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RESEARCH ARTICLE

Fabrication and characterization of PLGA polymeric nanoparticles containing berberine and its cytotoxicity on breast cancer cell (MCF-7)

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ABSTRACT

Breast cancer is one of the most common types of cancer in the world which threatening the lives of millions of people every year. Common treatments for cancer include surgery, chemotherapy, radiation and radiotherapy, which have been limited and sometimes ineffective due to the adverse side effects, multidrug resistance, prolongation of the treatment period and, most importantly, the ineffectiveness of these treatments. The use of modern medical therapies based on nanotechnology is one of the noteworthy ways to overcome these therapeutic limitations.

In this study, different formulations of PLGA polymer nanoparticles were synthesized using dual emulsion method (W1 / O / W2) and berberine was loaded into them. The optimal formula of PLGA containing berberine had 85.2% encapsulation efficiency, size 234 nm and surface charge-8.21. Also, the IC50 of PLGA containing berberine (42.39 µg / ml) compared to IC50 free drug (80.18 µg / ml) had higher cytotoxicity in killing cancer cells. The system had a slow release pattern and the maximum rate of release from nanoparticles was 61.46% in 48 hours. Also, the synthesized PLGA polymer system was approved for surface morphology and infrared spectroscopy.

Our findings show that PLGA polymer systems containing berberine have the potential to stop the growth of breast cancer cells that provide a promising future and an interesting therapeutic strategy in cancer treatment.

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INTRODUCTION

Cancer is one of the dangerous diseases in the world which killing millions of people every year. Cancer is one of the major global health concerns of the 21st century, and can affect any member of any population, anywhere (1). In 2018, Approximately 18 new cases of cancer were diagnosed, of which nearly 9 million were fatal (2). One of the main challenge of cancer treatment is to distinguish cancer cells from healthy cells in the body. In this regard, the main purpose of drug engineering is to detect cancer cells in order to reduce their growth

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and proliferation, while conventional cancer treatments such as chemotherapy cannot selectively target cancerous cells without impact with the normal cells of the body. There are numerous challenges in the treatment of cancer and the development of effective drugs against it, including the improper dose of the drug, adverse side effects due to the inappropriate drug distribution in tissues and the resistance of cancer cells to chemotherapy agents. Weak solubility of drugs in water is also a major problem in chemotherapy that unable drugs to penetrate biological membranes. Such challenges necessitate the effective development of targeted drug delivery (3-7). Nanotechnology is an interesting field of research that development in advanced technology (8, 9). Research in nanotechnology as an interdisciplinary technology is evolving but also the use of toxic chemicals and solvents in the synthesis of nanoparticles limits their use in clinical practice, thus the development of compatible, non-toxic and safe methods in the synthesis of nanoparticles are essential (10, 11). As a novel treatment for cancer, nanoparticles are sharply being examined and developed to overcome cancer limitations and upgrade targeted drug delivery systems. Gold nanoparticles, zinc oxide, titanium oxide, graphene magnetic nanoparticles, heparin-based nanoparticles, polymer nanoparticles, etc. are among the types of nanoparticles which have potential applications in the field of imaging, diagnosis and treatment of cancer. Targeted drug delivery, cytotoxicity and genetic toxicity, biosensors, antibiotic films and protein bonding and hypertrichosis of cancer (12). Targeted polymers based on biodegradable polymers are an attractive option for drug carriers. Polymer nanoparticles consist of nanocouples and nanocapsules and solid carriers of natural or synthetic polymers with a diameter of 10 to 1000 nm. Polymer-based nanoparticles are a good delivery system for delivering biomolecules, drugs, genes and vaccines. By encapsulating of drugs in these nanoparticles, the solubility and stability of the drugs will enhance (13). PLGA (poly lactic-co-glycolic acid) belongs to the family of hydroxy acids. This polymer can be considered as one of the most useful biological materials in the field of drug delivery. PLGA is a biodegradable and biocompatible polymer approved by the FDA and the European Medical Agency. Toxicological studies show that this polymer is an extremely safe material in macroscopic and microscopic systems. PLGA is soluble in a range of conventional solvents including chlorine; Tetra hydrofuran and acetone. PLGA could be in forms of PLGA-cysteine copolymers, PLGA-cell nano-hybrids or in combination with a variety of molecules such as bisphosphonates, Mannose lectin sialic acid, Biotin: folate, transferrin, peptides, nucleotides and antibodies (14).

Our knowledge of plants and their benefits is as great as human life. Man soon discovered the therapeutic properties of some plants. Numerous studies have shown that herbs have anti-cancer activity. Polyphenols, Folic acid, flavonoids, terpenes and alkaloids are among the compounds with medicinal properties in plants (15). Berberine, which is found in various plants including *Berberis vulgria* and belongs to the *Berberidaceae* family, is a natural isoquinoline colloid that is considered as an active molecule with hypoglycemic, antibacterial, antifungal, antiviral and anti-cancer properties. This active ingredient is present in the roots, limbs and bark of medicinal plants and has great potential for treating many physiological disorders, so it is widely used in Chinese traditional medicine. Also, the effects of Berberine on esophageal cancer and leukemic have been evaluated, which has shown significant mortality on cancerous cells (16). The aim of this study is to load Berberine in PLGA nanoparticles in order to investigate its effect on breast cancer cells.

MATERIALS AND METHODS

Synthesis of PLGA-BRB polymer NPs by using dual emulsion method (W1 / O / W2)

In the dual emulsion method, 1 mg of berberine (BBR) powder (Sigma, USA) was weighted and dissolved in a certain amount of distilled water, which is considered as the stock of the initial aqueous phase (W1). Then, weigh 0.01 g of PLGA polymer and dissolved with 1 ml of Dichloromethane (DCM) (Merck, German) and vortex until the polymer was completely dissolved, this was considered as stock of organic phase. The aqueous phase was then added to the organic phase (W1 / O) and sonicated with power of 110 w for 15 minutes. In the next stage and after the end of the first stage sonicate, the second aqueous phase of containing 2 ml of 5% PVA (Merck, German) was poured into beaker and then in order to form W1 / O / W2 emulsion, W1 / O emulsion were added during sonication. Then, the W1 / O / W2 emulsion is added to 5 ml of 0.1% PVA while gently straining

Table 1. Different synthesized formulations of PLGA-BBR polymer nanoparticles

Formula Code	Polymer/Drug	Solvent PLGA	PVA%	Sonication Time 1/2
F1	1/50	Chloroform	2%	5 min/ 2 min
F2	1/50	Chloroform	5%	10 min/ 3 min
F3	1/25	Chloroform	5%	5 min/ 2 min
F4	1/25	DCM	2%	10 min/ 3 min
F5	1/25	DCM	5%	10 min/ 3 min
F6	1/10	DCM	2%	5 min/ 2 min
F7	1/10	DCM	5%	10 min/ 3 min
F8	1/5	DCM	5%	10 min/ 3 min

so that in addition to the complete formation of nanoparticles, the DCM also evaporates completely. Finally, after 16 hours, the emulsion was centrifuged at 14,000 rpm for 15 minutes, the supernatant was removed, and the precipitate was rinsing for 3 times with deionized water. The nanoparticles were washed to separate the free drug. Different formulations of PLGA polymer containing beberin (PLGA-BBR) based on the Table 1, with different concentration ratio of drug to polymer, type of solvent used, different PVA% used in synthesizing nanoparticles and various durations of sonication were synthesized and the optimal formulation was selected from them.

Plotting standard curve

In order to evaluate the drug encapsulating rate and its release rate from nanoparticles, two standard curves with water and PBS (Phosphate Buffered Saline) (Merck, German) were plotted. For this purpose, first 0.001 g of berberine powder was dissolved in water / PBS solvent in different concentrations (1000,500,250,62.5,31.25 mg/ mL) and the adsorption was read at a wavelength of 345nm (maximum absorption wave length) by spectrophotometer (Epoch, USA) with three repetitions and its standard curves were plotted.

Investigation of encapsulation rate

To determine the encapsulation rate of nanoparticles after the centrifugation of nanoparticles in the first washing step, the supernatant was sampled and the absorbance of the supernatant was read at a wavelength of 345 nm and thus, the concentration of non-loaded drug in nanoparticles was obtained based on the standard curve BBR in water. Subsequently, subtract this concentration from the initial concentration of BBR to obtain the amount of drug loaded in the PLGA nanoparticles.

Evaluation of drug release profile from PLGA nanoparticles

1 mg of PLGA -BBR nanoparticles were poured into 1 ml microtubes and 1000 µL of PBS buffer was added to each. Then, samples were transferred to a dialysis bag and placed on a shaker at 37 ° C with gentle motion in a beaker containing 200 ml of PBS. Samples at regular intervals (30,60,120,180,240, ... min) were sampled by removing 200 µL of supernatant and replacing it with 200 µL of fresh PBS buffer. The absorption of samples was read at wavelength 345 nm by the spectrophotometer and then the concentration of the released drug was determined by using standard curve in PBS.

Investigating size and zeta potential of nanoparticle

Dynamic light scattering (DLS) (Brookhaven Instruments Corp) was used to evaluate the nanoparticle size and polydispersity index. The hydrodynamic diameter parameter (particle size) and the dispersion index were determined using dynamic laser diffraction technique by Zeta-sizer device (Malvern; Nanozeta-sizer ES) and particle size analyze at room temperature, diluted with deionized water. Each parameter was measured

3 times and after each measurement, the mean values and standard deviation were calculated. This was done at a temperature of 25 degrees Celsius, an angle of 90 degrees with a wavelength of 657 nm. Also, in order to investigate the zeta potential of nanoparticles, a Zeta Sizer apparatus (Malvern; Nanozeta-sizer ES) was used at room temperature.

Imaging of PLGA polymer nanoparticles

Scanning electron microscope (SEM) and Atomic force microscope (AFM) were used to image the nanoparticles. In order to analyzing the samples by SEM, a diluted sample (0.1 mg/mL) was coated with a layer of gold. Form, monotony and smoothness of nanoparticles were evaluated by AFM. Atomic force microscope is used to assay topography of samples in the nanometer range. In order to prepare the sample for AFM imaging, a suspension of the sample was first made with water at a concentration of 0.1 mg / mL. Then, with the help of freshly cut sheets of Mica, a bunch of tape was created. A drop of the suspension was poured on the mica and after 5 minutes with a steady stream of air, the drop was removed from the surface and then imaging was done.

Investigation of chemical bonds using FT-IR technique

Molecular interactions of compounds in polymer system formulations and BBR are evaluated using FT-IR method. In this method, using a centrifuge, the polymer nanocarrier is separated from the suspension containing the sample and its excess solution is evaporated. The isolated sample was mixed with KBr and its FTIR spectrum was scanned in the wavelength range (400-4000 cm^{-1}) by FT-IR spectrometer instrument (Model 8300, Shimadzu Corporation, Tokyo, Japan). At this stage, the absorption spectrum of BBR, a PLGA containing berberine and blank PLGA was obtained and used to evaluate the molecular interaction.

Cytotoxicity analyzing

MTT assay as a colorimetric method used to assay cytotoxicity of nanoparticles. Reduction of tetrazolium by mitochondrial reductase enzymes of cell is the basis of MTT assay. MCF-7 breast cancer cell line as a cancerous cell, MCF-10A normal breast cell as a normal cell were purchased from the Pasture instituted of Iran and healthy foreskin fibroblasts (HFF) cell line as a control cell were obtained from Stem Cell Biology Research Center (Yazd, Iran). Cells were grown in DMEM medium (Gibco, Grand Island, NY) containing 10% bovine fetal serum (Gibco, Grand Island, NY) and 1% Penicillin-Streptomycin under cellular conditions. To stick cells on the plates, cells were cultured in 96-well plates for 48 hours and after reaching the required number of cells in each well (10^4), the cells were treated for 48 hours with different concentrations (15.6, 31.25, 62.5, 125, 250, 500 µg/mL) of free form of BBR, blank PLGA and PLGA-BBR. After that, cells were washed and 20 µL of MTT solution with concentration 0.5 µL added to each well and incubated for 4 hours. After removing the supernatant, in order to dissolve Formazan crystals, 150 µL DMSO was added to each well and the incubation was performed for 30 minutes. At the end, by EPOCH microplate spectrophotometer (synergy HTX, Bio Tek, USA), the absorbance of each well was read at wavelength of 570 nm and cells viability rate was evaluated.

In vitro cellular uptake

The fluorescence intensity detection method used in order to figure out the uptake and distribution of nanoparticles in the cells. Shortly, MCF-7 and HFF cells were seeded in 6-well plates and treated with free form of BBR and PLGA-BBR and incubated for 4 hours and then, washed twice with PBS buffer (pH=7.4) to remove the effect of background color for microscopic examination. After that, cells were fixed with 4% paraformaldehyde solution (Sigma). The nuclei of cells were counterstained with DAPI (1mg ml_1) for 15 min. Images were obtained by fluorescence microscopy (BX61, Olympus, Japan).

Statistical analysis

Data analyzed by GraphPad PRISM version 8 (GraphPad, San Diego, CA). All results were collected with three repetitions and the values were display as the mean ± standard deviation. A Students t-Test and ANOWA test were used for comparing two independent groups and multiple groups respectively.

RESULTS

Berberin standard curves

The standard curves of BBR were obtained to calculate the drug loading rate on PLGA polymer nanoparticles. The standard curve of BBR in water and PBS (Fig 1A, B) was plot at wavelength

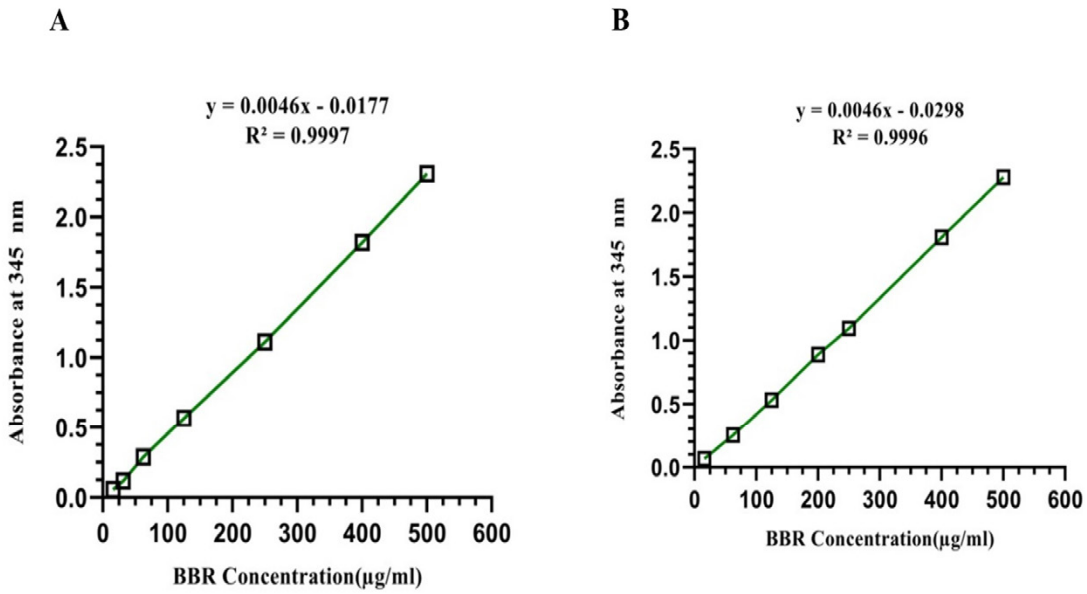

Fig. 1. A, Standard curve of Berberin in PBS buffer. B, Standard curve of Berberin in water

of 345 nm (maximum absorption wavelength of Berberin) using a spectrophotometer. Also, the BBR line equation and and R-squared value (R^2) was calculated in each graph to investigate the drug loading rate and release rate.

Selection the optimal formulation of PLGA polymer

In order to select the optimal formulation to achieve nanosystem with acceptable encapsulation efficiency (EE) and controlled release, various factors were investigated. The ratio of drug to polymer was one of the effective factors in EE. As the ratio of drug to polymer increased, the drug loading rate had a significant increase, but this factor remained constant after a certain amount. Polymer solvent was another factor which affecting the drug loading rate in PLGA polymer. The use of chloroform reduced the drug loading rate due to its rapid evaporation and volatility. However, the use of dichloromethane solvent showed an effective role in increasing the drug loading rate on polymer nanoparticles, so that this ration was doubled compared to chloroform. The sonicate time of the emulsion during the synthesis of nanoparticles was also an important factor in drug loading rate. Increasing the sonicate time of the polymer emulsion increased the drug loading rate. Also, the use of well-molar ratio of PVA in the synthesizing of polymer nanoparticles increased the cohesion and reduced the size of nanoparticles, which was an important factor in choosing the optimal formula. According to Table 2, F7 formula with an encapsulation efficiency 84.2±1.73, a %load 8.42 and a maximum drug release rate of 61.46±0.67 from nanoparticles in 48 hours was selected as the optimal formulation.

Size, polydispersity index (PDI) and zeta potential of nano particles

Based on the results obtained from Malvern Zeta Sizer, the average size of polymer nanoparticles in this study was 234 ± 2.48 nm and PDI was 0.324 and its zeta potential was -8 ± 1.35 mV after loading of bebrin into nanoparticles. Also, by comparing the average size of nanoparticle before and after BBR loading (Fig. 2C, D) and also the average of their zeta potential before and after BBR loading (Fig. 2A, B), it can be concluded that by loading BBR into nanoparticles, the average nanoparticle size and their surface charge slightly increased but this difference is not significant.

SEM and AFM imaging of PLGA polymer nanoparticles

In the images, imaged by SEM and AFM (Fig. 3A, B), it was determined that the nanoparticles had a smooth surface without any aggregation and spherical shape and in general it can be concluded that the nanoparticles had a suitable morphology to deliver the drug to the target tissue.

Table 2. Encapsulation Efficiency, load and release rate in 8, 24 and 48 hours in different synthesized formulations. *: optimal formula

FORMULA CODE	ENCAPSULATION EFFICIENCY (%EE MEAN SD)	%LOAD	%RELEASE (8H)	%RELEASE (24H)	%RELEASE (48H)
F1	10.1±0.06	0.1	13.2±1.11	20.4±0.85	27.1±1.01
F2	19.3±1.43	0.19	10.4±1.04	23.5±0.48	22.6±0.29
F3	32.5±2.07	0.13	11.8±0.5	30.3±0.92	33.28±0.57
F4	75.6±2.4	0.3	22.5±1.59	52.14±2.1	49.11±0.56
F5	77.5±1.18	0.31	19.2±0.96	42.8±0.68	48.27±0.99
F6	82.9±2.5	8.29	37.51±0.72	55.2±1.36	57.9±0.56
F7*	84.2±1.73	8.42	30.4±0.35	53.17±2.05	61.46±0.67
F8	80.4±2	8.04	33.45±0.71	49.18±0.57	58.31±0.52

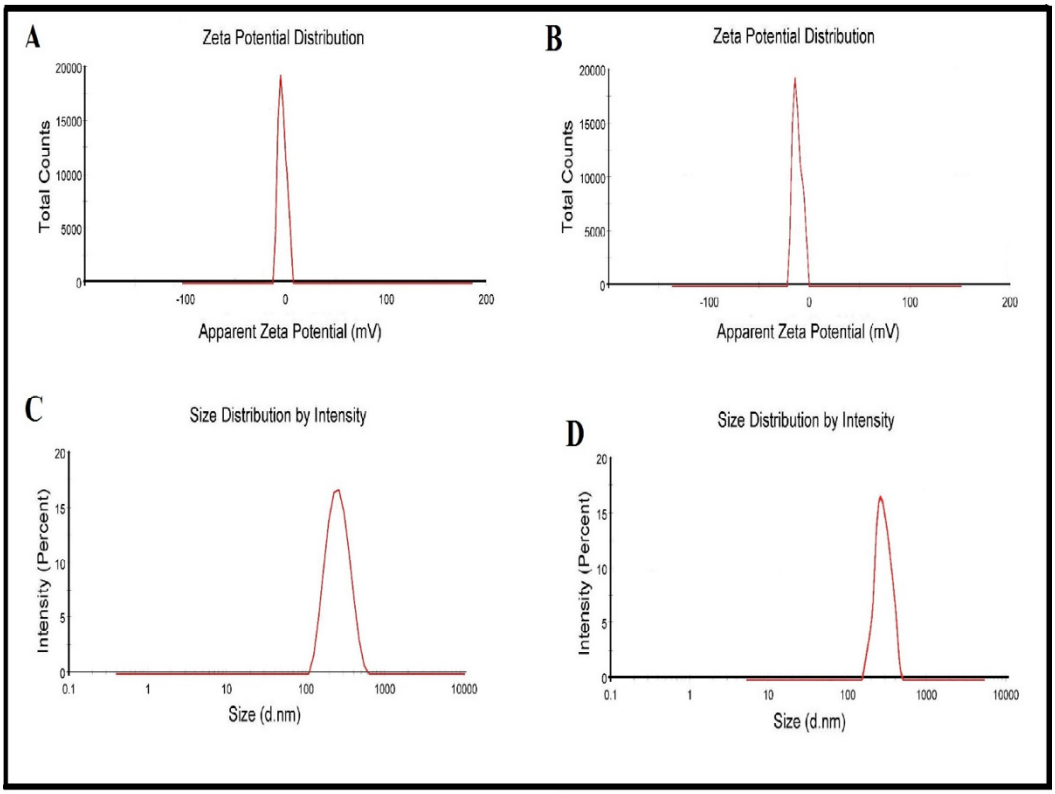

Fig. 2. A, zeta potential distribution of optimal formula before loading berberin. B, zeta potential distribution of optimal formula after loading berberin C, size distribution of optimal formula after loading berberin D, size distribution of optimal formula before loading berberin.

The data obtained from the SEM microscope also demonstrated that the mean diameter PLGA-BBR was coordinate with the results of DLS.

FTIR spectrum evaluation

To investigate the interaction between berberin and PLGA polymer FTIR technique was used. According to Fig 3, all characteristic peaks in blank PLGA spectrum repeated in PLGA-BBR with minor changes. For example, the 3450 cm^{-1} index peak, which represents the functional group O-H, was expressed after loading the BBR with a slight difference in the 3436 cm^{-1}. Also, peak 2925 cm^{-1}, which represents the alkaline group and is the result of rotation around axis C-H, was expressed after loading the drug in 2932cm^{-1}. So, these data prove the presence of berberin in PLGA nanoparticles and also demonstrate that there is no chemical interaction between the drug and the synthesized system and the drug maintained its natural nature.

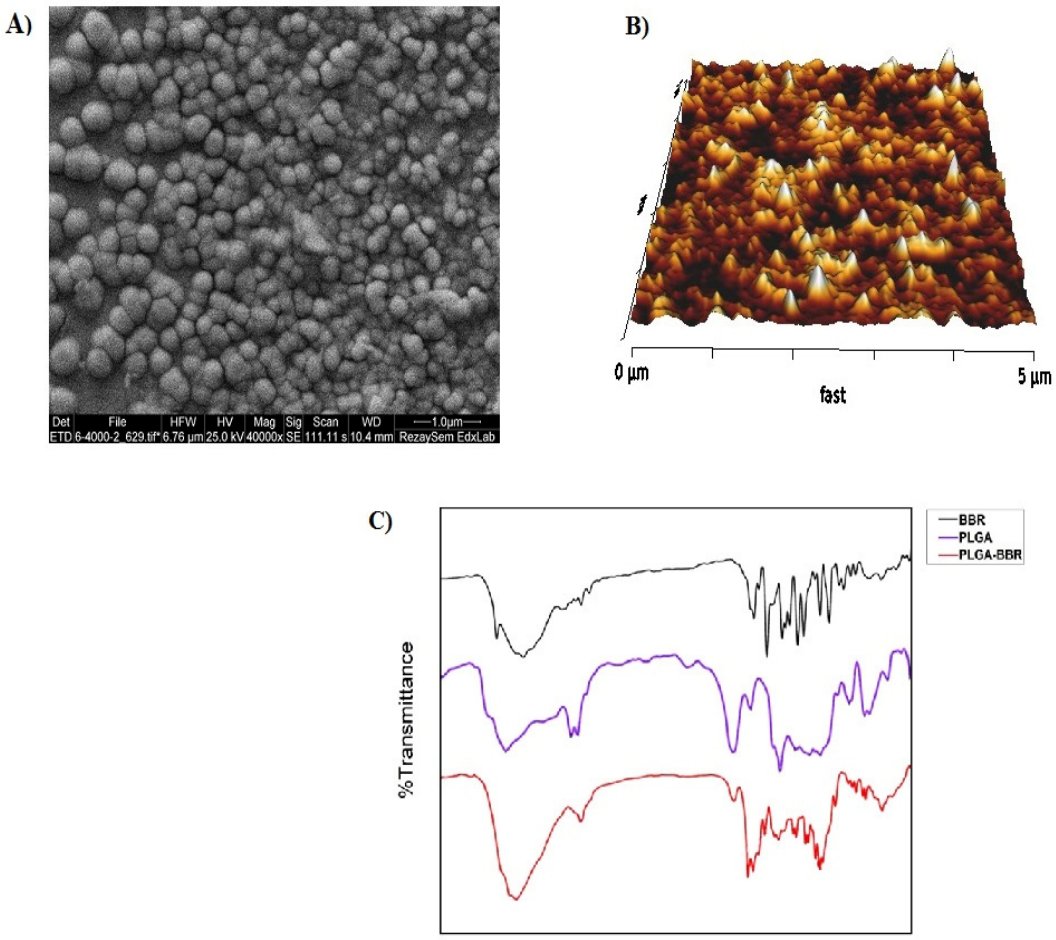

Fig. 3. A, Scaninig electron microscopy (SEM) photograpy PLGA-BBR. B, Atomic forces microscopy (AFM) photography of PLGA-BBR. FTIR spectra of free form of berberin, blank PLGA and PLGA-BBR from top to bottom, respectively.

In-vitro drug release study

Release pattern of BBR from PLGA polymer nanoparticles which was performed by dialysis method at 37° C and 42 ° C and at PH = 4.7 and PH = 5.2, which indicates normal and cancerous cell conditions, in two different time periods was shown in Fig 4A. Release pattern follows a two-phase pattern, which in the first phase had a rapid release and gradually enters to a slow release phase, which indicated the semi-targeted kinetics of PLGA polymer. Moreover, the release rate of the drug at 42 ° C is much higher than the release rate at 37 ° C (Fig 4B), which is important in controlled release into cancer cells.

Cellular uptake

To investigate the cellular uptake behavior of PLGA-BBR particles in on MCF-7 cells as a cancer cell model and HFF cells as a model for normal human cells, cellular uptake assessment was done by the fluorescence intensity detection method. Fig 5, demonstrates successful delivery of PLGA-BBR to normal and cancerous cells. DAPI (blue), was used in order to nucleus staining. Berberine, due to its fluorescent nature, causes the cellular cytoplasm to turn green on fluorescent imaging.

Cytotoxicity assay

The cytotoxicity of free form of BBR, blank PLGA and PLGA-BBR on MCF-7, HFF and MCF-10A cells was investigated by MTT assay. MCF-7 breast cancer cells were treated with different concentrations (15.6, 31.25, 62.5, 125, 250, 500 µg/mL) of free form of BBR and PLGA-BBR for 48 hours. The results showed that the IC_{50} of free form of BBR is 80.18 µg/mL on MCF-7 breast cancer cells, while the IC_{50} of PLGA-BBR is 42.39 µg/mL. The results showed that the IC_{50} of PLGA-BBR was

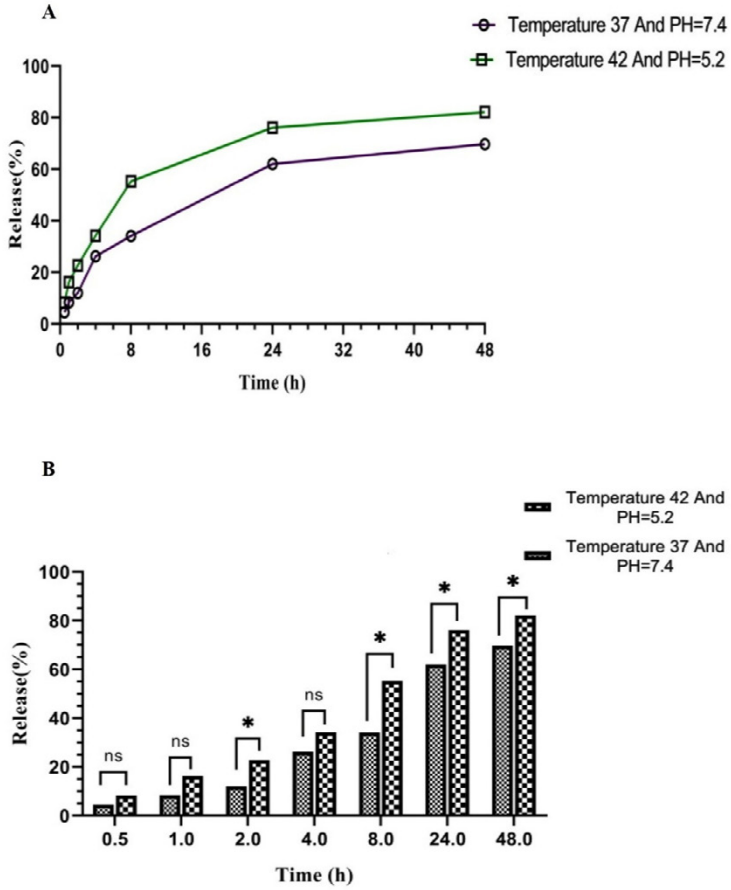

Fig. 4. A, The in vitro release profile of berberin from PLGA polymer nanoparticles in different conditions of temperature and pH. B, Differences in drug release rates from PLGA polymer particles at different times, in various different temperature and acidity conditions. *: P-value<0.05 And ns: there is no significant difference.

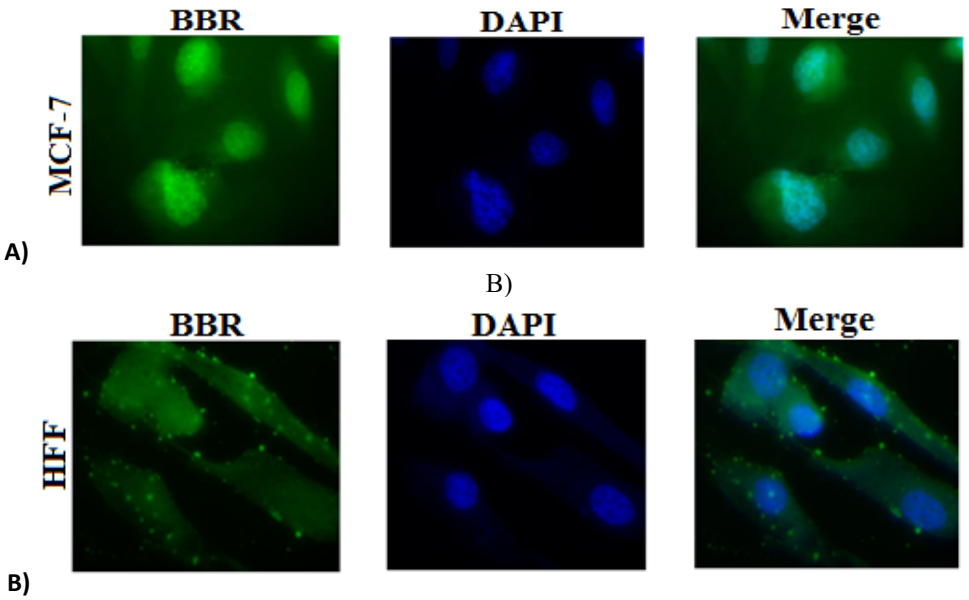

Fig. 5. Cellular uptake images of MCF-7 and HFF cells in order to investigate cellular uptake of PLGA-BBR

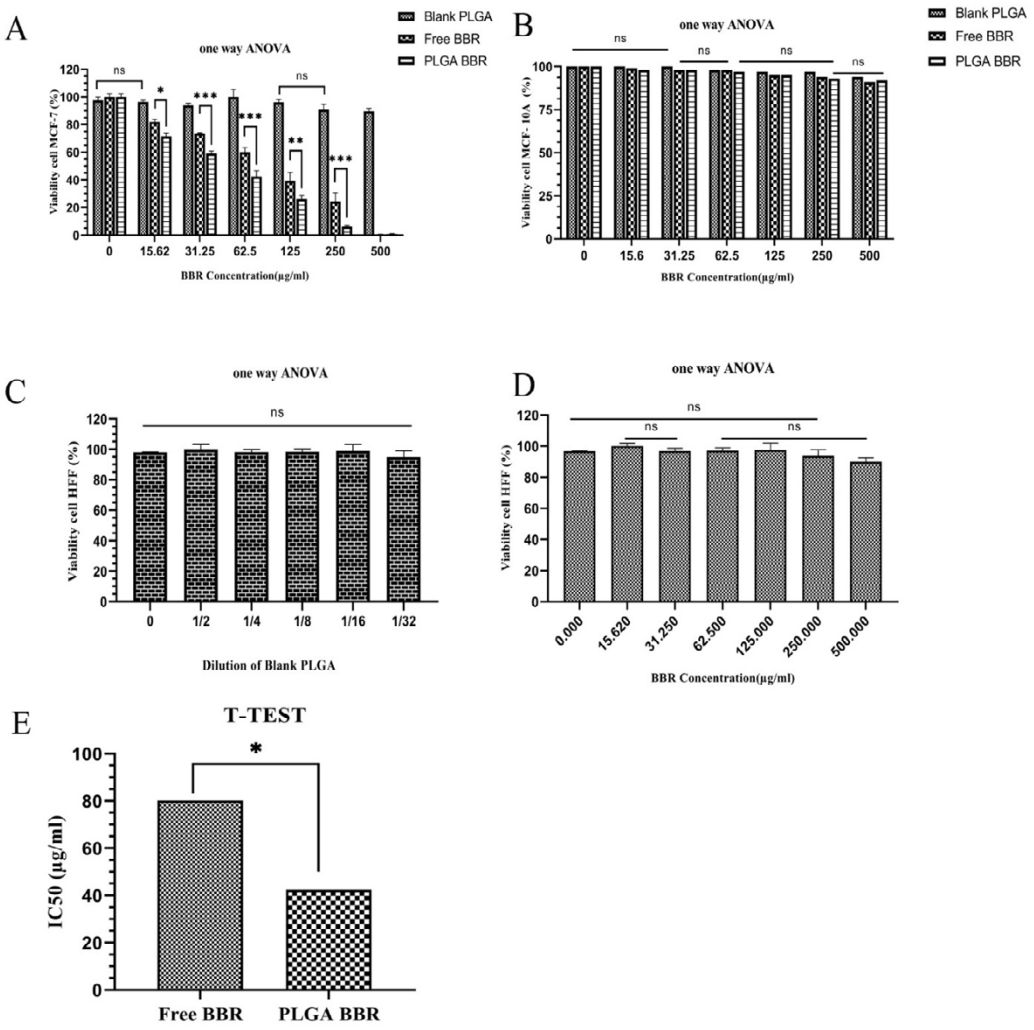

Fig. 6. A, Comparing Cell viability rate of MCF-7 breast cancer cells after treatment with various concentration of free BBR, blank PLGA and PLGA-BBR. B, Comparing cell viability rate of MCF-10A normal mammalian breast cells after treatment with various concentration of free BBR, blank PLGA and PLGA-BBR. C, Comparing cell viability of HFF cell after treatment with various dilution of blank PLGA. D, Comparing cell viability rate of HFF cells after treatment with various concentration of BBR. E, Comparing IC_{50} free form of BBR and PLGA-BBR on MCF-7 breast cancer cell. ns: no significant difference. *: P-value <0.05. ***: P-value <0.0001

lower than the IC_{50} of free form of BBR at specific concentrations. In other words, encapsulating BBR showed a greater effect on the mortality of MCF-7 cancer cells. Also, blank PLGA did not show any cytotoxicity effect on the cancerous cells. On the other hand, cytotoxicity study of BBR effects on HFF and MCF-10A cells in different concentrations showed that BBR had no negative effect on the growth of healthy human cells and was completely safe. Also, cytotoxicity study of blank PLGA effects at various concentration on HFF cells showed that blank PLGA has no toxic effects on normal cells (Fig. 6).

DISCUSSION

Cancer is the leading cause of death and one of the main obstacles to increasing life expectancy in any country in the 21st century (17). According to reports from the International Agency for Research on Cancer, based on geographical diversity in 20 regions of the world, it was found that in 2018, about 1.18 million new cases of cancer were detected, of which 6.9 million were fatal (18). Ineffectiveness of treatment methods in advanced stages of cancer is one of the main reasons for rising mortality rates of cancer. Chemotherapy and radiation therapy are the current treatments for

cancer with adverse side effects, because after metastasis, the removal of cancer cells using surgery is not targeted and reliable, and this approach can affect the normal tissues of the body. On the other hand, chemotherapy and radiation therapy, in addition to affecting cancer cells, also affect normal cells. Myeloid suppression and neurotoxicity, anaphylaxis, anemia, intoxication, cardiac toxicity, mucositis, pain, allotherapy, anorexia are some of the side effects of chemotherapy (19). In this regard, nanotechnology has entered in the field of cancer treatment. Nanotechnology is a promising window in science that focuses on targeted drug delivery systems and the transport of small and large effective molecules in the treatment and diagnosis of cancer. Recently, pharmaceutical companies have made large financially and temporally investments, to formulate herbal medicines and replace them with chemical drugs, because for the treatment of chronic diseases requires long-term drug therapy, which the side effects of chemical drugs are unpredictable and this is the reason that the WHO recommends the use of herbal medicines. Herbal extracts are widely used in traditional medicine due to their availability and affordable price (20). BBR extract may suppress tumor progression by suppressing abnormal cell proliferation, cell cycle arrest, and induction of apoptosis. Studies have also highlighted the function of BBR in inhibiting tumor cell invasion and angiogenesis, which in turn fight against cancer metastasis. BBR has also been shown to inhibit the growth of cancer cells more than normal cells. This function is due to various biochemical mechanisms, including: the induction of apoptosis and autophagy and interaction with DNA, which potentially leads to DNA damage and altered expression of cancer cell genes (21, 22). The aim of the present study was to synthesize PLGA polymer nanoparticles containing Berberine and evaluate its effect on MCF-7 cancer cells and healthy HFF cell and normal breast mammalian cell MCF-10A. Among the factors that has a great impact on the loading rate of nanoparticles is the suitable solvent. Using chloroform as a solvent in the synthesizing of PLGA nanoparticles containing berberine, we concluded that PLGA polymer dissolved easily in chloroform but due to the highly volatility of chloroform, it evaporated during synthesis. On the other hand, after a steering time of 24-16 hours, a large volume of emulsion evaporated and the remaining amount of emulsion containing nanoparticles was less than expected. Berberine loading rate in PLGA polymer nanoparticles was between 25% and 35%. Meanwhile, the use of dichloromethane solvent (DCM) in the synthesis of nanoparticles containing berberine showed a high loading rate. The volume of the final emulsion was also significant after the 24-hour gentle steering. The loading percentage of berberine in PLGA nanoparticles, based on the use of DCM, was 85%, which was acceptable. Another factor influencing the loading rate of berberine in PLGA nanoparticles is the drug polymer ratio. Accordingly, in this study, we considered the ratios of 1/50, 1/25,1/10 in synthesizing of nanoparticles. The results showed that the ratio of drug to polymer was directly related to the loading rate of berberine in nanoparticles, so that the loading rate of nanoparticles increased from 35% to 85%. The drug release profile from PLGA showed a stable, regular, and continuous two-phase release pattern after an initial explosive release phase in the first 8 hours. In the first phase, which lasted 24 hours, the drug release pattern showed a relatively linear pattern with increasing free drug concentration. This means that the release of the drug from the nanoparticles has been going on at a constant rate over this period of time. By the end of this step, approximately 62.18% of the total drug loaded in the nanoparticles was released into the buffer. After this phase, we were faced with a stage of slow release rate so that the release pattern gradually moves towards linearity. By the end of this phase, approximately 69.79% of the drug was released at 48 hours. Also, the size of PLGA polymer nanoparticles was in the nano-range and averaged 234 nm. Among the factors that have a great impact on the size and stability of nanoparticles, the coating of polyvinyl alcohol (PVA) emulsifier has a great impact on the size, charge and stability of PLGA polymer nanoparticles. PVA increases the cohesion of nanoparticles by creating a protective layer around them and prevents the rapid omission of the solvent, and of course, excessive use of PVA causes agglomeration of particles. On the other hand, it has been demonstrated that in addition to the PVA concentration, its molecular weight is also one of the determining factors in the size of nanoparticles. In addition to its stabilizing role, PVA also increases the viscosity of the solution (23). Therefore, with increasing PVA, the stability also increases and in turn reduces the size of nanoparticles. Another influential factor in

nanoparticle size is the duration of emulsion sonicate during nanoparticle synthesis (24). Ultrasonic is used to produce small sizes with homogeneous distribution and high encapsulation efficiency. Zeta potential changes were also monitored during the optimization steps. Theoretically, large negative or positive values (numerical value; less than 30 mV) of zeta potential means higher colloidal stability due to the higher electrostatic repulsion but biologically, particles with too high a negative or positive zeta potential are subject to detection and clearance from the body. A zeta potential of between 5 and 15 mV is usually considered optimal for nanomedical systems, because the zeta potential of most cells is in this range. The importance of the negative zeta potential is that it prevents non-specific interactions with blood components and thus reduces the risk of opsonization and scavenging by the immune system (25). In 2008, Ozkan et al. Showed that due to the treatment of mcf-7 breast cancer cells (which had a zeta potential of -20.3 mv) with nanoparticles with a zeta potential of -13.5 mv, the zeta potential of the cells became more negative after 30 minutes, 4 hours and 24 hours and decreased to -24.5, -25.4 and -26.3 mV, respectively. Electron microscopy images also confirmed attachment of nanoparticles to cells and showed nanoparticles entered to 7-MCF cells through endocytosis. They also observed the endocytosis of negatively charged nanoparticles into cells with negative surface charge, which logically should not be possible due to electrostatic repulsion, could be mediated by some proteins. Therefore, the negative charge of nanoparticles in the range close to the surface charge of target cells can be favorable for their cellular uptake (26). The average zeta potential of optimal formulation in this study was (8-), which is in the desired range. During the optimization, although with decreasing the size of nanoparticles, the numerical value of their negative charge almost doubled, but this increase did not follow a regular pattern at different stages. However, this effect may be related to the be more density and more cohesive polymer network in the structure of these nanoparticles. In general, encapsulation of effective materials greatly enhances its effectiveness for greater bioavailability. In this study, encapsulation of berberine in PLGA polymer nanoparticles increased the cytotoxicity of berberine in MCF-7 cancer cells compared to the free form of drug. Various researches have proven that encapsulation of anti-cancer agents within PLGA can improve their anti-cancer effects. For example, a study on curcumin encapsulation in PLGA nanoparticles showed that curcumin-encapsulated PLGA nanoparticles significantly improved healing of normal and diabetic wounds by twice as much as free form of curcumin, ie encapsulation of curcumin into PLGA, in addition to light protecting, increases stable release and solubility of curcumin and thereby increasing its effectiveness (27). In another study, the effect of quercetin encapsulation in PLGA polymer system was investigated. Quercetin is a highly hydrophobic substance and has limited biological use due to its poor permeability and rapid deposition. In this study, demonstrated that charged PLGA-quercetin nanoparticles can overcome low hydrophobicity of quercetin and improve its effects. NPs of PLGA containing quercetin can significantly inhibit activation of COX2 and NF-KB pathway in the Hacat cell line. In other words, these findings indicate a positive effect of encapsulating on effective substances with low bioavailability (28). In one study, the effect of purified berberine was examined on mcf-7 cancer cells and normal human epithelial cells (mcf-12F). Then, its cytotoxicity was evaluated using MTT assay and it was shown that berberine had a significant effect in inhibiting prolifration of cancer cells while it had no effect on healthy breast epithelial cells and berberine was able to kill half of cancers cells at concentration of 25 mM (29). The anti-cancer effects of berberine have been well demonstrated in many different types of human cancers. In general, the results have shown that the concentration of berberine has key role in the survival rate of cancer cells (30). In the present study, we compared the effect of free form of berberin and PLGA polymer nanoparticles containing berberin on MCF-7 cancer cells and healthy foreskin fibroblasts (HFF) cells using MTT assay. In this study, we treated MCF-7 and HFF cancer cells with the same concentrations of free form of berberine and PLGA containing berberine. The results showed that encapsulation of berberine in PLGA polymer nanoparticles improved its effects on MCF-7 cancer cells, so that, IC50 of berberine was reduced from 76 mg in the free form to 40 mg in the encapsulated form. On the other hand, free form of berberine and PLGA-BBR showed the lowest cytotoxicity on healthy foreskin fibroblast (HFF) cells, which confirms the safety of berberine and PLGA polymer nanoparticles on

normal cells. The effect of blank PLGA nanoparticles on MCF-7 cancer cells was also investigated, which showed low effect of nanoparticles on cancer cells and thus confirmed the cytotoxicity of PLGA-BBR associated with the effect of berberine on cancer cells.

CONCLUSION

The aim of this project was to synthesize and characterize PLGA polymer nanoparticles containing berberine to increase its effectiveness on MCF-7 cancer cells. Based on the results, it was found that loading berberine on PLGA polymer nanoparticles is much more effective than free form of berberine on MCF-7 cancer cells, more ever, this formulation did not cause a negative effect on normal breast mammalian cells and human foreskin fibroblast cells. In other words, encapsulating effective herbal anti-cancer ingredients can be a beneficial and promising way to improve their effects and also increase their bioavailability and reduce drug resistance and drug toxicity in modern cancer chemotherapy.

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CONFLICTS OF INTEREST

There is no any conflicts of interest to disclose.

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