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Preparation of silibinin loaded pegylatedniosomal nanoparticles and investigation of its effect on MCF-10A human breast cancer cell line

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ABSTRACT

Silibinin possesses a broad spectrum of biological applications such as anticancer activities; however, poor bioavailability reduces its efficacy at the tumour sites. In the current study, silibinin was encapsulated in nanoniosomal particles in the presence of polyethylene glycol and estimated its efficacy against breast cancer in vitro. Nanoniosomalsilibinin was synthesized using the reverse phase evaporation method and characterized for shape morphology, particle size, zeta potential and drug-release characterises. In the next step, MCF 10A breast cell lines were used to evaluate the rate of nanoniosomalsilibinin cytotoxicity. In these investigation, the particle size and zeta potential of the niosomal nanoparticles were calculated 322.3±17.6nm and -18.4±1.1mV, respectively. Drug loading and encapsulation efficiency were evaluated 92.8±7.3 and 14.3±1.3, respectively. The drug release study confirmed the power of nanoparticles to drug retention by 64.1± 5.9 release in a period of 34 hours. MTT assay revealed higher cytotoxic efficacy of silibininnanoniosomal particle than free silibinin on MCF 10A cell lines. Taking collectively, the present study suggests that silibinin-loaded nanoniosomes can be applied as an effective drug delivery system to cause a usefully chemopreventive response in order to the treatment of breast cancer.

Keywords: Breast cancer, Silibinin, Niosomal nanoparticles, MCF-10A

INTRODUCTION

Breast cancer is the most popular cancer among women which evaluated for 23% of the total cancer cases and 14% of the cancer deaths [1].Currently, there are wide spectrums of cancer therapy, the most prevalent of which are radiotherapy, chemotherapy, and surgery or a combination of these techniques. Their effectiveness, as a result of systemic toxicity of radiotherapeutic drugs or chemotherapy, in cancer treatment has been a limitation [2]. Chemotherapy or chemoprevention via non-toxic factors could be one approach for reducing the incidence of cancers. Many natural agents have exhibited chemotherapeutic potential in a variety of bioassay systems[3]. Therapeutic intervention by developing novel phytochemicals which are cost-effective and non-toxic in cancer management [4]. Bioflavonoids are a common category of polyphenolic substances that are present in most plants [5]. Niosomes are microscopic structure of two layers. These non-ionic vesicles possess a structure consisting of hydrophilic and hydrophobic moieties together, thereby be able to accommodate drug molecules. Niosomes have been implemented in many pharmaceutical applications. In such therapeutic applications, significant advantages of using niosomescould to decrease systemic toxicity by encapsulation of therapeutic agents and minimize clearance of such factors from the blood stream by slow drug release [6].To develop novel strategies that enhance the therapeutic

efficacy and minimize the systemic toxicity of chemotherapeutic agents, more attempts are being directed towards investigating dietary supplements and other phytotherapeutic factors for their synergistic efficacy with anticancer drugs [2, 7, 8]. Silibinin is one of these plants derived flavonoid present in silymarin extracted from the seeds and fruits of the milk thistle (Silybummarianum) [5]. One of the most potent effects observed in preclinical investigation of silibininis G1 arrest and apoptosis. Silibinin increases dramatically the efficacy of several chemotherapy factors both in vitro[9]and in vivo [10]. Based on the successes and the advantages of niosomes[6],we attempted to investigate the efficiency of nanoparticles in delivering silibinin and improving its anti-proliferative effect on the breast cancer cell line MCF-10. To this, silibinin was encapsulated into pegylatedniosomal nanoparticles by thin film hydration technique and then evaluated its inhibitory effect on MCF-10 cell line and compare with inhibitory effect of free drug. Polyethylene glycol (PEG) was utilized in order to its efficacy on the developing of stability.

MATERIALS AND METHODS

Span 60, cholesterol, and polyethylene glycol 2000, poly sorbet-80 and silibinin from Sigma Company (Germany) were obtained; MCF 10A cell line was purchased from cell bank of Pasteur Institute in Iran.

Preparation of nanoparticles containing drug

Briefly, approximately 7 mg of span 60, 6 mg of poly sorbet-80, 30 mg of cholesterol, and 13 mg of silibinin were dissolved in 40 ml chloroform by stirring for 2 hours at 120rpm. After perfect dissolving, the solvent was removed using rotary evaporation instrument in 60˚C and 150 rpm. Then, 14 mg of polyethylene glycol 2000 was dissolved in 20 ml Phosphate Buffer Saline (PBS, pH=7.2).One hour after the formation of gel layer at the bottom of flask, the gel layer was dissolved in buffer by stirring at 220rpm, perfectly. Finally, in order to homogenize prepared vesicles, the resultant suspensions were sonicated at 55 HTz and room temperature for 5 min. Blank nanoparticles were prepared with the same technique without adding of the drug (silibinin).

Size determination of nanoparticles:

The size and zeta potential of nanoparticles were determined by Zetasizer instrument (Nano ZS3600, Malvern Instruments, UK). In order to this purpose, 1 mg of the formulation was dissolved in 100 ml of PBS. After determination of its absorption in 633 nm, the zeta potential and mean diameter of the nanoniosomes were measured using a Zetasizer instrument.

Surface morphology

Scanning electron microscopy (SEM) (KYKY-em3200) was utilized to determine the morphology of the prepared nanoniosomes.

Encapsulation yield

The amount of encapsulated drug was determined as follows: The suspension containing nanoniosomes was centrifuged for 30 min at 21000 rpm and 4˚C.Then, the supernatant was separated and the absorbance of each drug formulation was read at λ 290nm.The amount of encapsulation and drug loading was determined according to the below formulae.

$$\text{Drug encapsulation efficiency (\%)} = \frac{\text{Initial drug concentration}\left(\frac{\text{mg}}{\text{ml}}\right) - \text{supernatant drug concentration}\left(\frac{\text{mg}}{\text{ml}}\right)}{\text{Initial drug concentration}\left(\frac{\text{mg}}{\text{ml}}\right)} \times 100$$

$$\text{Drug loading efficiency (\%)} = \frac{\text{The amount of drug into nanoparticle}\left(\frac{\text{mg}}{\text{ml}}\right)}{\text{Weight of nanoparticle}\left(\frac{\text{mg}}{\text{ml}}\right)} \times 100$$

Drug release studies

The amount of released silibinin was carried out through dynamic membrane diffusion technique. Niosomal suspension containing 6 mg silibinin was poured into a dialysis bag with cut off 10,000 Da. The dialysis bag containing above suspension was floated in a flask containing 24 mL phosphate buffer, pH 7.2. The flask was hold on a magnetic stirrer at 25°C and 120 rpm for 34 hours. At the time interval of 2, 5, 8, 11, 17, 27 and 34, samples of 2 mL was withdrawn and replaced with fresh PBS. The absorbance of each sample was read at 290 nm. The concentration of the free silibinin in each period was determined by using the standard curve.

Cellular cytotoxicity

In order to investigation of cytotoxicity effect of silibinin loaded noisome, 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay was performed and the effect of them compared to cytotoxicity effect of free drug and blank noisomes. For this purpose, MCF 10A cell line was cultured under humidified atmosphere containing 5% CO2 in RPMI-1640 cell culture supplemented by 10% Fetal Bovine Serum (FBS), 100 µg/ml streptomycin, and 100U/ml penicillin. In brief, to each cell line's culture subsequently, 100µl of a suspension containing 12,000 cells was placed in the wells of a 96-well plate and incubated at 37°C with 5% CO2. Cells were investigated at the same concentrations of the nanoniosomalsilibinin formulation, control, and also standard silibinin drug. After 24 hour, the supernatant of the cells was removed, and 100µlRPMI 1640 medium was added. After 48 hours of incubation, the medium containing drug formulation was removed and MTT solution (0.5 mg/ml PBS) was added into each well and incubated at 37°C for 1 hour. The formazan crystals formed were dissolved in 100µlisopropanol 100% and the absorbance was read at 570 nm using ELISA reader (Bio Tek Instruments, VT, U. S.A).This process was repeated three times. Cell viability was evaluated by following formula

$$\%\,\text{Cell Viability} = \frac{\text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{Control}}} \times 100$$

Statistical Analysis

For statistical analysis, SPSS software version 11 was used and P values < 0.05 was considered as significance.

RESULTS

Characterization of nanoparticles

In this study, we achieved considerable success as the silibinin loaded niosome nanoparticle formulation prepared via reverse phase evaporation method. The size and zeta potential of drug loaded nanoparticles were calculated 322.3±17.6nm and -18.4±1.1mV, respectively.

Morphology instruction

As shown in figure 1, SEM images exhibited the nanoniosome containing silibinin formed nanoparticles in crystal shape.

Drug loading and encapsulation efficiency

The results of encapsulation and loading efficiency were evaluated 92.8±7.3 and14.3±1.3, respectively. In other words, 92% of used drug become associated with nanoparticles and silibinin accounts for 14.3% of nano drug weight.

Cytotoxicity assay and viability

As shown in Figure 2, cell viability was significantly decreased in a dose-dependent manner after exposure of breast cell lines to the free drug and its nanoliposomal formulation using the MTT assay. Also, the amount of IC_{50} of nano drug was lower than free drug on the MCF 10A cell lines. This result indicated that liposomal nanoparticles enhanced the efficacy of silibinin in comparison to the free drug.

Drug release

The results of drug release demonstrated a sustained release pattern. After 34 hours, 64.1±5.9 percent of niosomes were released. Regarding the figure 3, the drug release formulations takes place in ascending order; however, time after drug release is slower.

DISCUSSION

Nanotechnology isbeing carried out in the treatment of different diseases through nanoparticulate drug delivery system, because it supplies several benefits over conventional drug delivery system[11].This study reported that the niosomal formulation could be one of the promising delivery systems for the breast cancer treatment by using drug silymarin. Reverse phase evaporation technique is a suitable method for preparation of silibinin loaded niosomal nanoparticles which was confirmed by appropriate properties of nanoparticles. PEG was applied in this investigation by reason of proper stability in blood circulation, low immunogenicity, water solubility and antigenisity and also the ability of extend the period of drug release [12].Drug release is a strongly influence factor in drug delivery systems[13]. Ina survey results which was performed by Dadgar et al. on the subject of toxicity effect of pegylatednanoliposomalartemisinin on breast cancer cell line, was observed that the amount of drug release was estimated about 5.17% during 48 hour. Their results illustrated that pegylatednanoliposomal increased artemisinin cytotoxicity compared with standard drugs[14]. In this research, a sustained release of silibinin from nanoparticles was here perceived. The release was initiated with a burst release indicating the release of adsorbed drug from

nanoparticles. While 16 percent of drug was released after 34 hours, this value was found to be 25 percent for free drug. As mentioned above, the power of retention capability may be partially come from the existence of PEG in the formulation. Moreover, augment the stability of particles enhance the efficacy of drug by develop the drug delivery to cancer cells.

Particle size as well as zeta potential (ZP) of nano drug have a significant effect on the various properties of nanomedicine delivery systems [15].Zeta potential reveals information about the surface charge and stability of the nanoparticle formulation. The positive charge of nanoparticles can be easily absorb to negatively charged cellular lipid bilayer and then cooperates to efficient intracellular trafficking [16]. In the case of negatively charged in comparison with positively charged (up to 3/6% of the injected dose)nanoparticles, studies on liposomes illustrated that the negatively charged liposomes does not exhibit proper accumulation in the lymph nodes (1/2 % of the injected dose) and also negatively ones reveal lower medicine entrapment [17], and some degree of medicine release during storage [18]. In addition, the negatively charged nanoparticles compared to positively charged nanoparticles, disappear more slowly from the blood stream and remained in blood stream for a longer time [19]. Also, negatively charged nanoparticles had lower cytotoxicity compared with positively nanoparticles. Asobserved in present study, zeta potential values of the silibinin nanoparticles possessed negative potential of -18.4±1/1 mV. Hence, both positive and negative zeta potential have own their efficiencies. In general, the surface charge density of nanoparticles should be optimized for beneficial intracellular delivery of encapsulated medicine and low toxicity [15].

Drug encapsulation efficiency equal to 92.8±7.3percent proved the appropriate efficiency of technique. High encapsulation efficiency is advantageous, because it transports proper amount of drugs at the target position and boosts the homing and residence time of the medicine. The therapeutic effectiveness of the nanoparticles would mainly rely on duration of their accessibility and the dose on the intracellular target. A carrier could slowly release the nanoparticle at the target site of action as well as could undergo its prolonged therapeutic effects. The sustained release pattern of the medicines from the nanoparticles might be due to the disordered crystalline shape of the drug into the formulation[20].

The cytotoxicity effects of the nano drug in comparison with free silibinin illustrated the higher efficiency of nanoniosoamlsilibinin in destruction breast cancer cells. This fact may be due to the amphipathic structure of nanoniosoaml drug that is similar to the bilayer structure of cell membrane. Therefore, nanoniosoaml drug can easily penetrate to tumour cell and release the drug directly into the target cell, and causing the death of MCF 10A cell lines [6].

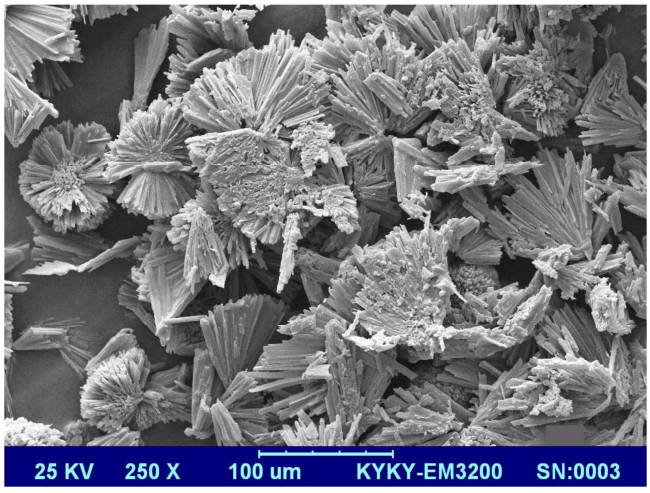

Figure 1: scanning electron microscope images of nanoparticles silibininniosomal

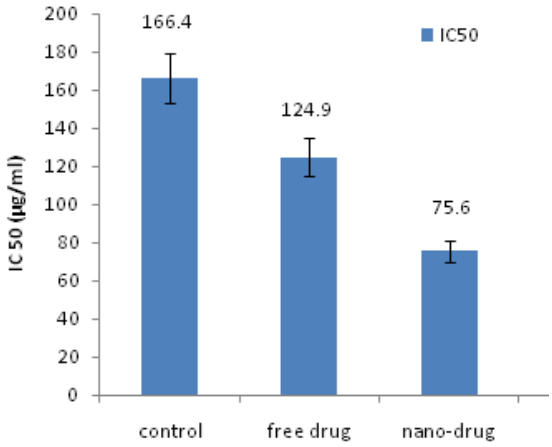

Figure2: The cytotoxicity Nanoniosomesilibinin, silibinin freed and control over cell lines fromhuman breast cancer MCF 10A

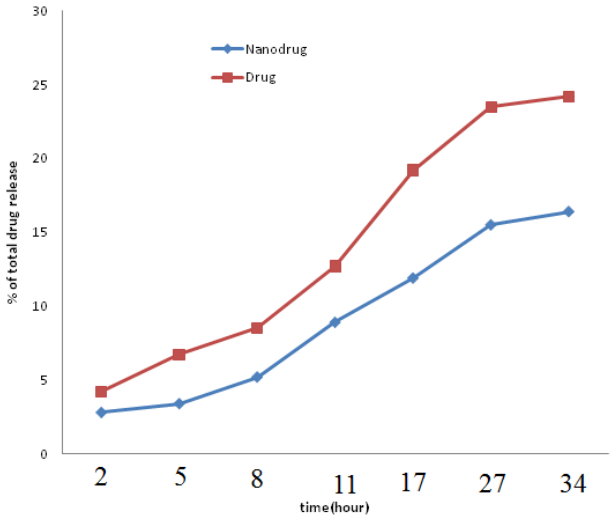

Figure 3: The release of nanoparticles silibininniosomal

CONCLUSION

In this current study, thin film hydration technique was used successfully for preparation of silibinin loaded niosomal nanoparticles. The size, zeta potential, drug loading, encapsulation efficiency and drug retention capability of nanoparticles containing drug were calculated and recognized appropriate. The investigation was followed by evaluation of the efficacy of nanodrugon MCF 10A cell line which demonstrated superior cytotoxicity of nanoniosomal particle compared to free drug. The result showed that the pegylatedniosomal nanoparticles were suitable carriers for silibinin delivery to this cell line.

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