBS buffer (Figure 1B) is a straight line with equation Y = 0.0394X + 0.0053 and R-squared value (R2) = 0.9997. This standard curve in isopropanol buffer is also a straight line with equation Y = 0.164X + 0.0957 and R2= 0.9999 (Figure 1C).

The optimal formulation selection

In order to select the optimal formulation of nanonisomes containing curcumin, the effect of different molar ratios of cholesterol and span60 on the size, zeta potential, dispersion index (PDI) and encapsulation rate of synthesized nano-nisomes was investigated. The results are listed in Table 2. As shown in the Table 2, encapsulation efficiency decreases with increasing cholesterol. Formula 1 (F1) was selected as the optimal formulation due to the higher encapsulation rate and more

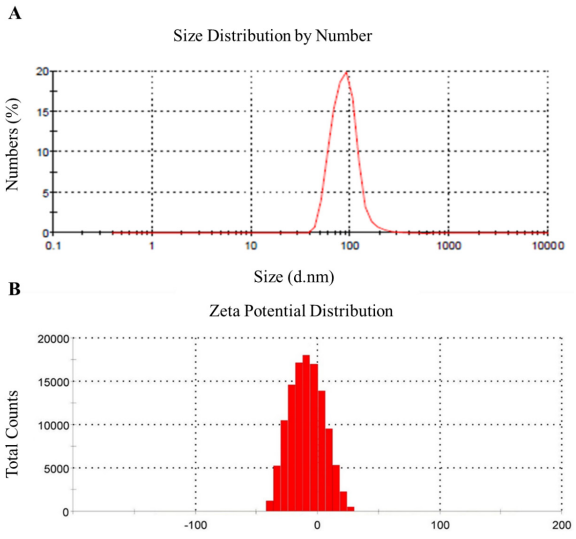

Fig. 2. A) Data obtained from the analysis of a niosomes by a zeta-sizer. According to these data, the average size of nanoparticles is 90.08 nm and their dispersion index is 0.236. B) Average of zeta potential of niosomes. The data show that the zeta potential of nanoparticles is -8.21 ± 3.46 mV

Table 2. Effect of the SPC: cholesterol with various molar on size, PDI, Zeta potential and entrapment efficiency

Formulations	Encapsulation Efficiency (% EE Mean± SD)	Size (12)	PDI	Zeta potential (mV)	%Release (72 hr)
Formulation 0 (F0)	81±1.3	87.3±2.12	0.248±0.52	-8.42 ± 2.37	32.6±5.8
Formulation 1 (F1*)	73.5±1.8	90.8±2.41	0.236±0. 1	-8.9 ± 3.46	60.08±4.3
Formulation 2 (F2)	71.3±2.4	92.4±1.81	0.241±0.31	-9.31± 2.74	51.3±4.8

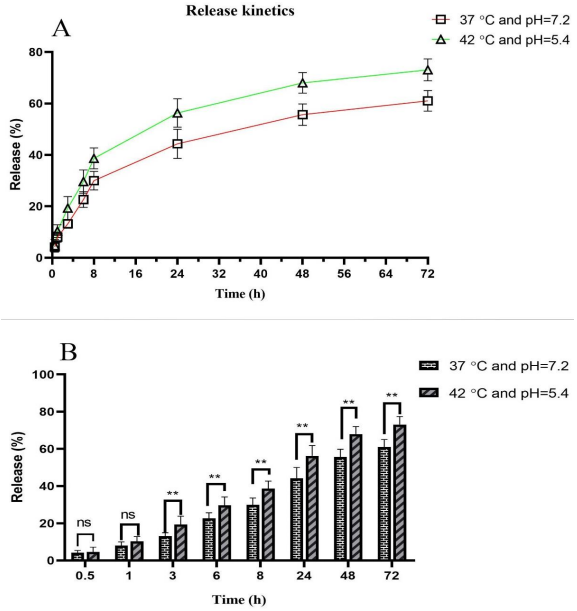

Fig. 3. A) *In vitro* kinetic release of curcumin in different temptures. B) Differences in drug release from Niosomes at various times, in different temperature and pH. *: *P*-value<0.05 And ns: there is no significant difference

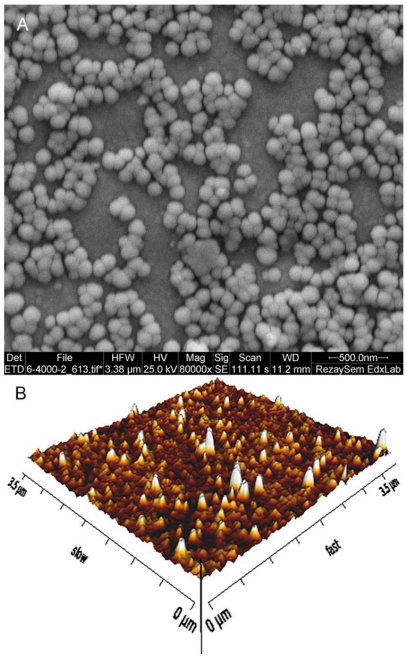

Fig. 4. A) Scanning electron microscopy (SEM) photography of niosome containing curcumin. B) Atomic forces microscopy (AFM) photography of niosome containing curcumin

drug release within 72 hr. The size, encapsulation efficiency, PDI and zeta potential of F1 formula as optimal formula was 90.8±2.41 nm, 73.5±1.8, 0.236 and -8.9 ± 2.37 respectively (Figure 2A, B).

Drug release pattern from niosomal formulation

Drug release pattern from nano-niosome based on curcumin standard curve in PBS buffer (Fig. 3) at specified intervals (0.5 to 72 hr) in two different conditions (physiological condition and cancerous cell condition) was drawn. The kinetic study of curcumin release from nanosystems showed that the release rate of the drug at 37 °C and 42 °C for 72 hr was 60.31% and 71.32%, respectively. As shown in Figure 6A, the release profile of curcumin was biphasic consisting of a rapid release in the early stage, followed by a slower slope showing a sustained release process. As expected, rapid drug release took place at a higher temperature range in which cancer cells efficiently grow (T=42°C). This phenomenon demonstrated that the synthesized nanoniosomes are well-designed to fight effectively against cancerous cells.

AFM and SEM imaging of nano-niosome containing curcumin

The surface morphology of nisomes containing curcumin was evaluated by AFM and SEM imaging. According to Fig. 4, the niosomes were uniform spherical in shape, almost identical in size, and without any aggregate formation.

Investigation of FTIR spectra: The graphs shown in Fig. 5 are obtained from the FTIP analysis. It shows (A) blank niosome (B) noisome containing curcumin. By comparing the two diagrams, it can be observed that neither the new peak nor the index peak has been removed, which can be concluded that there is no link between the curcumin and the niosome and no interaction is achieved. In fact, the curcumin retained its chemical nature and was stable at the time of formulation, which can be concluded that the curcumin still has its biological activity. The presence of the index peaks of diagrams A and B indicates that the system does not interact with the curcumin. For example, the 3445 index peak represents the OH group with a slight difference in the system containing the extract at 3447 cm^{-1}. The peak 2925 cm^{-1}, which represents the alkane group and is formed by rotation around the C-H axis, is also created in diagram B with a slight difference at 2922 cm^{-1}.

Cellular uptake behavior

Fig. 6 illustrate the uptake of nanosystems by

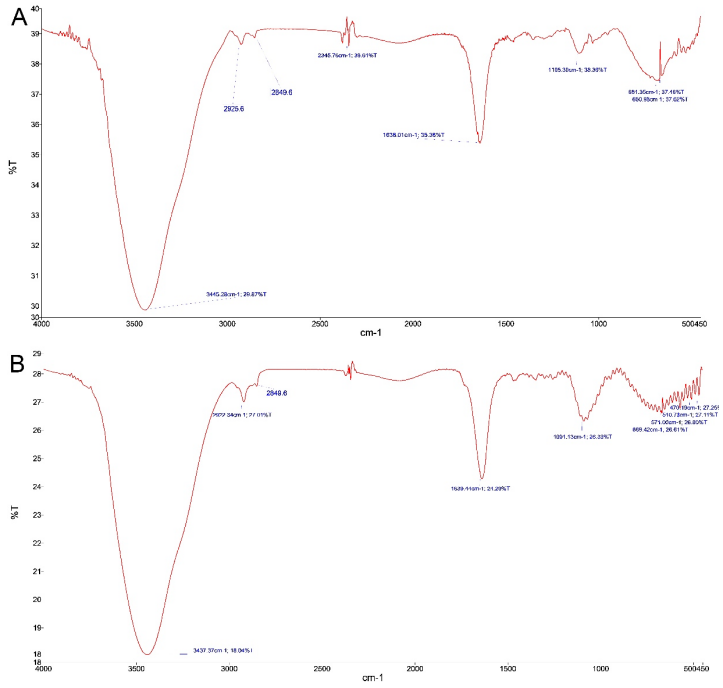

Fig. 5. FTIR spectra of optimum formula (F1) in before (A) and after curcumin loading (B)

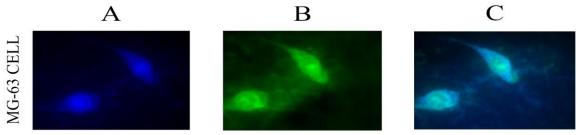

Fig. 6. Cellular uptake of nanoniosome containing curcumin by MG-63 cancer cells, A) cell nucleus counterstained with DAPI. B) Curcumin stained cytoplasm. C) Merge of A, B

cancerous cells. According to this image, A, B, and C, respectively, represent the nucleus stained with DAPI below the blue filter. Curcumin, which due to its fluorescent nature causes the cytoplasm to stain. The merge, which is the result of the overlap of the green and blue filters and indicates the uptake of nanosystems by the cancerous cells successfully.

Cell viability assay

MTT assay was used to investigate cell viability. As shown in Fig. 7, blank niosomes had no toxic effect on cancer cells, which indicating that blank synthesized niosomes had no side effects on normal cells. The results of MTT assay on MG-63 cells after 48 hr of treatment with different concentrations of free form and niosomal form of curcumin show that at concentrations of more than 20 µg/mL, cell survival rates in free form are

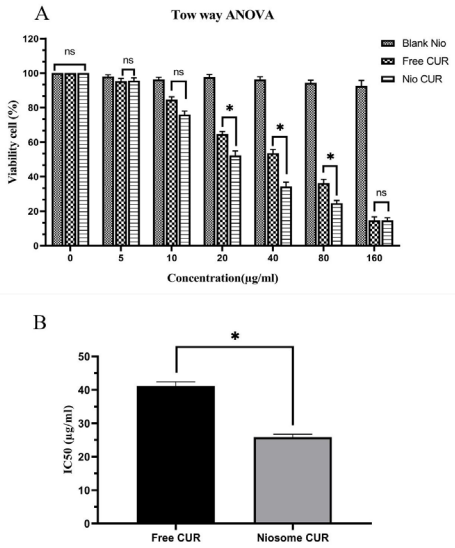

Fig. 7.(A) Cell viability on MG-63 cell line of niosomal formulations with various concentrations of curcumin, determined after 48 hr using MTT assay. (B)The IC_{50} values of curcumin on MG-63 cells administered in the forms of free curcumin and curcumin niosomal form. Data expressed as mean± SD from three independents experiments (ns= not significant difference, *= *P*< .05)

significantly different from niosomal form. These findings demonstrate that free form of curcumin inhibits the growth of cancer cells at higher doses

within 48 hr, while the niosomal form of curcumin at a same dose and same time can cause more toxicity in cancer cells than in free form and thereby inhibiting cell growth. The results showed that the mortality of cancerous cells treated with noisome containing curcumin increased significantly compared to free form pf curcumin at all different concentrations of curcumin, (P<0.05). Also, IC_{50} calculated on MG-63 bone cancer cells for free form of curcumin and encapsulated curcumin were 42.04 µg/mL and 26.48 µg/mL respectively, (Fig. 7A, B), which shows, curcumin in niosomal form with lower concentration and dose can causes more toxicity in cancer cells than the its free form.

DISCUSSION

In this study, nanotechnology and in particular nanoparticle technology have been evaluated for new treatments in osteosarcoma. We succeeded synthesizing of nano-niosomes containing curcumin with a size of 90.08 nm, zeta potential of -8.9 mV and polydispersity index of 0.236 with an encapsulation rate of 73.5% of the slow release type, which can reduce the dosage of the drug compared to its free form in MG-63 cell line of bone cancer that supposed to be have more resistance than other types of bone cancer cells lines to chemotherapy (33). Current treatments for osteosarcoma appear to be ineffective, particularly in the treatment of metastatic diseases which it could be due to the occurrence of multiple drug resistance, activation of anti-apoptotic pathways and insufficient accumulation of drug agents. It has also been observed that metastatic osteosarcoma generally shows a weaker response to chemotherapy than primary tumor, and although metastatic cells have properties that only cause metastasis; but they also refuse cell death after cytotoxic treatment. Overall, improving conventional therapies for osteosarcoma is an urgent and unfulfilled need. In addition to this therapeutic intent, the practical goal of treating osteosarcoma is to suppress the disease to a subclinical state, fight cancer cells to prevent or stop metastatic spread, and maintain good quality of life (QoL) as much as possible (34, 35). It is also one of the few times that that a totally herbal treatment combined with nanotechnology as an advanced technology has been used to improve the treatment process of osteosarcoma.

In 2016, Peng Chen et al reported that curcumin decreased the expression of estrogen-dependent alpha receptors (ERRα) in osteosarcoma cancer cells, which in turn increased the sensitivity of cells to curcumin and thus induced Apoptosis through the production of reactive oxygen species in osteosarcoma cells. They also reported that curcumin could activate the ERRα / miRNA-125a pathway by affecting miRNA-125a, thereby modulating the apoptotic pathways in cancerous cells (36). In Another study by Yaowu Zhang et al in 2017 founded that curcumin could increase apoptosis and autophagy in MG-63 bone cancer cells through mitochondrial signaling pathways. They reported that curcumin could induce apoptosis and autophagy in osteosarcoma cancer cells by affecting the signaling pathway of c-Jun N-terminal kinase (JNK) (37). In 2018, Muhammad Nazirul Mubin Aziz and his colleagues reported that curcumin increased mortality in MG-63 and SAOS cancer cells by changing their morphology. They also stated that the expression of many apoptotic genes and proteins increases after the effect of curcumin on these cells, including caspase-3, caspase-9, and BAX, which indicates that induction of apoptosis in these cells, could be through the effect of curcumin on mitochondrial signaling pathways (38). Also, at study in 2015 by Dazhi Yu et al on MG-63 bone cancer cells reported that curcumin could reduce the expression level of miRNA-138 target genes Smad4, NFκB p65 and cyclin D3, which in turn can reduce the proliferation of these cells (39). Zhanpeng Luo and colleagues reported in 2018, the expression of CLTC and ITPR1 mRNA in curcumin-treated cells increased significantly compared to the control group, which could induce apoptosis (40). In line with these studies, our study also showed that the survival rate of MG-63 bone cancer cells decreased compared to control group, after curcumin treatment, which could indicate the induction of apoptosis in these cells after curcumin treatment. Lack of investigation of genes involved in apoptosis signaling pathways was one of the disadvantages of our study compared to the above studies, but the use of niosomes to deliver curcumin to target cells and compare it with the free form of the drug is as this study advantages.

So far, several studies have been performed on the synthesizing and characterization of drug delivery nanocarriers. As mentioned, the encapsulation efficiency is one of the important parameters in the selection of drug nanocarriers,

which depends on important factors such as particle size, nature of embedded material, type of lipids used and their concentration in nanoparticle structure (41). Drug release also is another of the important features of a system in choosing it as a drug carrier, which is directly related to the amount of cholesterol used in the structure of nanolipids (42, 43). In designing and synthesizing a suitable nanoparticle for drug delivery to cancer cells, four critical criterial should be considered: 1, suitable capacity for drug loading 2, low nanoparticle toxicity 3, appropriate physicochemical properties for drug loading and delivery, and 4, low-dose effect. Niosomes are nanoparticles with high encapsulation ability, biocompatibility, easy and low cost design, which are proposed as a new alternative to phospholipid particles that are increasingly used in clinical stages (44, 45). In this study, we succeeded in synthesizing niosomes with high encapsulation efficiency and suitable physicochemical properties for the delivery of curcumin to cancer tissue cells. Spen-60, non-ionic surfactant used in the chemical structure of synthesized niosomes in this study, had low toxicity and high degradability. Cholesterol was also added to increase the stability of nanoparticles. Previous studies have demonstrated that increasing cholesterol in the structure of nanoparticles can increase drug release rate from the system, but excessive cholesterol causes instability of synthesized nanoparticles (46). According to Tab 1, 2, with increasing cholesterol from 20% to 30%, the encapsulation rate of curcumin decreased, but the release rate of curcumin from the niosomes changed with the change in the concentration of cholesterol used in the structure of the niosomes. This decrease in curcumin encapsulation rate with increasing cholesterol content may be due to the hydrophobic nature of these substances, which causes competition between cholesterol and curcumin for loading in noisome bilayer. This Furthermore, increasing the cholesterol content increased the size of nanoparticles. sonication is also another factor in determining size of nanoparticle. The surface charge of nanoparticles is also one of the important factors in their use as drug carriers. The zeta potential is a good indicator to measure it. The zeta potential of nanoparticles in this study was -8.9 ± 2.37 mV, which indicates that these nanoparticles are anionic, which increases their stability because in addition to preventing the deposition of nanoparticles, it also prevents opsonization and scavenging them by macrophages because it, in turn, prevents their non-specific interaction with blood components (47, 48). Also, the pattern of drug release from the non-system in this study was a two-phase pattern with a rapid release phase in the first stage and a slow release pattern in the second phase. It was also found that the pattern of curcumin release from niosomes changes in response to stimuli, which is an important factor in the use of nanoparticles as drug carriers, and so far there have been many studies and efforts to achieve this goal, because with using these stimulants could differentiate drug release in cancer cells in compared to healthy cells and reduced toxicity in healthy cells, and increased the effect of nanosystems on cancer cells (45, 46). In this study, we succeed in developing a temperature and pH-sensitive nanosystem that could improve the therapeutic effect of curcumin under cancerous cells condition. Also, by evaluating the images from fluorescent microscopy, it can be concluded that the uptake of nanoniosomes by cancer cells is well done and consequently this causes more toxicity of encapsulated curcumin compared to its free form on cancerous cells. Numerous studies, such as the study of Haghir al-Sadat et al. in 2019, showed that by encapsulating drugs, the stability and cellular uptake of these drugs in the body increases, which in turn improves the function of the drug (49). This study also showed that the toxicity of curcumin on Bone cancer cells have increased in encapsulated form. In this study, almost all of the beneficial benefits of using drug delivery carrier have proven and can conclude that nanoniosomes are able to enhance therapeutic effects of curcumin.

CONCLUSIONS

The resistance to chemotherapy and its side effects are among main barriers for successful treatment of OS. Using herbal medicine could be a new window for overcome these problems, but it is a vital importance to introduce improved drug delivery system in order to enhance efficiency of herbal as drug. to achieve these goals, we synthesized different niosomal formulation and chose the optimal formulation from them. Our successful finding proposed novel anionic niosomal formulation for administration of curcumin. The encapsulation rate and release profile of curcumin was tremendously appropriate. Spherical shape,

nanoscale size, suitable negative charge and no chemical interaction of curcumin with the system was confirmed by different characterization methods. Also, by using cytotoxicity assessment methods on MG-63 bone cancer cell line, we concluded that The IC_{50} drug is significantly lower in the niosomal form than in the free form of drug. So the nanoniosomal form can dramatically improve the therapeutic effect of curcumin in the treatment of cancer. Experimental evidence from our study demonstrated that the use of a curcumin-carrying niosomal drug delivery system was effective in combating cancerous cells *in vitro*, but there is a long way to go before it can be used in the clinical course of treatment as a safe and reliable drug. In this study, almost all of the beneficial benefits of using drug delivery carrier have proven and can conclude that nanoniosomes are able to enhance therapeutic effects of curcumin.

ACKNOWLEDGEMENTS

The authors thank the participants of this study. Special thanks also go to the staff of the NBTF Company, Yazd, Iran. The current study was funded by NBTF Company, Yazd, Iran and Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

REFERENCES

1. Durfee RA, Mohammed M, Luu HH. Review of osteosarcoma and current management. Rheumatology and Ther. 2016;3(2):221-243.
2. Harrison DJ, Geller DS, Gill JD, Lewis VO, Gorlick R. Current and future therapeutic approaches for osteosarcoma. Expert review of anticancer therapy. 2018;18(1):39-50.
3. Czarnecka AM, Synoradzki K, Firlej W, Bartnik E, Sobczuk P, Fiedorowicz M, et al. Molecular biology of osteosarcoma. Cancers. 2020;12(8):2130.
4. Isakoff MS, Bielack SS, Meltzer P, Gorlick R. Osteosarcoma: current treatment and a collaborative pathway to success. J Clin Oncol. 2015;33(27):3029-3035.
5. Simpson S, Dunning MD, de Brot S, Grau-Roma L, Mongan NP, Rutland CS. Comparative review of human and canine osteosarcoma: morphology, epidemiology, prognosis, treatment and genetics. Acta Vet Scand. 2017; 59(1): 1-11.
6. Smrke A, Anderson PM, Gulia A, Gennatas S, Huang PH, Jones RL. Future Directions in the Treatment of Osteosarcoma. Cells. 2021;10(1):172-183.
7. Omidi M, Malakoutian M, Choolaei M, Oroojalian F, Haghiralsadat F, Yazdian F. A Label-Free detection of biomolecules using micromechanical biosensors. Chin Phys Lett. 2013;30(6):068701.
8. Zhang Y, Yang J, Zhao N, Wang C, Kamar S, Zhou Y, et al. Progress in the chemotherapeutic treatment of osteosarcoma. Oncology letters. 2018;16(5):6228-6237.
9. Li S, Sun W, Wang H, Zuo D, Hua Y, Cai Z. Research progress on the multidrug resistance mechanisms of osteosarcoma chemotherapy and reversal. Tumor Biol. 2015;36(3):1329-1338.
10. Spínola V, Castilho PC. Evaluation of Asteraceae herbal extracts in the management of diabetes and obesity. Contribution of caffeoylquinic acids on the inhibition of digestive enzymes activity and formation of advanced glycation end-products (in vitro). Phytochemistry. 2017;143:29-35.
11. Jiang Y, Peng T, Gaur U, Silva M, Little P, Chen Z, et al. Role of corticotropin releasing factor in the neuroimmune mechanisms of depression: Examination of current pharmaceutical and herbal therapies. Front Cell Neurosci. 2019; 13: 290-302. 2019;13:290.
12. Sivaraman D, Anbu N, Kabilan N, Kumar MP, Shanmugapriya P, Christian G. Review on current treatment strategy in Alzheimer's disease and role of herbs in treating neurological disorders. Int J Trans Res Ind Med. 2019;1(1):33-43.
13. Mansoori B, Mohammadi A, Doustvandi MA, Mohammadnejad F, Kamari F, Gjerstorff MF, et al. Photodynamic therapy for cancer: Role of natural products. Photodiagnosis Photodyn Ther. 2019;26:395-404.
14. Akhlaghi M, Ebrahimpour M, Ansari K, Parnian F, Zarezadeh Mehrizi M, Taebpour M. Synthesis, study and characterization of nano niosomal system containing Glycrrizha glabra extract in order to improve its therapeutic effects. New Cell Mol Biotech. 2021;11(42):65-82.
15. Babazadeh A, Zeinali M, Hamishehkar H. Nano-phytosome: a developing platform for herbal anti-cancer agents in cancer therapy. Curr. Drug Targets. 2018;19(2):170-80.
16. Hewlings SJ, Kalman DS. Curcumin: a review of its effects on human health. Foods. 2017;6(10):92.
17. Nelson KM, Dahlin JL, Bisson J, Graham J, Pauli GF, Walters MA. The essential medicinal chemistry of curcumin: miniperspective. J Med Chem. 2017;60(5):1620-1637.
18. Hussain Z, Thu HE, Amjad MW, Hussain F, Ahmed TA, Khan S. Exploring recent developments to improve antioxidant, anti-inflammatory and antimicrobial efficacy of curcumin: A review of new trends and future perspectives. Mater Sci Eng C. 2017;77:1316-1326.
19. Fadus MC, Lau C, Bikhchandani J, Lynch HT. Curcumin: An age-old anti-inflammatory and anti-neoplastic agent. J Tradit Complement Med. 2017;7(3):339-346.
20. da Silva AC, de Freitas Santos PD, do Prado Silva JT, Leimann FV, Bracht L, Goncalves OH. Impact of curcumin nanoformulation on its antimicrobial activity. Trends Food Sci Technol. 2018;72:74-82.
21. Zhang W, Chen C, Shi H, Yang M, Liu Y, Ji P, et al. Curcumin is a biologically active copper chelator with antitumor activity. Phytomedicine. 2016;23(1):1-8.
22. Rahmani AH, Alsahli MA, Aly SM, Khan MA, Aldebasi YH. Role of curcumin in disease prevention and treatment. Adv Biomed Res. 2018;7.
23. Salehi B, Stojanović-Radić Z, Matejić J, Sharifi-Rad M, Kumar NVA, Martins N, et al. The therapeutic potential of curcumin: A review of clinical trials. Eur J Med Chem. 2019;163:527-545.
24. Oroojalian F, Rezayan AH, Shier WT, Abnous K, Ramezani M. Megalin-targeted enhanced transfection efficiency in cultured human HK-2 renal tubular proximal cells using aminoglycoside-carboxyalkyl-polyethylenimine-containing nanoplexes. Int J Pharm. 2017;15(1):102-120
25. Ghasemiyeh P, Mohammadi-Samani S. Solid lipid nanoparticles and nanostructured lipid carriers as novel drug delivery systems: applications, advantages and disadvantages. Res Pharm Sci. 2018;13(4):288-303.
26. Kadian R. Nanoparticles: A promising drug delivery approach. Asian J Pharm Clin Res. 2018:30-35.
27. Rashidi A, Omidi M, Choolaei M, Nazarzadeh M, Yadegari A, Haghierosadat F, Oroojalian F, Azhdari M.

Electromechanical properties of vertically aligned carbon nanotube. Adv Mat Res. 2013; 705: 332-336.
28. Rahimizadeh M, Eshghi H, Shiri A, Ghadamyari Z, Matin MM, Oroojalian F, et al. Fe (HSO 4) 3 as an efficient catalyst for diazotization and diazo coupling reactions. J Korean Chem Soc. 2012;56(6):716-719.
29. Akhlaghi M, Taebpour M, Lotfabadi NN, Naghib SM, Jalili N, Farahmand L, et al. Synthesis and characterization of smart stimuli-responsive herbal drug-encapsulated nanoniosome particles for efficient treatment of breast cancer. Nanotechnology Reviews. 2022;11(1):1364-1385.
30. Mohseni M, Dehghani AM, Akhlaghi M, Ansari K. A New Therapeutic Approach For The Treatment Of Breast Cancer Using Synthesis Of Liposomes Scan Online To View This Article Containing Silybinin And Their Characterization. 2021.
31. Hemati M, Haghiralsadat F, Jafary F, Moosavizadeh S, Moradi A. Targeting cell cycle protein in gastric cancer with CDC20siRNA and anticancer drugs (doxorubicin and quercetin) co-loaded cationic PEGylated nanoniosomes. Int J Nanomedicine. 2019;14:6575.
32. Nia AH, Behnam B, Taghavi S, Oroojalian F, Eshghi H, Shier WT, Abnous K, Ramezani M. Evaluation of chemical modification effects on DNA plasmid transfection efficiency of single-walled carbon nanotube–succinate–polyethylenimine conjugates as non-viral gene carriers. Med Chem Comm. 2017;8(2):364-375.
33. Roundhill EA, Jabri S, Burchill SA. ABCG1 and Pgp identify drug resistant, self-renewing osteosarcoma cells. Cancer Letters. 2019;1:142-157.
34. Tang ML, Bai XJ, Li Y, Dai XJ, Yang F. MMP-1 over-expression promotes malignancy and stem-like properties of human osteosarcoma MG-63 cells *in vitro*. Curr. med. sci. 2018;38(5):809-817.
35. Ferrari S, Smeland S, Mercuri M, Bertoni F, Longhi A, Ruggieri P, et al. Neoadjuvant chemotherapy with high-dose Ifosfamide, high-dose methotrexate, cisplatin, and doxorubicin for patients with localized osteosarcoma of the extremity: a joint study by the Italian and Scandinavian Sarcoma Groups. J Clin Oncol. 2005;23(34):8845-8852.
36. Chen P, Wang H, Yang F, Chen H, He W, Wang J. Curcumin promotes osteosarcoma cell death by activating miR-125a/ERRα signal pathway. J Cell Biochem. 2017;118(1):74-81.
37. Zhang Y, Chen P, Hong H, Wang L, Zhou Y, Lang Y. JNK pathway mediates curcumin-induced apoptosis and autophagy in osteosarcoma MG63 cells. Exp Ther Med. 2017;14(1):593-599.
38. Aziz MNM, Hussin Y, Che Rahim NF, Nordin N, Mohamad NE, Yeap SK, et al. Curcumin analog DK1 induces apoptosis in human osteosarcoma cells *in vitro* through mitochondria-dependent signaling pathway. Molecules. 2018;23(1):75.
39. Yu D, An F, He X, Cao X. Curcumin inhibits the proliferation and invasion of human osteosarcoma cell line MG-63 by regulating miR-138. Int J Clin. 2015;8(11):14946.
40. Luo Z, Li D, Luo X, Li L, Gu S, Yu L, et al. Curcumin may serve an anticancer role in human osteosarcoma cell line U-2 OS by targeting ITPR1. Oncology Letters. 2018;15(4):5593-5601.
41. Honary S, Zahir F. Effect of zeta potential on the properties of nano-drug delivery systems-a review (Part 1). Trop J Pharm Res. 2013;12(2):255-264.
42. Danhier F, Feron O, Préat V. To exploit the tumor microenvironment: passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. J Control Release. 2010;148(2):135-146.
43. Malekimusavi H, Ghaemi A, Masoudi G, Chogan F, Rashedi H, Yazdian F, et al. Graphene oxide-l-arginine nanogel: a pH-sensitive fluorouracil nanocarrier. Biotechnol. Appl. Biochem. 2019;66(5):772-780.
44. Ghafari M, Haghiralsadat F, Khanamani Falahati-pour S, Zavar Reza J. Development of a novel liposomal nanoparticle formulation of cisplatin to breast cancer therapy. J Cell Biochem. 2020;121(7):3584-3592.
45. Akhlaghi M, Taebpour M, Sharafaldini M, Javani O, Haghiralsadat BF, Oroojalian F, et al. Fabrication, characterization and evaluation of anti-cancer and antibacterial properties of nanosystems containing Hedera Helix aqueous extracts. Nanomed J. 2022; 9(1): 43-56.
46. Shehata TM, Ibrahim MM, Elsewedy HS. Curcumin Niosomes Prepared from Proniosomal Gels: *In vitro* Skin Permeability, Kinetic and *In vivo* Studies. Polymers. 2021;13(5):791.
47. Azadmanesh F, Pourmadadi M, Zavar Reza J, Yazdian F, Omidi M, Haghirosadat BF. Synthesis of a novel nanocomposite containing chitosan as a three-dimensional printed wound dressing technique: Emphasis on gene expression. Biotechnology Progress. 2021;37(4):3132-3145.
48. Mirhosseini M, Shekari-Far A, Hakimian F, Haghiralsadat BF, Fatemi SK, Dashtestani F. Core-shell Au@ Co-Fe hybrid nanoparticles as peroxidase mimetic nanozyme for antibacterial application. Process Biochem. 2020;95:131-138.
49. Haghiralsadat F, Amoabediny G, Naderinezhad S, Zandieh-Doulabi B, Forouzanfar T, Helder MN. Codelivery of doxorubicin and JIP1 siRNA with novel EphA2-targeted PEGylated cationic nanoliposomes to overcome osteosarcoma multidrug resistance. Int J Nanomedicine. 2018;13:3853.