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ORIGINAL ARTICLE

Niosomes Containing Isomeldenin Plus Lupeol Induce Apoptosis of SK-OV-3, MCF-7, and 3SKBr Cell Lines and Alter the Expression of Apoptotic Genes

Alaa Naseer M. Ali[1], Hadeel A. Omear[2], Shaima Rabeea Banoon[3], Abdolmajid Ghasemian[*4], M.A. Abdelzaher[5,6]

[1]*Department of Biology, University of Mustansyria, Baghdad, Iraq*

[2]*College of Science, Tikrit University, Iraq*

[3]*Department of Biology, College of Science, University of Misan, Misan, Iraq*

[4]*Noncommunicable diseases Research Center, Fasa University of Medical Sciences, Fasa, Iran*

[5]*Environmental Science and Industrial Development Department, Faculty of Postgraduate Studies for Advanced Sciences, Beni-Suef University, Beni-Suef 62511, Egypt*

[6]*Anesthesia Techniques Department, College of Health and Medical Techniques, Al Mustaqbal University, 51001, Babylon, Iraq*

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KEYWORDS

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ABSTRACT: Cancer treatment is a concern due to side effects, the resistance of tumor cells, and costs. Application of alternative compounds particularly delivered by nanocarriers is a paramount approach for the efficient treatment of cancer cells. Our objective was to kill ovary and breast cancer cells using nanoniosomes containing isomeldenin plus lupeol. Herein, nanoniosomes were synthesized using the thin-layer hydration method and their physical and chemical traits were assessed using scanning electron microscopy (SEM) and Dynamic Light Scattering (DLS) analysis. The dialysis bag was also used the entrapment rate and release pattern. Various concentrations of lupeol and isomeldenin were prepared for the assessment of anticancer effects against ovary and breast cancer cells (SK-OV-3, MCF-7, and 3SKBr cell lines) using the MTT assay and gene expression. The results were compared to each bioactive compound singly and free forms of them. The expression of Bax and Bcl2 genes was examined at low concentrations of each isomeldenin and lupeol (25µg mL^{-1}) singly and in combination with gemcitabine (25µg mL^{-1}). The size of synthesized nanoniosomes included 203.2 nm size and the entrapment rate was 72.6%. The death rate of SK-OV-3 in exposure to isomeldenin (100 µg mL^{-1}) included 76.34% and for MCF-7 and 3SKBr cell lines included 64.66% and 62.99%, respectively. The lupeol (100µg mL^{-1}) killing rate against SK-OV-3, MCF-7, and 3SKBr cell lines respectively included 42.36%, 40.26%, and 39.96%. The nanoniosomes containing isomeldenin killed SK-OV-3, MCF-7, and 3SKBr cell lines at 89.6%, 78.3%, and 69.6%, respectively. Moreover, nanoniosomes containing lupeol killed SK-OV-3, MCF-7, and 3SKBr cell lines at 88.9%, 79.2%, and 66.9%, respectively. The expression of the Bax gene was decreased 3.2 fold and the Bcl2 gene was increased 2.6 fold in exposure to nanoniosomes containing both gemcitabine and lupeol being significantly higher than those of control. The combination of each isomeldenin and lupeol with gemcitabine significantly increased the death of ovary and breast cancer cells in niosomal form. Herbal bioactive compounds nano-formulation is promising for cancer therapy.

*Corresponding author: majidghasemian86@gmail.com (A.Ghasemian)
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INTRODUCTION

Ovary and breast cancers are among the leading causes of mortality among women. [1, 2]. Breast cancer is the second leading cause of cancer death in women worldwide. Approximately 81% of breast cancers are invasive. [3-5]. The number of deaths caused by this malignant disease is increasing and one-third of patients die from this disease. Conventional treatments for breast cancer include surgery, radiation therapy, chemotherapy, and hormone therapy. However, it is associated with serious side effects and costs for patients. [6-9]. Traditional cancer therapies have been associated with various drawbacks such as off-target toxicity, high costs, inefficient treatment, and resistance of cancer cells. [10-13]. One of the latest expanding sciences is nanotechnology, which is the result of extensive studies related to the movement of subatomic particles. The dimensions of these particles in nano are between 1 and 100 nm [14, 15]. Nanotechnology is available at the nanometer scale; for this reason, it has provided access to new tools for the treatment, diagnosis, control, and monitoring of biological systems. There are various delivery systems with advantages and challenges in which system, chemotherapeutic agents stability, high load, efficient delivery, controlled release and Efficient cancer cell treatment is the main purpose. [16-19]. Niosomes are among promising vesicular nanoparticles and bilayer vesicles, composed of non-ionic surfactants. [20-23]. Niosomal formulation of therapeutic agents increases drug protection and retention time, hydrophobic drug solubility in aqueous solution, bioavailability, drug penetration, and specific delivery to target cells and tissues. [23]. The anti-inflammatory, antioxidant, and antitumor activities of some medicinal plants and their bioactive compounds have been determined. Terpenoids are among the promising anticancer bioactive compounds. [24-26]. Lupeol and isomeldenin are examples of anticancer compounds acting via various molecular pathways. [27, 28]. The aim of our study was niosomal loading of isomeldenin and lupeol in single and combination forms with gemcitabine and assessment of their anticancer effects against SK-OV-3, MCF-7, and 3SKBr cell lines.

MATERIALS AND METHODS

Cell lines

Cell lines including SK-OV-3, MCF-7, and 3SKBr were prepared from the University and taken from -70°C and cultured in DMEM containing 10% fetal bovine serum and antibiotics.

Herbal bioactive compounds

Bioactive compounds isomeldenin and lupeol were purchased from Sigma Aldrich. Various concentrations of each compound were prepared including 0.5-200 µg mL^{-1} for the MTT test on cancer cell lines.

Nanoniosome synthesis and evaluation

For the nanoniosomes preparation [29], cholesterol and span 60 were solubilized into chloroform and methanol (2:1 ratio) and merged completely. Next, the solution was transferred into a specialized rotary balloon under vacuum conditions of 60°C and 150 rpm for entire solubilization. At the hydration stage, 10 mL of each compound was added to the lipid film and rotated for 30 min distiller device at vacuum conditions. Then, the sonication was performed to decrease the particle size. The entrapment efficacy (EE %) is defined based on the ratio of compounds within the niosome to the free forms. For the separation of free compounds from the entrapped forms, centrifugation at 14000 g for 45 min was performed resulting in the precipitation of the entrapped form. The absorbance was read at 540 nm and the following formula was used:

$$EE\ \% = \text{free form concentration-primary concentration/initial concentration} \times 100$$

Various formulations of niosomes were prepared according to the span60/Tween60 molar ratio and the encapsulation rate and niosome sizes were determined. The physical and chemical features, sizes, and particle distribution were determined using a zeta-sizer device. In addition, the niosomes morphology was determined using a scanning electron microscope (SEM). The

releasing rate of compounds was assessed using a dialysis bag test.

Cell cytotoxicity and anticancer effects

The anticancer effects of free forms and nanoniosome formulated compounds was evaluated using the MTT assay [30]. After treatment of cell lines SK-OV-3, MCF-7, and 3SKBr into 96-well plates, the plates were incubated for 24 hrs. Next, the solution was taken and microculture Tetrazolium Test dye was added and incubated for 4 hr at 37°C in CO2 conditions. Then, the dye was removed and the formazan crystals formation by living cells was assessed using the isopropanol solubilization and read at 570 nm.

Survival rate= absorbance of treated cells/ absorbance of control cells × 100

Gene expression

Total RNA was extracted from the treated and control SK-OV-3 cell line using TRIzol reagent (ThermoScientific, USA) and cDNA was synthesized (BioFact™ RT Series cDNA synthesis kit). The RNA quality was evaluated via absorbance at 260/280 nm using NanoDrop. The expression of Bax and Bcl2 genes was evaluated using specific primers as follows:

Bax- F: 5'-GGCGAATTGGAGATGAACTG-3' and R: 5'-TTCTTCCAGATGGTGAGCGA-3', Bcl2- F: 5'-CTTTGCAGAGATGTCCAGTCAG-3' and R: 5'-GAACTCAAAGAAGGCCACAATC-3' and Gapdh-F: 5'-GCAGCTCCTTCGTTGCCGGT-3' and R: 5'-CCCGCCCATGGTGTCCGTTC-3' [31]. The Gapdh gene was considered as the test control. Real-time PCR (RT-qPCR) was implemented using the LightCycler®96 device (Roche, Germany).

RESULTS

Synthesis and characterization of niosomes

By the application of optimized formulation of the nanoniosomes using a molar ratio of surfactant: cholesterol (1:1) and Span 60: Tween 60 (50:50) and 1 mg of each compound and sonication of 7 min, acceptable characters were obtained. Additionally, the EE % was highest at the nanoniosomes size of 203.2 nm 72.6%. The accumulated releasing rate pattern of isomeldenin in free and entrapped forms during 72 hrs, demonstrated a significant lower rate in nanoliposomes-formulated form (32%) compared to the free form (94%) ($p < 0.0001$). Additionally, regarding lupeol, nanoniosome-formulated form release (28%) was significantly lower than that of free form (92%) after 72 hrs ($p<0.0001$) (Figure 1).

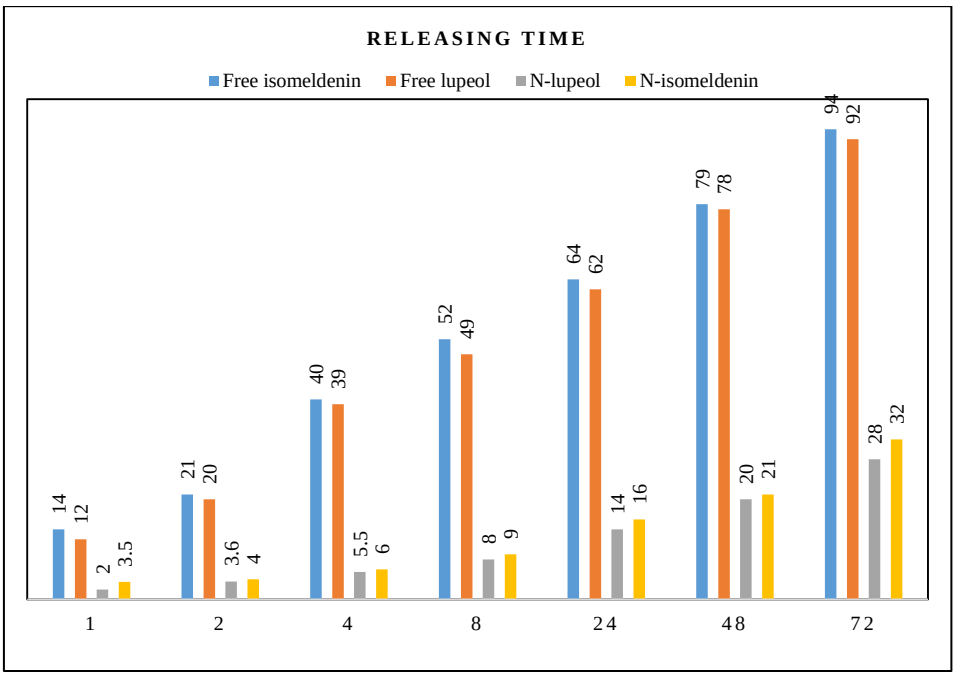

Figure 1. The releasing rate of compounds

Cell cytotoxicity

The death rate of SK-OV-3 in exposure to isomeldenin (100 µg mL^{-1}) was 76.34% and for MCF-7 and 3SKBr cell lines included 64.66% and 62.99%, respectively. The lupeol (100 µg mL^{-1}) killing rate against SK-OV-3, MCF-7, and 3SKBr cell lines respectively included 42.36%, 40.26%, and 39.96%. The nanoniosomes containing isomeldenin killed SK-OV-3, MCF-7, and 3SKBr cell lines at 89.6%, 78.3%, and 69.6%, respectively. Moreover, nanoniosomes containing lupeol killed SK-OV-3, MCF-7, and 3SKBr cell lines at 88.9%, 79.2%, and 66.9%, respectively (Table 1).

Table 1. The death rate of SK-OV-3, MCF-7, and 3SKBr cell lines in exposure to isomeldenin and lupeol.

Cell line	Isomeldenin % death rate	Lupeol % death rate	values-*value*
SK-OV-3	76.34	42.36%	0.04321
MCF-7	64.66	40.26%	0.05136
3SKBr	62.99	39.96%	<0.001

Gene expression

The expression of Bax and Bcl2 genes in exposure to nanoliposomes-loaded isomeldenin respectively altered 1.6 (decrease) and 1.9 fold (increase). Their expression in exposure to lupeol included 1.8 fold and 1.6 fold, respectively. In addition, the expression of the Bax gene was decreased 3.2 fold and the Bcl2 gene was increased 2.6 fold in exposure to nanoniosomes containing both the isomeldenin and lupeol being significantly higher than those of control (Figure 2).

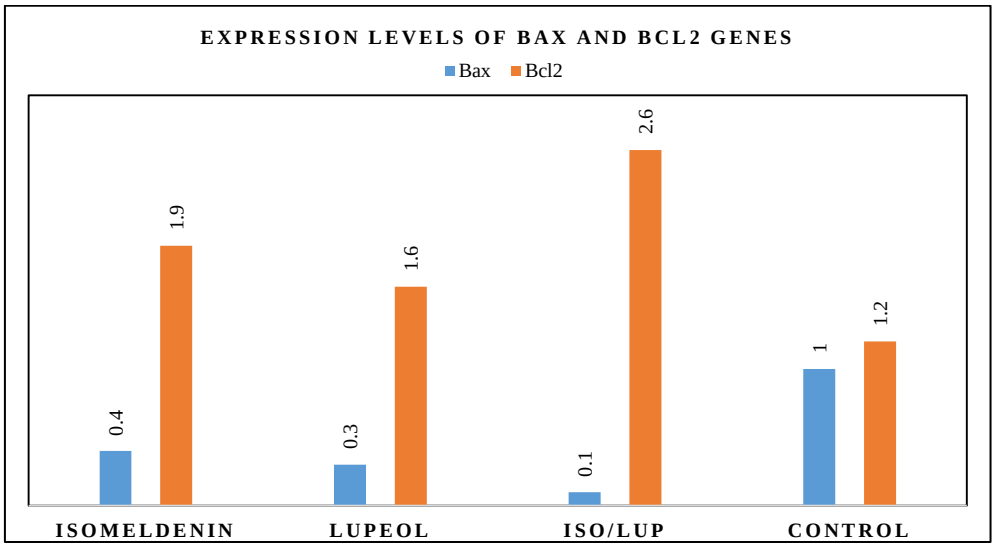

Figure 2. The expression levels of Bax and Bcl2 genes in exposure to a single and combination of isomeldenin and lupeol compounds

DISCUSSION

Accurate and efficient cancer treatment is a considerable insight that needs investigation of proper delivery systems and novel anticancer agents. [16, 17, 32, 33]. The encapsulation of drugs by using vesicular nanoparticles is promising in this regard which can be associated with controlled therapeutic agent release, mitigation of off-target toxicity, and enhanced concentration at the target site. [34, 35]. Another concern is the evolution of the non-susceptibility of cancer cells and hence one of the suitable alternatives for combating cancer cells includes herbal bioactive compounds. [36, 37]. In this study, to increase the anticancer effects of herbal compounds, nanostructured niosomes were used for compound loading and drug delivery. Niosomes can change the pharmacokinetic profile, drug distribution in the body, and even drug metabolism. By the application

of optimized formulation of the nanoniosomes using a molar ratio of surfactant: cholesterol (1:1) and Span 60: Tween 60 (50:50) and 1 mg of each compound and sonication of 7 min, acceptable characters were obtained. Additionally, the EE % was highest at the nanoniosomes size of 203.2 nm being 72.6%. The accumulated releasing rate pattern of isomeldenin in free and entrapped forms during 72 hrs, demonstrated a significant lower rate in nanoniosome-formulated form (32%) compared to the free form (94%) ($p < 0.0001$). Additionally, regarding lupeol, nanoniosomes-formulated form release (28%) was significantly lower than that of free form (92%) after 72 hrs ($p < 0.0001$) (Figure 1). The death rate of SK-OV-3 in exposure to isomeldenin (100 µg mL^{-1}) was 76.34% and for MCF-7 and 3SKBr cell lines included 64.66% and 62.99%, respectively. The lupeol (100 µg mL^{-1}) killing rate against SK-OV-3, MCF-7, and 3SKBr cell lines respectively included 42.36%, 40.26%, and 39.96%. The nanoniosomes containing isomeldenin killed SK-OV-3, MCF-7, and 3SKBr cell lines at 89.6%, 78.3%, and 69.6%, respectively. Moreover, nanoniosomes containing lupeol killed SK-OV-3, MCF-7, and 3SKBr cell lines at 88.9%, 79.2%, and 66.9%, respectively.

The expression of the Bax gene was decreased 3.2 fold and the Bcl2 gene was increased 2.6 fold in exposure to nanoniosomes containing both gemcitabine and lupeol being significantly higher than those of the control. The rate of release of niosomes nanocarrier extract was significantly slower than the extract alone, which is very important from the point of view of pharmacology. Various studies have been carried out for the synthesis and niosomal formulations of herbal compounds and essential oils [20, 38-40]. Balanocarpol was encapsulated in the niosome structure and the cytotoxicity effects of human ovarian and breast cancer lines were evaluated. Niosome nanoparticle formulation for encapsulation and delivery of balanocarpol showed superior anticancer effect compared to free balanocarpol, and improved water solubility [41]. The henna extract was encapsulated in a niosome structure and its antitumor activity was evaluated against the MCF_7 breast cancer cell line [42, 43]. The niosomes had a spherical shape and the particles had a size of about 250 nm in diameter. The encapsulation efficiency was about 70%. Therefore, this study showed that the niosomal formulation or encapsulation of henna extract significantly increases its cancer cell toxicity effects compared to the free extract. The pegylated green tea extract was encapsulated by (polyethylene glycol or PEG) coated with niosomes and the anticancer effects were evaluated against different cell lines. The size of niosomes increased significantly after loading with the extract. The encapsulation efficiency varied between 70 and 80%. In this study, the method of extraction was different from the present study, and niosome was covered [43]. Coating of niosomes using PEG, and the culture medium cell toxicity by MTT was similar to the present study. Diosgenin was encapsulated by niosome structure, but the cytotoxicity increase against HepG2 cells was not significant [44]. Also, lycopene extract was encapsulated in a niosome structure and it was investigated against MCF-7 breast cancer cells it was shown that the particle size and niosomes containing lycopene extract ranged from 170 to 230 nm. Nanoniosomes containing lycopene extract inferred a wide range of anti-cancer properties [45]. Encapsulation of pomegranate and chamomile extracts as nanoniosome increased the anticancer efficacy against breast cancer cells and also changed the expression of apoptotic and anti-apoptotic genes [46, 47].

CONCLUSIONS

The combination of isomeldenin and lupeol significantly increased the death of ovary and breast cancer cells. Herbal bioactive compounds nano-formulation is promising for cancer therapy. The nanoniosomes containing isomeldenin killed SK-OV-3, MCF-7, and 3SKBr cell lines at 89.6%, 78.3%, and 69.6%, respectively. Moreover, nanoniosomes containing lupeol killed SK-OV-3, MCF-7, and 3SKBr cell lines at 88.9%, 79.2%, and 66.9%, respectively. The expression of the Bax gene was decreased 3.2 fold and the Bcl2 gene was increased 2.6 fold in exposure to nanoniosomes containing both gemcitabine and lupeol being significantly higher than those of control.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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