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RESEARCH PAPER

Enhanced *in vitro* cytotoxicity and intracellular uptake of Genipin via loaded on Nano-Liposomes made from Soybean lecithin in MCF-7 cells

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ABSTRACT

Objective(s): As an alternative to chemical drugs, natural compounds such as Genipin can reduce toxicity and side effects. In recent years, Genipin's antioxidant properties have been considered a potential cancer treatment. Therefore, the present study investigated anti-cancer activity of newly formulated nano-liposomal loaded Genipin, made from soy lecithin, against MCF-7 cancer cell line.

Materials and Methods: After synthesis, the physicochemical properties of the liposomes were confirmed by Dynamic light scattering (DLS), Scanning Electron Microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and UV-vis spectrophotometry. The *in vitro* cytotoxic activity of nano-liposomal loaded Genipin in comparison with free Genipin, was explored on MCF-7 cell line using MTT colorimetric assay.

Results: Our results showed that the prepared nano-liposome had a diameter of 166.2 nm. Its Zeta potential was -25.4 mV which indicates the good electrostatic stability of nano-liposomes. Also, a slight size distribution (PDI 0.2870) and a high encapsulation efficiency (EE% >82% and DL>28%) are other features of synthesized nano-liposomal loaded Genipin. The in vitro result of release profile demonstrated that the drug-controlled release from Genipin loaded-liposomal is 65% during 70h. Our results revealed that the IC50% (cytotoxicity) of MCF-7 cells treated with nano-liposomes loaded Genipin were higher than those treated with free Genipin (about 2.4 orders of magnitude). Additionally, cell uptake studies evidenced a higher uptake of negative nano-liposomal loaded Genipin.

Conclusion: In a nutshell, newly formulated nano-liposomal is an ideal vehicle for negative targeting (anticancer effect) of drugs to tumor cells that may result in improved efficacy and reduced toxicity of encapsulated drug moiety.

Keywords: *Anticancer activity, Genipin, Intracellular uptake, Liposomes, Soybean lecithin*

How to cite this article

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INTRODUCTION

A range of natural compounds are potentially effective treatments for breast cancer due to their toxic effects on tumor cells that lead to programmed cell death [1]. Genipin is a gardenia-derived herbal medicine and has been used as a traditional Chinese medicine for decades [2, 3]. The latest reports suggest that Genipin has a drug with medicinal and biological properties including anti-antigenic [4], anti-proliferative [1], and antioxidant [5]. Many studies have shown that it inhibits the proliferation of cells through apoptosis and induces cell death in many cell lines [6, 7], including gastric cancer [8], breast cancer [9], and prostate cancer [10]. Also, it has been demonstrated that Genipin regulates Bcl-2, Bax, caspase-3, JNK, p38MAPK, and reactive oxygen species (ROS) in breast cancer cell lines

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[7]. It also has anti-proliferative properties in the MDA-MB-231 breast cell line [1]. Genipin is a chemo-preventive agent in preventing metastatic breast cancer [6]. In addition, it induces apoptosis in the MDA-MB-231 cell line by decreasing the regulation of Bcl-2 and increasing the regulation of caspase-3 and Bax [10]. It was also expected to reduce the expression of UCP2 in cancer cells [5]. Since Genipin is a hydrophobic and fat-soluble compound, nano-drug delivery systems could improve its efficiency. Today, liposomes are mostly used as carriers of drugs and genes, as well as for modeling cell membranes in animals and humans rather than other nanocarriers. The advantages of liposomes, including reduced side effects of chemotropic drugs, high efficacy and low toxicity, have attracted the attention of researchers. Other advantages of liposomes include ease of production in industrial volumes, excellent manufacturing quality, and variety in sizes, chemical and bar electric composition [11]. Liposomes are made from macro-sized to nano, but in the pharmaceutical industry, nano liposomes (less than 200 nm in size) were mostly used. Liposomes have an aqueous nucleus that is surrounded by one or more layers of phospholipid groups. Phospholipid layers are composed of two parts including the hydrophilic head and the hydrophobic tail. The hydrophilic head is made up of glycerol, phosphate, and usually choline, and the hydrophobic tail is made up of a saturated or unsaturated hydrocarbon chain. When the phospholipid layer is dispersed in water, it forms a bilayer membrane in which non-polar tails and polar heads come into contact with the aqueous medium. The size of liposomes can be between 5 nanometers and 50 micrometers in diameter [12].

Thus in the present study for the first time, Genipin was encapsulated in the bilayer of a nano-liposome made from soy lecithin to deliver the Genipin into cell mechanisms and comprehensive incorporation and acceptable cytotoxicity of newly formulated nano liposome-Genipin compared to free Genipin in the MCF-7 cell line.

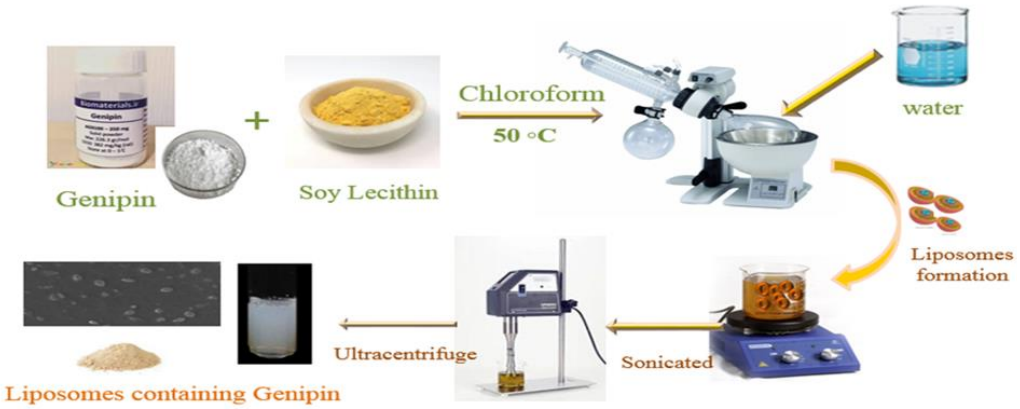
MATERIALS AND METHODS

Materials

Genipin was obtained from Challenge Bio-products Co., Ltd. Soy lecithin was obtained from German-grail and chloroform purchased from the German Mark. From Gibco BRL, USA, we obtained cell culture chemicals such as Dulbecco's modified Eagle's medium (DMEM), fetal calf serum, trypsin, penicillin and streptomycin antibiotics. Additional compounds were obtained from Sigma Aldrich and used devoid of further purification.

Preparation of Genipin-loaded liposomes (liposomal-Genipin)

Preparation of nano liposomes containing Genipin was performed using a thin layer hydration, and ultrasound method [13]. First, lecithin and Genipin were dissolved in chloroform and then the organic solvent was evaporated using rotary evaporator. The aqueous phase was added to the thin film at the bottom of the balloon. Finely multilayered liposomes were performed. To remove the extra layers and reduce the size of the liposome, sonication was performed with an ultra-probe system. The liposomes synthesized by centrifugation were then separated (Scheme1).



Scheme 1. Schematic preparation of liposome cantoning Genipin

Determination of hydrodynamic diameter and Zeta potential of particles by dynamic light scattering

The average diameter of liposomes was measured using a model dynamic light scattering (DLS) device (Malvern Instruments Ltd. Worcestershire, UK) made in Germany. After the synthesis of nano-liposomes, 1 ml of the solution was dispensed into 1.5 ml of the cuvette and placed in the sample reading place inside the device to measure the average size and PDI of nanoparticles. A DLS device was used to evaluate the Zeta potential of nano-liposomes containing Genipin. To measure the surface charge of nano-liposomes, 1 ml of the solution was dispensed into 1.5 ml of the cuvette and placed in the sample reading place inside the device.

Measurement of morphology of nano-liposomes-loaded Genipin

Scanning Electron Microscopy (SEM) (KYKY-EM3200, China) was used to observe the microstructure and morphology of the nano-liposomal loaded Genipin. After synthesizing and separating the nano-liposomes, the solution was diluted 10 times with distilled water, placed in a sonic bath for 5 minutes until the solution was uniform, then the solution was poured onto a slide and placed in a desiccator for 24 h. After drying, the entire surface of the lamellar was covered with a very thin layer of gold and evaluated with SEM microscope at a magnification of 1 µM.

Determination of entrapment efficiency and drug loading

UV-vis spectrometer was used for evaluating the entrapment efficiency (EE %) and drug loading (DL %) efficiency of nano-liposomes. The concentration of Genipin in nano-liposomes was calculated using the calibration curve.

$y = 0.8895x + 0.0024$, $R2 = 0.98$ at 241 nm

The calibration curve was achieved from the UV absorbance of different standard Genipin solutions *(dissolved in ethanol).* The suspension of freshly prepared nano-liposome was centrifuged at 14800 rpm for 30 min. The supernatant was then eliminated for the determination of free Genipin in the suspensions. Calculation of EE and DL was performed by the formula given below:

EE%X 100

DL%: [Total drug −Free drug / Total weight nanoparticles] ×100.

Fourier transforms infrared spectroscopy (FTIR) analysis

The interactions between empty liposome, Genipin compounds and the liposome loaded-Genipin were assessed by Fourier transform infrared spectroscopy (FT-IR). FT-IR spectra were detailed via FTIR set (model FTIR prestige-21, Shimadzo Co., Japan) between a variety of 200-4000 cm^{-1} through a spectral resolution of 4cm^{-1}.

In vitro drug release of nano-liposomes loaded Genipin

In vitro release study of nano-liposomes loaded Genipin was done [14]. Briefly, nano-liposomes loaded Genipin were placed in a dialysis bag (cut off 12 kDa), which was placed in 50 mL of phosphate buffer saline (PBS; pH 7.4), and maintained at temperature room with constant stirring at 550 rpm. In timely (0, 2, 4, 6, 8, 10, 12, 24, 36, 72) intervals, 2 mL aliquots of the release medium were detached, and the alike volume of new phosphate buffer saline solution was added to the system. The concentration of released Genipin in the medium was calculated by the Genipin calibration curves with absorption at 241 nm.

FITC-Labeling of nano-liposomes loaded Genipin

For fluorescent labeling of nano-liposomes loaded Genipin, we re-dissolved 200 µL of nano-liposomes loaded with Genipin into 500 µL fluorescein isothiocyanate (FITC) solution (5 mg mL^{-1} in ethanol, Sigma Aldrich), and then stirred the solution in the dark at 1000 rpm for 12 hrs at room temperature. Then suspension centrifuged at 7000 RCF and washed several times with sterile PBS. The achieved FITC- nano-liposomes loaded Genipin were re-suspended and stored at 4˚C in the dark, until use (final concentration: 1 mg mL^{-1}).

Cell culture

MCF-7 cells were cultured as a monolayer in a 25 cm^2 culture flask (Orange Scientific, Belgium) under a standard humidified incubator (37˚C, 5% CO_2). MCF-7 cells were maintenance in DMEM medium complemented with 5% FCS, 100 UmL^{-1} penicillin and 100 μgmL^{-1} streptomycin and, then were incubated (37 °C, 5% CO_2, and 95% humid air) for 24 and 48 h.

Cell viability/cytotoxicity studies

To determine the IC_{50} values, the yellow tetrazolium salt, 3-(4, 5-dimethylthiazol-2-yl)-2,

5-diphenyltetrazolium bromide (MTT) viability assay, was used with some modifications. Briefly, in 96-well plates (Fisher Scientific, Loughborough, UK), MCF-7 cells were seeded in a growth medium at 1×104 cells/well. Previous to apiece cytotoxicity test, cells were acceptable to follow for 24 h. Different doses of nano-liposomes loaded Genipin were addition to the dishes, and protected in 5% CO_2, at 37 °C. After treatment for 0, 24 or 48 h, MTT (concentration of 0.5 mg/ml in Dulbecco's Phosphate Buffered Saline (DPBS, Sigma)) was addition to wells, separately, and protected for 4 h at 37 °C. The supernatant was detached and the precipitated formazan crystals were then solubilized with 100 µL pure DMSO. Absorbance (optical density; OD), were measured at 560 and 630 nm via a Microplate Auto reader device (BIO-TEK Instruments, Winooski, VT, USA). Cell viability was considered as a compared with control by Graph Pad Prism v.5.0.

Cellular uptake of nano-liposomes

A breast cancer cell line (MCF-7) was used for the Cellular uptake assay. MCF-7 cells were incubated in medium (DMEM) containing 10% FBS (GIBCO 16000-044), 2 mmol/l glutamine, and 50 IU/ml penicillin and 50 g/ml streptomycin (GIBCO 15140-122) at 37 ◦C, 5% CO_2. Subsequently the early passage in culture plate, MCF-7 cells were full-grown to 70-80-%-confluence in the culture medium containing 10% FBS in 24-well tissue culture plates CO_2 incubator. After filtration, the FITC-Labeling of nano-liposomes loaded Genipin mixture (200 µg/ml) was hatched with MCF-7cells at 37 ◦C, 5% CO_2 for 4h. Then MCF-7 cells were wash away three times with PBS and immediately subjected to fluorescence microscopy imaging (Eclipse TE 2000U microscope; Nikon, Tokyo, Japan) at 40× magnification. Image investigation was performed via the Image-Pro Plus software (Media Cybernetics, Silver Spring, MD, USA).

Immunofluorescence microscopy

After treated cells with FITC-Labeling of nano-liposomes loaded Genipin, morphology of apoptotic cells reports the topographies of apoptosis after 4',6-diamidino-2-phenylindol (DAPI) staining. For DNA staining and microscopic examination, cells were cultured in a 24-well Petri dish with 150,000 cells/well and followed for 24 h and treated with blank-Nano liposome, free Genipin and nano-liposomes loaded Genipin before DAPI staining. Then the medium was completely removed, and stained with (DAPI; 1 µg mL^{-1} in staining buffer). After 15 min incubated at 37 ◦C, cells mounted in 80% glycerol and were experimental using an Eclipse TE 2000U microscope (Nikon) at 40× magnification and images were investigated by the Image-Pro Plus software (Media Cybernetics).

Statistical analysis

All information were definitude as means ± standard deviation (SD). Statistical judgements between the treatments were studied by ANOVA, Post Hoc analysis, Tukey's HSD test (SPSS for Windows, version 18.0). A *P*-value of ≤ 0.05 was measured to be statistically significant. Each test was accepted out five times.

RESULTS

Physicochemical characterization of nano-liposomes loaded genipin

Dynamic light scattering (DLS) is a physical, non-destructive and fast method that is used for determining particle size distribution in solution and suspension. DLS result showed an average size of 168.2 nm and slight size distribution (PDI <0.02870; Fig. 1A), which represents that the particles have uniform and excellent distributions [15].

According to Fig. 1B, Zeta potential of nano-liposomes loaded Genipin was -25.3 mV. The zeta potential is a key physicochemical property for the deposition of nano-liposomes and an important factor in the stability of nano-liposomes.

The microstructure of nano-liposomes was confirmed at the nanometer scale by SEM. After the ultrasound process, the size of the vesicles was reduced to the nanometer level and the images of the nano-liposomes loaded Genipin was shown in Fig. 2. According to Fig. 2, nano-liposomes loaded Genipin viewed as regular circular vesicles in the size range of 141nm that the presence of a bilayer membrane around the career is also well known.

UV–vis spectroscopy and FT-IR analysis

Usaged FT-IR and UV−vis spectroscopy of empty liposomes, Genipin compounds and nano-liposomes loaded Genipin were studied to get visions into the occurrence of contact among them (Fig. 3 & 4).

UV−vis spectroscopy of empty liposomes, Genipin and nano-liposomes loaded Genipin in the range of 200−400 nm were clarified (Fig. 4). The

single peak at 230 is allocated to Genipin, which was with a moderately thin value in liposome loaded-Genipin (235). These overlying spectrum display that Genipin structure was well preserved throughout the liposomal process.

FTIR spectra of free Genipin contains principle bands at 3400, 2856, 2512, 1685, 1677, and 1119 (Fig. 4A), and empty liposomes comprises peaks at 3410, 2924, 2854, 1735, 1651, 1465, 1095 and 709 (Fig. 4B). The FTIR spectra of liposome loaded-Genipin (Fig. 4C) contains principle bands at, which were alike peaks to individuals confined in both free Genipin and empty liposome spectrum, 3439, 2922, 2858, 1735, 1699, 1656, 1539, 1458, 1107, and 701 representative that the liposomal capsulation of Genipin complexes did not outcome in the new linkages creation, therefore certifying effectual drug unpacking at the target places.

According to FTIR result, in empty liposome spectrum, the comprehensive peak placed at 3410 cm^{-1} resembles to O-H vibrating, at stretches 2858 cm^{-1} and 2854 cm^{-1} resemble to C-H stretching, the peaks placed at 1095 cm^{-1} and 709 cm^{-1} relate to C=C=C symmetric stretch. In Genipin, the comprehensive peak positioned at 3400 cm^{-1} resembles to O-H vibrating, at stretches 2856 cm^{-1} and 2512 cm^{-1} relate to C-H widening, the peaks adjusted at 1685 cm^{-1} and 1677 cm^{-1} resemble to widening ambiances of carbonyl groups, the peaks at 1119 cm^{-1} match to primary O-H groups. In liposome loaded-Genipin the band placed at 3339 cm^{-1} represents the O-H widening, the principal bands at 2922 cm^{-1} related to the C–H functional group, the strong band addressed at 2858 cm^{-1} inferring the CH stretching of CH 3 and CH 2 groups, the bands centered at 1735 and 1699 cm^{-1} are related to C = O widening, and the scissoring ambiances of the C-C collections are signified by the 1647 cm^{-1}peak. Vibrational band

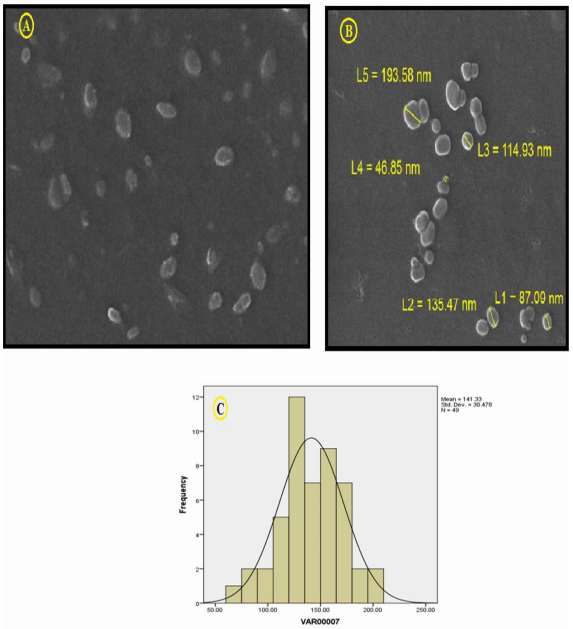


Fig 2. Morphology (A), size (B) and Size frequency (C) of nano-liposomes loaded Genipin using the SEM

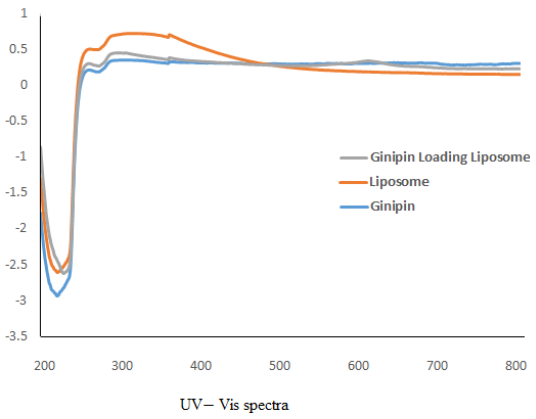


Fig 3. UV-Vis absorption spectra of empty liposome, Genipin and nano-liposomes loaded Genipin

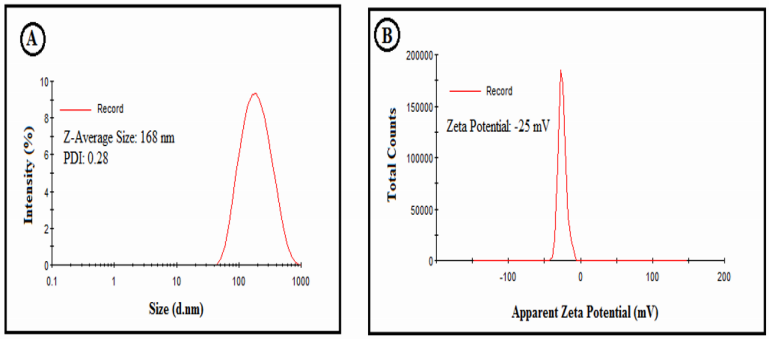


Fig 1. (A): Determination of hydrodynamic diameter and PDI, and (B): Zeta potential of nano-liposomes loaded Genipin by DLS

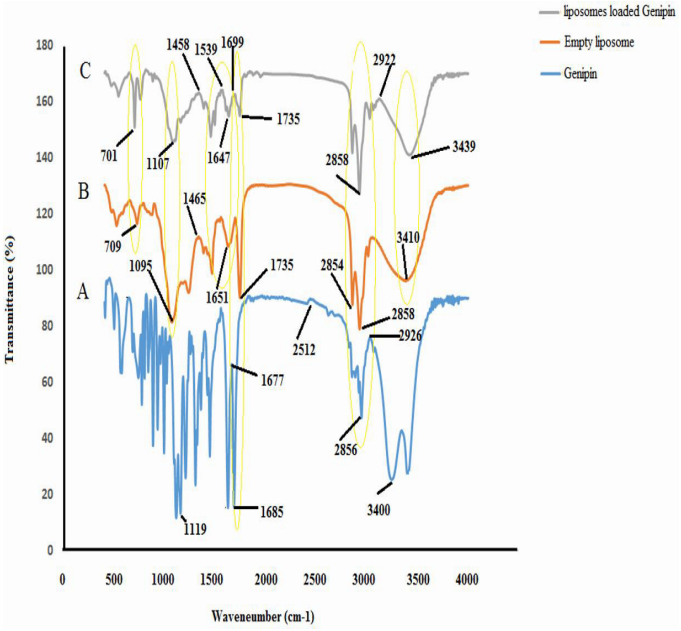


Fig 4. FT-IR diagrams of the empty liposome, Genipin and nano-liposomes loaded Genipin

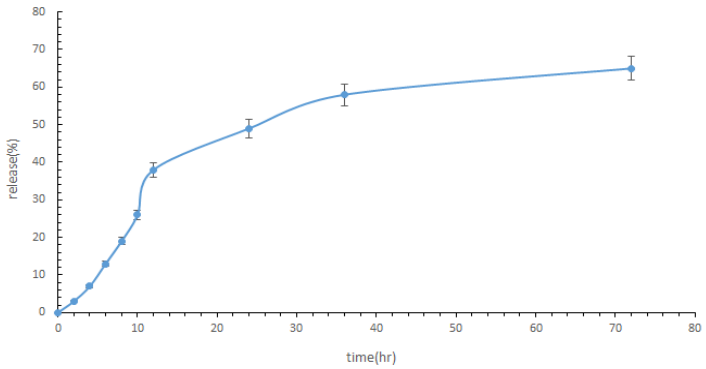


Fig 5. The in vitro drug-release profile of nano-liposomes loaded Genipin at pH 7.4

characteristic of phosphate groups distributed to the PO_2− symmetric and PO_2− anti-symmetric widening style are placed at 1107 cm^{-1} and 1075 cm^{-1}, respectively. As a result of incorporation of Genipin into phospholipids from liposomes, we observed an Significant infrared absorption spectrum changes in liposome loaded-Genipin FTIR; the comprehensive peak conforming to O-H collections is lifted from 3400 cm^{-1} to 3439 cm^{-1}, the O-H peaks at 2856 cm^{-1} and 2926 cm^{-1} is lifted to 2922 cm^{-1}, the peak related to C - H widening in phospholipids is moved since 1685 cm^{-1} to 1735 cm^{-1}, the peak at 1677 cm^{-1} is lifted to a higher rate (1699 cm^{-1}), and the bands at 1119 cm^{-1} correspond to prime C=O collections, is change from 1119 and 1095 cm^{-1} to 1107 cm^{-1}. Polar heads of phospholipids are the region of spectral change that is the most definite. This may be the result of hydrogen bonds between phospholipid polar heads and hydroxyl groups in Genipin. This change liposome loaded-Genipin FTIR spectrum confirms the creation of a liposome containing -Genipin.

In vitro release profile

In vitro release study of nano-liposomes loaded Genipin was achieved by the dialysis bag diffusion technique at pH 7.4 and 25 °C. The *in vitro* drug release results demonstrated that the nano-liposomes loaded Genipin provided drug-

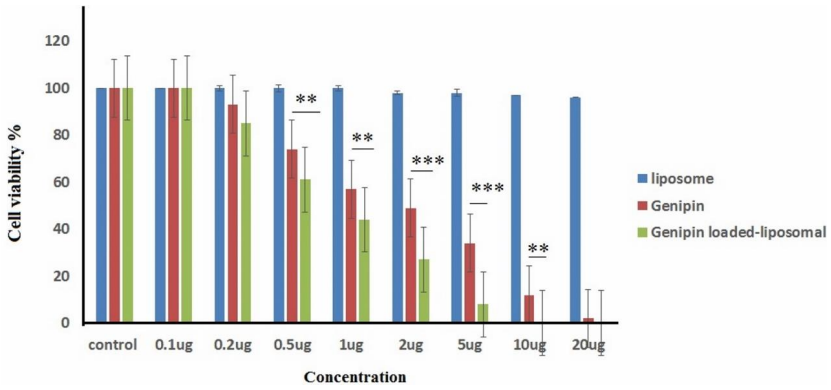


Fig 6. MTT assay for cell viability measurement

controlled release from Genipin (65%) (Fig. 5). And a high encapsulation efficiency (EE% >82% and DL>28%) are other optimal features of synthesized nano-liposomal loaded Genipin.

Cytotoxicity and viability assays

The data presented in Fig. 6 also shows the sensitivity of MCF-7 cells against liposomes without Genipin, free Genipin and nano-liposomes loaded Genipin groups. After a 48 h incubation, MTT assay showed an anti-proliferative effect in MCF-7 cells in a dosage-dependent manner in three groups. Initially, 0.1 to 0.2 µg concentrations showed limited toxicity to breast cancer cells in nano-liposomes loaded Genipin and free Genipin groups. The percentage of cell viability for 0.1 to 20 µg concentration at liposome without Genipin groups were 100%, 100%, 100%, 99%, 99%, 98%, 98% and 96%, respectively. The cell viability for 100 ng to 20 ug concentration at free Genipin group, were decreased compared with control and liposome without Genipin Groups (P<0.05). Thus the percentage of cell viability for all concentration at free Genipin group were 100%, 92%, 73%, 57%, 48%, 31%, 14% and 1%, respectively. The cell viability for 100 ng to 20 ug concentration at nano-liposomes loaded Genipin group, were decreased compared with control cells (0g), free Genipin and liposome without Genipin Groups (P<0.05). The percentage of cell viability for all concentration at nano-liposomes loaded Genipin group, were 100%, 85%, 62%, 46%, 27%, 7%, 0% and 0%, respectively. Thus, the maximum inhibitory effect of nano-liposomes loaded Genipin was found with the increase of Genipin concentration to 5 µg for MCF-7 cells.

Comparison between the three groups' shows that, in nano-liposomes loaded Genipin and free Genipin groups (at 100 ng to 20 ug concentration) the cell survival rate decreased compared to the liposome without Genipin and the control groups. Comparison between the two groups (in nano-liposomes loaded Genipin and free Genipin groups) shows that the rate of survival reduction in nano-liposomes loaded Genipin group was much greater than free Genipin group. Where cell

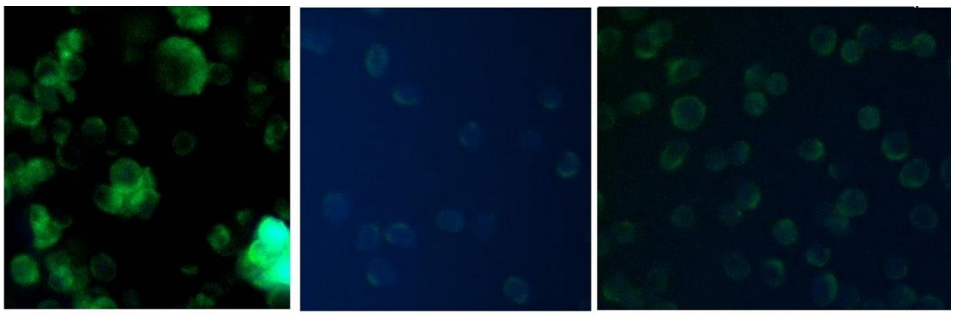


Fig 7. (a) Fluorescent microscopy images of the MCF-7 cells with Immunofluorescence staining by FITC (green) and DAPI (blue) dyes. Scale bars are 200 µm

viability reached 7% for MCF-7 cells at a maximum drug concentration of 5 µg. The lowest IC_{50} for nano-liposomes loaded Genipin group was at 5 µg drug concentration while for free Genipin group theses IC_{50} was observed at 20 µg concentration.

Cellular uptake kinetics of the nano-liposomes loaded genipin monitored by microscopy

As shown in Fig.7, cellular uptake of FITC-Labeling of nano-liposomes loaded Genipin was confirmed via confocal microscopy in MCF-7cells. After 4 h incubated with the MCF-7 cells, it was obviously that FITC-Labeling of nano-liposomes loaded Genipin centered in the perinuclear area. Moreover, the subdivisions presented a coronoid organization round the cell nucleus, and the nuclei area remains free. During the course of this study, the cells proved to be viable and stained with DAPI as such these FITC-Labeling of nano-liposomes loaded Genipin did not overtly cytotoxicity. By staining the nucleus with DAPI, blue fluorescence was exhibited, supporting the hypothesis that green fluorescence results from cellular internalized particles rather than surface adherent particles. The subsequent outcomes display the upsurge of the DAPI sign on alive cells in the cells treated with FITC-Labeling of nano-liposomes loaded Genipin with 0.2 µg/ml concentration (Fig. 7). The features appear more prominent in the cells treated with nano-liposomes loaded Genipin.

DISCUSSION

Breast cancer is one of the principal reasons of death among women universal, in both industrialized and developing nations. Chemotherapy is the standard and most common treatment for cancer, however, low performance and high side effects can limit its applications. Today, the use of a nano-drug delivery system such as liposome to deliver the optimal amount of anti-cancer medicine to cancer cells and thus better cure cancer cells has been confirmed *in vitro* and *in vivo*. The anti-cancer properties of several compounds obtained from plant extracts have prompted researchers to focus their attention on these compounds for the treatment of various cancers. Therefore, one of the most effective and alternative therapies for chemotherapy is the use of natural compounds with moderate toxicity and fewer side effects. Gardenia fruit-derived Genipin has been used as a traditional Chinese medication for numerous decades, and current studies have confirmed its medicinal and biological properties [3]. Based on recent research, Genipin is a prophylactic chemical to prevent metastatic breast cancer [16-18].

In the present study, Genipin was loaded into liposomal nanocarriers made from soybean lecithin. Measurement of zeta potential is a key physicochemical property for controlling the mystification and deposition of nano-liposomes, which are important factors in the stability of nano-liposomes. In the nano-liposome loaded Genipin, the surface charge was negative; this anionity is due to the fact that soybean lecithin is a natural phospholipid, consisting mostly of phosphatidylcholine. This compound is similar to natural membrane phosphatidylcholine because of its advantage over egg lecithin and synthetic phospholipids. Another important physicochemical property to increase intracellular uptake nano-liposomes into a cancer cell line, is their size and shape. According to the DLS and SEM evaluation, the nano-liposome loaded Genipin is a regular circle shape with uniform dispersion and an average size of <200 nm. Nano-liposomes loaded Genipin have a diameter of 141.33 nm, which is clearly smaller than the size measured by Zetasizer (168 nm). Because, Zetasizer determines the hydrodynamic diameter with the largest value because of the solvent effect, whereas SEM size is determined in the dried state. Imaging using SEM can provide a more accurate measure of surface morphology and size distribution [19, 20]. Also, a high encapsulation efficiency (EE% >82%) and a Genipin-controlled release from nano-liposome (Fig. 5) are other excellent features of synthesized newly formulated nano-liposomal loaded Genipin.

According to the MTT assay results, the cell viability was decreased in different doses of nano-liposome loaded Genipin compared with similar doses of free Genipin groups (P<0.05). The higher cytotoxicity of the nanoliposomes loaded Genipin indicated that the nanoliposomes were able to overcome Genipin's poor solubility in water and penetrated cell membranes effectively. In contrast, free Genipin showed very little cytotoxicity against MCF-7 compared with nanoliposomes loaded Genipin (Fig. 6), which can be explained by higher cellular uptake and effectiveness of nano-liposomal loaded Genipin compared with free Genipin groups. Additionally, these results clearly showed that liposomes were not cytotoxic to MCF-7 cells, which indicates a high degree of

biocompatibility for the constituent soybean lecithin in our newly formulated nano-liposomal.

In the absence of fluorescent labels in unmodified nano-liposomes loaded Genipin, a second labeling process was required. FITC, a standard labeling molecule that emits fluorescence, was therefore adsorbed on the outward of the nano-liposomes loaded Genipin. Following treatment with FITC- nano-liposomes loaded Genipin, the aggregates of particles were tracked with green fluorescence (Fig. 7). Labeling the nano-liposomes loaded Genipin with FITC had no statistically significant effect on their subdivision size and ZP. We chose MCF-7 cells line owing to its obtainability and capacity to arrangement a supporter cell mono-layer. As FITC-labeled nano-liposomes loaded with Genipin were incubated with cells for 4 h, it was evident that located in the peri-nuclear area for the nano-liposomes loaded with Genipin. The particles did not surround the nuclei of the cells, instead forming a coronoid arrangement around the nucleus. Internalization of Genipin-labeled nano-liposomes into cells may be modeled as an adsorption process, shadowed by the creation of vesicles and then the internalization of the vesicles containing nano-liposomes [21]. After 24h, we detected the nano-liposomes designate current mostly into the cells. Upon endocytosis, the nanoparticles were mainly located in the cytoplasm near the nuclear membrane. Even though the nano-liposomes had negatively charged surfaces, we observed that a considerable proportion were assumed through the cells and continued unchanging throughout vesicular passage. However, the exact mechanism of our nano-liposomes' internalization is still unclear. The cells keep on feasible through the development of this homework and stained with DAPI by way of these FITC-Labeling of nano-liposomes loaded Genipin did not any obvious cytotoxicity. DAPI is a common DNA-stain dye for Apoptosis cell discrimination. Blue fluorescence was revealed owing to cell nucleus stain via DAPI, that olive fluorescence was qualified by reason of cellular assumed subdivisions slightly than outward obeyed subdivisions [21]. This discovered smoothed and unbroken nuclei in the cells treated with FITC-Labeling of nano-liposomes loaded Genipin. So they had nuclei that performed extra radiantly when tainted and display hemispherical molded round the nucleus edge (apoptosis). The subsequent outcomes display the upsurge of the DAPI sign on alive cells in cells treated with FITC-Labeling of nano-liposomes loaded Genipin with 0.2µg/ml concentration (Fig. 7). The topographies appear additional projecting in the cells treated with nano-liposomes loaded Genipin.

CONCLUSION

In this study for the first time, thin layer hydration and ultrasound were used to prepare Genipin conjugated nano-liposome as a desirable targeted drug delivery system. As a result of that, the nano-liposome carriers are improved the drug therapeutic effects and decreased its complication effects. Thus loaded of Genipin into the thin layer of nano-liposome carriers led to enhancement of its solubility, stability, and effectiveness. Besides, cell uptake readings demonstrated an advanced uptake of negative nano-liposomal. In conclusion, new formulated nano-liposomal are a perfect careers for negative directing of medicines (anticancer effect) to cancer cells that might consequence in enhanced effectiveness and abridged harmfulness of capsulated medicines.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no competing interests.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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