

Original Article

Evaluation of the Efficacy of Niosomal Curcumin Nanoformulation in Cancer Therapy

Ashraf Alemi^{1*}, Mohammad Farrokhifar², Mojtaba Haghi Karamallah³, Majid Farrokhifar⁴, Zakieh Entezari Nasab⁵, Ali Farrokhifar⁶

ABSTRACT

During the past decade vesicles as a tool to improve drug delivery, has created a lot of interest amongst the scientist working in the area of drug delivery systems. Based on their biodegradable, biocompatible and non immunogenic structure, niosomes are promising drug carriers that are formed by self-assembly of nonionic surfactants and cholesterol in an aqueous phase. Curcumin (Cur), a natural polyphenol found in *Curcuma longa*, has been utilized in multiple medicinal areas from antibiotic to antitumor treatment. However, the chemical structure of curcumin results in poor stability, low solubility and rapid degradation in vivo, limiting its clinical utilization. To address these problems, we have prepared a niosome system composed of nonionic surfactants polyoxyethylene sorbitan monostearate and cholesterol by thin film hydration method. The niosomal curcumin was evaluated for anti-cancer efficacy in prostate cancer cell line (PC-3) by MTT assay. Cur was encapsulated in the niosomes with a high entrapment efficiency of $98.4 \pm 0.4\%$. Average particle size was found to be 127.5 ± 1.2 nm. Niosomal curcumin (Nio -Cur) exhibited enhanced cytotoxic activity against PC-3 cells compared with free Cur. These results demonstrated that the Nio -Cur system is a promising strategy for the delivery of Cur and prostate cancer therapy.

Keywords: Prostate Cancer, Niosomal curcumin, Cancer Treatment, Chemotherapy

Author Information

1. Department of Clinical Biochemistry, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. Kar Higher Education Institute, Rafsanjan, Iran
3. Biotechnology Research Center, International Campus, Shahid Sadoughi University of Medical Science, Yazd, Iran
4. Department of Pediatrics, Sabzevar University of Medical Sciences, Sabzevar, Iran
5. Department of Pediatrics, Semnan University of Medical Sciences, Semnan, Iran
6. Faculty of Medicine, Aja University of Medical Sciences, Tehran, Iran

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Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other sections of the body (1). Cancer is one of the main causes of mortality worldwide and demonstrates a serious health problem (2). By 2050, according to the World Health Organization (WHO),

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it is anticipated that 17.5 million cancer deaths and 27 million new cancer cases will happen annually (3). Many cancers can be prevented by not drinking too much alcohol, maintaining a healthy weight, eating plenty of vegetables, fruits and whole grains, vaccination against certain infectious diseases, not eating too much processed and red meat, not smoking and avoiding too much sunlight exposure (4).

The prostate is below the bladder and in front of the rectum. The size of the prostate changes with age. In younger men, it is about the size of a walnut, but it can be much larger in older men (5). Prostate cancer is the development of cancer in the prostate. Prostate cancers are slow growing; however, some grow relatively quickly. The cancer cells may spread from the prostate to other region of the body, particularly the bones and lymph nodes. It

may initially cause no symptoms. In later stages it can lead to difficulty urinating, blood in the urine, or pain in the pelvis, back or when urinating (6,7). Older age, a family history of the disease, and race are factors that increase the risk of prostate cancer (8, 9). Prostate-specific antigen (PSA), blood test and a digital rectal exam (DRE) and finally a prostate biopsy are the main methods of diagnosis of prostate cancer (7). Prostate cancer is the most frequently diagnosed non-cutaneous neoplasm in and the second leading cause of cancer-related mortality in men (10,11). Prostate cancer is frequently diagnosed in men between 45 and 89 years of age (12). It is a major cause of morbidity and mortality in Iran. Prostate cancer is becoming an increasingly important public health problem, particularly in countries with trends toward an aging population (13).

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Routine screening, early diagnosis, newer treatment options and the possibility of cure have increased prostate cancer survivorship impressively. There is a huge difference in the rate of incidence of prostate cancer between Western (120 per 100,000 in Northern America) and East Asian countries (less than 10 per 100,000 in Asia). Current therapies (radical prostatectomy, chemotherapy, local radiotherapy or hormone therapy), although successful to treat localized, androgen-dependent prostate cancer, are of limited efficacy against androgen-independent, metastatic disease. Primary prevention appears as an attractive strategy to eradicate prostate cancer. Chemoprevention is a prophylactic method using non-toxic natural or synthetic compounds that reverse, inhibit, or prevent the development of cancer by inhibiting specific molecular steps in the carcinogenic pathway. Curcumin (Cur) is a promising chemopreventive compound (14). Cur (1,7-bis (4-hydroxy-3-methoxy-phenyl)-1,6-heptadiene-3,5-dione; diferuloylmethane) is a natural, yellow

compound found in *Curcuma longa* that is regarded as a natural polyphenolic antioxidant presented in many kinds of herbs (15). This natural compound used as curry ingredient and is used since centuries in Ayurvedic, Chinese, and Hindu medicine systems as a potent anti-inflammatory agent. Cur has been showed various pharmacological activities including anticancer, anti-inflammatory, antioxidant, antimicrobial and anti-rheumatic effects (16). Particularly, Cur has been demonstrated efficacy as an antineoplastic factor for many types of malignancies, including prostate, gastric, colorectal, breast, lung and pancreatic carcinoma (17). Despite of the well-received pharmacological properties, the therapeutic application of Cur has been hampered due to its deficits such as low aqueous solubility at acidic and physiological pH and its degradability in alkaline conditions. Moreover, poor absorption and rapid metabolism of Cur severely limit its bioavailability (18, 19). As a result, researchers have been exploring new strategies for the effective delivery

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of Cur with novel formulations including liposomes, micelles, conjugates, nanoparticles, nanoglobules, solid dispersions, polymeric nanoparticles and micelles (20, 21). Recent advances of nanotechnology have generated many promising drug delivery systems. Niosomes are nonionic surfactant vesicles with a bilayer structure, which have been used to deliver various drug elements such as chemotherapeutic agents, genes, hormones, antigens, and peptides (16, 17). Niosomes have some similarities with liposomes but are composed of nonionic surfactants such as polyoxyethylene sorbitan monostearate (Tween 60), sorbitan monooleate (Span 80), sorbitane monostearate (Span 60) and polyoxyethylenesorbitan monooleate (Tween 80), instead of phospholipids utilized in liposomes. This vesicular carrier system provides protection of the drug from degradation and inactivation caused by immunological and pharmacological effects. Furthermore, niosomes surpass liposomes through low production cost and room-temperature chemical storage requirements. In recent years, niosomes are becoming increasingly accepted as an alternative method of drug delivery to liposomes,

particularly for drugs with poor stability, low solubility, or rapid degradation (22-24). The present study investigated to effectively deliver Cur to tumor cells using niosomes composed of nonionic surfactants polyoxyethylene sorbitan monostearate and cholesterol, in order to improve the solubility and the therapeutic effects of Cur. The synthesis of Cur -loaded niosomes (Nio -Cur) and the physicochemical properties in terms of particle size, zeta potential, and polydispersity index (PDI) were evaluated. Moreover, the in vitro cytotoxicity of free curcumin and Nio -Cur against human prostate cancer cell line (PC-3) was investigated.

Materials and Methods

Materials

Polyoxyethylene sorbitan monostearate, cholesterol and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) were purchased from Sigma Chemical Co (St Louis, MO, USA). Curcumin was obtained from Sigma. Dialysis bags (MWCO 12000-14000) were supplied by Jingkehongda Biotechnology Co., Ltd. (Beijing, China). DMEM medium was purchased from InoClon Company, Iran. Chloroform, methanol and

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other chemicals utilized in this study were analytical grade. PC-3 cell line was supplied from the Iranian Biological Resource Center (Tehran, Iran).

Preparation of niosome and encapsulation efficiency of Cur by the thin film hydration method

The thin film hydration method employed to prepare niosome (25). Polyoxyethylene sorbitan monostearate and cholesterol (80:20 molar ratios) were dissolved in chloroform. Then 0.5 mg.mL⁻¹ Cur was added and the mixture was warmed to 50 °C for 40 min. In continuation, solvent phase was evaporated on rotary evaporator until a thin-layered film formed. The thin film was hydrated with 3000µl phosphate buffer saline. In order to decrease niosomal particle size, the resultant suspensions were sonicated (model UP200St, Hielscher Ultrasonics GmbH, Germany) at 60 HTz and 4 °C for 20 min. Afterwards, free Cur (unloaded) was separated from niosomal Cur (Nio -Cur) by dialysis bags that had a cut-off of 12 kDa.

Size and polydispersity index determination of Nanoparticles

The particle size distribution and polydispersity

index (PDI) of the niosome particles was determined by dynamic light scattering (DLS) using a ZetaPALS zeta potential and particle size analyzer (Brookhaven Instruments, Holtsville, NY, USA).

Analysis of encapsulation efficiency

In order to evaluate the entrapment efficiency, spectroscopic measurements were performed. The Nio -Cur particles were lysed with isopropanol to analyze Cur concentration. The amounts of Cur in niosome formulation were analyzed with a UV spectrophotometer (model T80+, PG Instruments, United Kingdom) at 429 nm (λ_{max}) (26).

Cell lines and culture conditions

PC-3 cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS), and penicillin/streptomycin (1 mg/mL) at 37°C and 5% CO₂ in a humidified incubator.

Cytotoxicity assays

The cytotoxicity of niosomal formulations was determined by the MTT assay (27, 28). Briefly, PC-3 cells were seeded in 96-well plates at 2×10⁴ cells per well. Following attachment 24 hours, the cells were treated with 200 µl fresh medium containing

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serial dilutions of free- Cur solution and niosomal Cur. After incubation for 72 h, 20 μl MTT (5 $\text{mg}\cdot\text{ml}^{-1}$ in PBS) was added into each 96- well plate and incubated for three hour at 37 °C. Finally, the medium was carefully removed and 150 μl of DMSO was added to the each well to dissolve the formazan crystals formed. Absorbance of each well was recorded by EPOCH Microplate Spectrophotometer (synergy HTX, BioTek, USA) at 570 nm. The cytotoxicity of the different formulations was expressed as the Inhibitory Concentration (IC_{50}) value defined as the drug concentration required inhibiting cell growth by 50% relative to the control. The IC_{50} values of free Cur and Nio -Cur were calculated using GraphPad Prism software.

Statistical analysis

Statistical data analyses were performed via GraphPad Prism software and expressed as mean \pm

SD. The student t-test was used when comparing two independent groups. P-Value < 0.05 was considered significant.

Results

Characterization of niosome formulation

The PDI, mean size diameter and zeta potentials of niosomal Cur nanoparticles prepared with Polyoxyethylene sorbitan monostearate and cholesterol were found to be 0.247 ± 0.028 , 127.5 ± 1.2 nm and -6 ± 0.21 , respectively.

Encapsulation efficiency

Encapsulation efficiency of formulation was determined through Cur standard curve in isopropanol (Fig. 1). Percent encapsulation of Cur in niosome nanoparticles was determined to be 98.4 ± 0.4 %

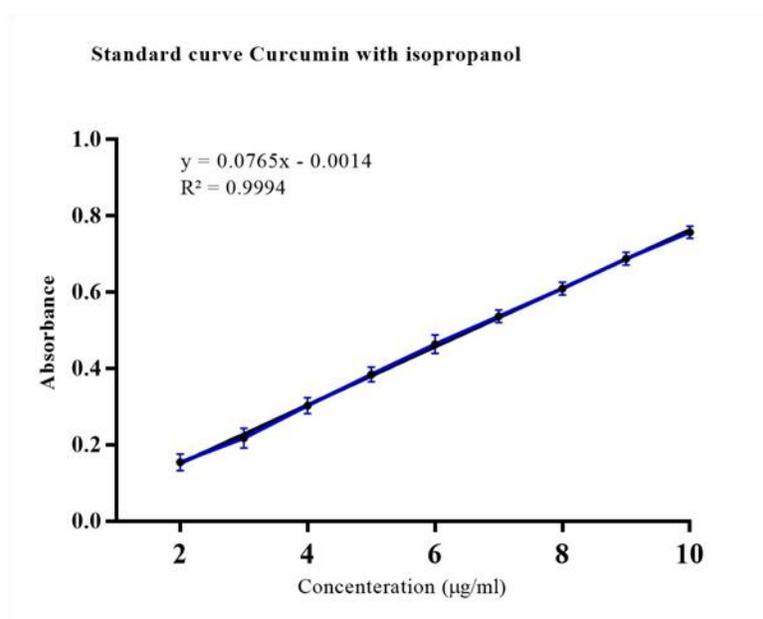


Fig. 1. Curcumin standard curve in isopropanol

Evaluation of cellular cytotoxicity effect

It was observed that toxicity of Cur in niosome formulation prepared with Polyoxyethylene sorbitan monostearate and cholesterol was higher than toxicity of free Cur. Furthermore, determined

IC₅₀ for niosome formulation was 46.37 µg/ml, while the IC₅₀ for free drug was determined to be 60.28 µg/ml (Fig. 2 and Fig. 3, respectively). The results showed that the Nio -Cur formula displayed significantly greater cytotoxic activity on PC-3 prostate cancer cell line in comparison to free Cur.

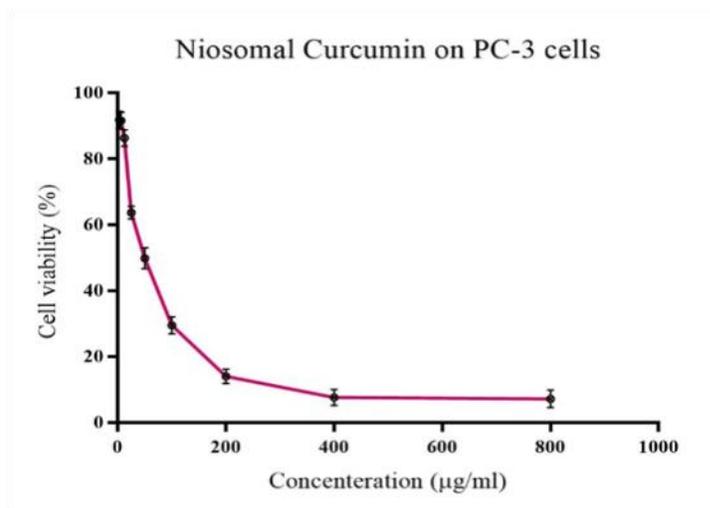


Fig. 2. Cell viability assay of PC-3 cell line after 72 hours of treatment with various concentrations of entrapped curcumin.

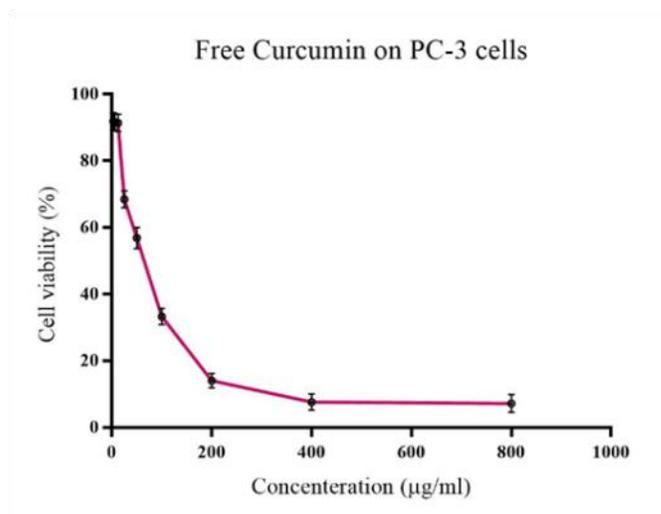


Fig. 3. Cell viability assay of PC-3 cell line after 72 hours of treatment with various concentrations of free curcumin.

Discussion

In the past few decades, considerable attention has been performed to the development of a novel drug delivery system. The aim of novel drug delivery systems is that it supplies the drug at a rate directed by the needs of the body, over the period of treatment, and it carries the drug directly to the tissues. Conventional delivery systems are unable to meet none of these. Novel drug delivery system sustains drug action at a predetermined rate, efficient drug level in the body and simultaneously minimizes the unpleasant side effects. It can also localize drug action in the diseased tissue by targeted drug delivery using carriers or chemical derivatization. It is specified that approximately 40% of the identified chemistry compounds such as Cur are poorly water soluble, and this is a challenging issue in their formulation (6). Usage of drug delivery systems is a well-known method to improve the solubility and retain the pharmacological activity of such drugs (29). Different types of pharmaceutical carriers such as polymeric micelles, particulate systems, and macro- and micromolecules are introduced in the form of novel drug delivery system for drug delivery

(30,31). Particularly, vesicular systems are drawing attention in this area, such as liposomes, niosomes, transferosomes and pharmacosomes. Among these drug delivery systems, niosomes have gained considerable importance. The disadvantages of liposomes such as necessity of an inert atmosphere during production, changeable phospholipid purity and high production cost can be eliminated by niosomes (29). Niosomes have several advantages such as high patient compliance, accommodate drug molecules with a wide range of solubilities, variable and controllable characteristics, controlled drug release, increasing the stability of encapsulated drug, improving oral bioavailability of poorly absorbed agents, enhancing skin penetration of drugs, and reaching the site of action by oral, parenteral and topical routes (32-34). In this study, a poorly water-soluble anticancer agent Cur was chosen as a model drug to be encapsulated in niosomes to examine the effects of niosome components on Cur properties. Nanoparticles average diameter, size distribution and zeta potential were obtained. The thin film hydration method used in this study to prepare niosomes was easy, appropriate encapsulation efficiency and low cost benefit. Measurement results in nanoniosomal

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Cur affirmed the particle size in nano scale. Karewicz et al. (35) prepared Cur -encapsulated liposomes consisting of dihexyl phosphate (DPH), egg yolk phosphatidylcholine (EYPC) and cholesterol by the film evaporation technique, allowing Cur to be soluble in the lipophilic bilayer owing to its lipophilicity. EYPC/DPH/cholesterol liposomal bilayer enabled improving the stability of Cur and served as a favorable vehicle for Cur. Xu et al. (32) prepared curcumin-encapsulated niosome containing span 80, tween 80, and poloxamer 188 by the film evaporation technique. This system provided controlled release of curcumin, thereby improving its therapeutic capability. The results of studying encapsulation efficiency showed that amount of Cur entrapped in nanoniosome formulation was very high. Sonication processes

not only reduced the size of particles, but also caused particles to be more homogeneous by reducing the size distribution. Considering the competencies of niosome overwhelming liposome, we prepared a niosome-based system for Cur delivery to improve its cytotoxic efficacy. Nio -Cur showed enhanced cytotoxicity effect when compared to free curcumin treatment of the PC-3 cancer cell line, indicating that niosomes promoted cellular uptake and the protection of the bioactive, but biochemically unstable chemical, curcumin. Xu et al. (32) prepared a niosome containing curcumin and evaluated its cytotoxicity effects on ovarian cancer A2780 cells. They found Nio -Cur exhibited enhanced cytotoxic activity and apoptotic rate against ovarian cancer A2780 cells compared with freely dispersed Cur.

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Conclusion

In this study, a novel Cur -loaded niosome system was developed to effectively deliver Cur for the treatment of prostate cancer. It was demonstrated that niosomes provided a high entrapment efficiency of Cur. The results of study affirmed the positive effect of sonication process to prepare the smaller particles. The results indicated that most of the drug is encapsulated into the carrier. Moreover, Nio -Cur exhibited enhanced cellular cytotoxic activity against prostate cancer PC-3 cells. According these results, the prepared Nio -Cur indicated to be promising as a nano-size carrier for the delivery of Cur and prostate cancer therapy.