194

Egypt. J. Chem. **Vol. 67,** No. 4 pp. 543 - 557 (2024)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Superiority of Curcumin over Gallic acid nanoliposomes in restraining the growth of Breast or Hepatic Cancer Cell Line

Nada Osama Mohamed, Omnia A. Abd El -Ghaffar, Medhat W. Shafaa, Mohamed S. El-Nagdy

Physics Department, Faculty of Science, Helwan University, Cairo, Egypt

Abstract

Transmission electron microscopy (TEM), particle size, polydispersity, zeta potential, differential scanning calorimetry (DSC), and Fourier transform infrared (FTIR) spectroscopy were used to physically characterize the interactions of curcumin or gallic acid with liposomes as model membranes. All liposomes showed distributions for contained and empty vesicles that were less consolidated, roughly spherical in shape, and dispersed. The values of the zeta potential tend to rise when gallic acid is incorporated into liposomal membranes. The primary peak temperature of empty liposomes moved to a greater level when curcumin was added. which indicates a conformational order within the phospholipids**.** The primary distinguishing endothermic peak of pure liposomes was reduced by gallic acid. FTIR study confirmed the interaction of curcumin or gallic acid with the liposome's moieties. At the highest liposomal curcumin or gallic acid concentration (1500 µg/ml), about 7% of cell viability was seen in MCF-7-treated cells. while for free curcumin or gallic acid treated cells the cell viability was approximately the same 22 %. HEPG-2 treated cells demonstrated cell viability at the maximum Curcumin concentration or its liposomal form (1500 µg/ml) of about 17% and 13%, respectively 72 hours after incubation. This study showed that the cytotoxicity effect of curcumin is more than that of gallic acid. The information gathered enables the formulation of a potential anticancer regimen that would administer free curcumin or its liposomal form to improve the efficacy of liver or breast cancer treatments.

Keywords: Curcumin; Gallic acid; Liposomes; FTIR; DSC; Cytotoxicity

Introduction

Cancer is a condition that can invade or spread to different sections of the body and is characterized by the unchecked growth and multiplication of aberrant cells. Death may follow from the spread if it is not controlled. The following therapies are available: surgery, radiation, chemotherapy, hormone treatment, immunological, and targeted therapy. **(1).**

Nanocarriers used to cure cancer have made great progress in lipid-based nanocarriers. Different forms of lipid formulations are currently available, such as lipid nanoparticles or nanostructured lipid carriers. Because of their bio compliance and biodegradability, these lipid-based models are less toxic than other drug delivery systems, such as polymeric nanoparticles.

Liposomes are phospholipid bilayers that form artificial membranous vesicles. Consequently of their biocompatibility and good ability to contain compounds that are hydrophobic and hydrophilic, liposomes have gotten a lot of interest in recent years. Liposomes are a great tool for researching how anticancer drugs interact with membranes due to the similarity between the cores of membrane bilayers and liposomes

By improving drug absorption, decrease metabolism, extending biological half-life, or decreasing toxicity, liposomes have been applied to increase the therapeutic efficacy of new and old pharmaceutical products. Then, rather than just the physico-chemical properties of the drug ingredient, the carrier's attributes play a major role in controlling how much of a drug is distributed. **(2,3).**

Curcumin (CUR; diferuloylmethane), is natural yellow pigment compound extract from the turmeric plant. It has a wide range of biological activities and pharmacological effects, including anti-inflammatory properties, anti-carcinogenic, and anti-virus capabilities, Additionally, Additionally, due to its low toxicity, it has intriguing therapeutic uses, it has prospective clinical uses., it is a lipophilic molecule that can permeate the cell membrane easily. **(4,5).**

Curcumin has anticancer activity against various cancers, such as breast cancer, ovarian cancer, head and neck squamous cell carcinoma, lung cancer, sarcoma, leukemia, and lymphoma, as well as gastrointestinal cancers (colon, stomach, and pancreatic), genitourinary cancers (prostate), **(6, 7).** However, curcumin has limited effectiveness because to its weak capacity to dissolve in water, low bioavailability, quick metabolism, and systemic exclusion **(7).** Using the benefits (such as anticancer efficacy) while trying to outweigh the drawbacks (such as solubility or toxicity) **(8)**, Recent research has revealed that curcumin inhibits cell growth in a range of human cancer cell lines when tested in vitro **(11).**

Gallic acid is a type of hydroxybenzoic acid that is primarily found in red fruits, black radish, and onions. Tea with up to 4.5 grams of gallic acid per kilogram of fresh weight of tea leaves is another significant source of the acid **(12).** It has been demonstrated that gallic acid has biological effects, such as anticancer effects. The chemopreventive effect has been verified by numerous investigations in a variety of cancer cell lines. Gallic acid has been discovered to selectively kill cancer cells while not damaging healthy cells, causing cancer cells to undergo apoptosis through mitochondria-mediated pathways **(12).** Additionally, it has been shown that gallic acid has anti-invasive and anti-metastatic properties in a variety of cancer cells. It might be an agent for both prevention and treatment of metastases from stomach cancer. Gallic acid dramatically reduces cell viability, invasion, and tube formation in human glioma cells, according to a different study **(12).** All of these data imply that additional research into the use of gallic acid as a chemopreventive and therapeutic agent is necessary.

Natural therapies may be the best alternative to chemotherapy and radiation therapy for the treatment of cancer because it has fewer physical adverse effects. This therapy can successfully prevent the destruction of good, normal cells that are close to cancer cells. **(14).**

In order to better understand how phospholipids behave in thermotropic phases and to identify changes in acyl chain conformations and distinctive PO_2^- bands in their polar heads, very little research has been done on the interactions between curcumin or gallic acid with phospholipids.

The present research targets to examine how curcumin or gallic acid influence the physical structural properties of model lipid membranes and to estimate the subtle perturbation of the lipid bilayer structure using transmission electron microscopy (TEM), Zeta potential, Dynamic light scattering (DLS), differential scanning calorimetry (DSC), and Fourier transform infrared (FTIR) spectroscopy. The work also assesses the cytotoxic effectiveness of curcumin or gallic acid and their combination with liposomes against human breast or hepatocellular carcinoma cell line (MCF-7 or HEPG-2) (in-vitro investigation).

Materials and Methods
Chemicals

From EIPICO (Egyptian International Pharmaceutical Industries Co, Egypt), curcumin or gallic with molecular weights of 368.38 and 170.12, respectively, were bought. In Figure 1, the molecular makeup of gallic or curcumin is seen. In Seohaean-ro, Gyeonggi-do, Korea, we purchased pure 99.9% ethanol from DaeJung Chemicals. In Figure 2, soy bean lecithin lipid with a molecular weight of 760 and purity of ≥98% in powder form acquired from Sigma (Carl Roth, Karlsruhe, Germany) is shown. In addition, tris base in powder form with a molecular weight (MW) of 121.1 was bought from CDH in New Delhi, India. RPMI-1640 medium, sodium bicarbonate, dimethylsulphoxide (DMSO), Indigo Trypan: It was purchased from Sigma Chemical Co., St. Louis, Missouri, USA, who also supplied trypan blue, penicillin/streptomycin, trypsin, acetic acid, foetal bovine serum (FBS), sulphorhodamine-B (SRB), 0.4% SRB dissolved in 1% acetic acid, trichloroacetic acid (TCA), and 100% isopropanol in an isotonic solution. Distilled highly pure water was used to create solutions. All other materials utilized in this experiment were of HPLC quality.
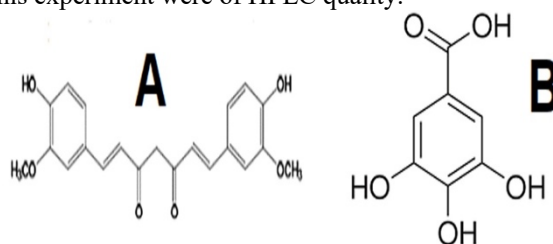

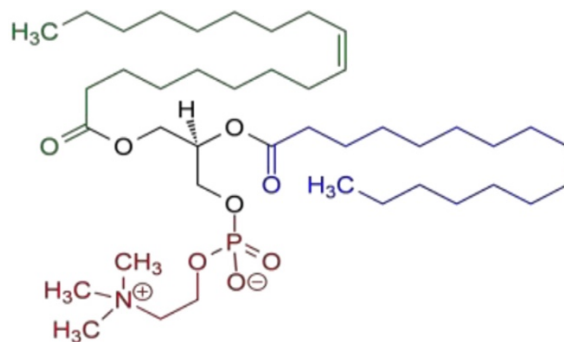**Fig.1: The molecular structure of gallic acid (B) and curcumin (A).**

Fig.2. Schematic chemical structure of Soy Lecithin Lipid.
Preparation of liposomes (soy bean lecithin)

Soy Lecithin: curcumin or gallic acid at molecule ratio of 7:2 is employed to make balanced Multilamellar vesicles (MLVs) utilizing the Bangham thin-film hydration technique **(15)**. An amount of 30 mg soy lecithin and 4.2 mg of curcumin or 2 mg of gallic acid at molecule ratio of 2:7 to soy lecithin were moved to 100 ml cylindrical flask. The flask was then shaken as 20 ml of ethanol (EtOH) was added, and the mixture was thoroughly dissolved. Using a vacuum rotary evaporator in a warm water bath (50 °C), the organic solvent was progressively extracted to create a roughly thin lipid film coating the flask's interior wall. A Tris buffer (0.2 M, pH 7.2 at 37 °C) was used to hydrate the lipid layer to create multilamellar vesicles for 15 minutes in a boiling water bath at 50 °C (MLV). The flask was then subjected to mechanical shaking at 50 °C for 1 hr and then nitrogen stream was used to flush the flask and closed immediately. The same method as described above was used to construct an empty liposome that acts as a control, utilizing only the mass aliquots of soy lecithin previously utilized in the manufacture.

Dynamic light scattering and Zeta potential

Zeta potential, average particle size and size distribution of curcumin- or gallic-acid-loaded liposomes, or a combination of the two and blank liposomes were calculated by using "Nanotrac Wave II, Microtrac, USA" (particle sizing method) for DLS (dynamic light scattering) in 7.4 PH of tris buffer at 25 °C. All results are reported as mean standard deviation and the experiment was run in triplicate.

FT-IR spectra

Lyophilized samples were made into KBr disks by combining 1 mg of the lyophilized compounds with 100 mg of dried and crushed KBr. The compounds' vibrational spectra were then recorded using an IR spectrometer of the Jasco-6300 FT-IR plus, Japan, type. The background spectra was that of KBr, and the samples were conducted over a wavenumber frequency range of 400–4000 cm^{-1}.

Differential scanning calorimetry (DSC)

Japanese-made DSC-50 Shimadzu scanning calorimeter was used to take DSC scans. DSC samples, reference cells, and degassed lyophilized substances weighing 0.5 mg were all assessed at room temperature. The scan was initiated (2 °C /min scan rate), and throughout the equilibration phase, the chemicals should fall to the bottom of the thermometer cell. The scan was performed at 25–200 °C.

Cell culture

Human Breast cancer cell line was isolated from breast mammary gland of 69 years old white female with breast cancer catalog number (MCF-7HTB-22) or Hepatocellular carcinoma cell line was derived from a hepatocellular carcinoma of a 15-year-old white male kid with liver cancer catalog number (HEPG2 HB-8065). were put in a -180 °C (liquid nitrogen). Then they were kept by serial sub-culturing in "Vacsera Vaccination Center, Cairo, Egypt". At 37 °C, cells are kept alive in DMEM media with 100 mg/mL streptomycin, 100 units/mL penicillin, and 10% heat-inactivated fetal bovine serum in a humidified, five percent (v/v) CO_2 atmosphere.

Cytotoxicity assay

The SRB assay was employed to assess the viability of cells. a portion of 100 µl cell media for 24 h. a suspension of (5×10^3) cells had been placed in 96-well plates and incubated for 24 hours before being treated with another portion of 100 µl media contains drugs at varying concentrations starting from 0 to 1500 µg/ml. 72 hours after drug exposure, Cells have been fixed by replacing the media with 150 µL of 10% TCA and incubating for 1 hour at 4 °C. After removing the TCA solution, the cells were washed five times with distilled water. Aliquots of 70 µl SRB solution (0.4% w/v) were added and incubated in the dark for 10 minutes at room temperature. Plates had been washed three times in 1% acetic acid and dried in the air overnight. The protein-bound SRB stain was then dissolved in 150 microliter of TRIS (10 mM), and the intensity of absorption at 540 nm was measured using a BMG LABTECH®-FLUO microplate reader (Ortenberg, Germany).

Results and Discussion

As illustrated in **Figure 3**, TEM scans revealed that, all liposomes generated in this study had a nearly spherical form, were effectively diffused, and were less aggregated for empty (**Figure 3A**) and encapsulated vesicles. TEM findings showed that the encapsulation of Curcumin can be physically associated with altering the surface membrane packing characteristic of liposomes (**Figure 3B**). The presence of gallic acid in liposomes (**Figure 3C**) increased the distance between neighboring bilayers, resulting in larger liposomes than controls. The larger particle size could be attributed to stronger pharmacological interactions with the lipid bilayer of liposomes through hydrogen bonding. It is supported that both Curcumin or Gallic acid may be inserted into the bilayer's hydrophobic region, and these findings are consistent with the DSC and FTIR data.
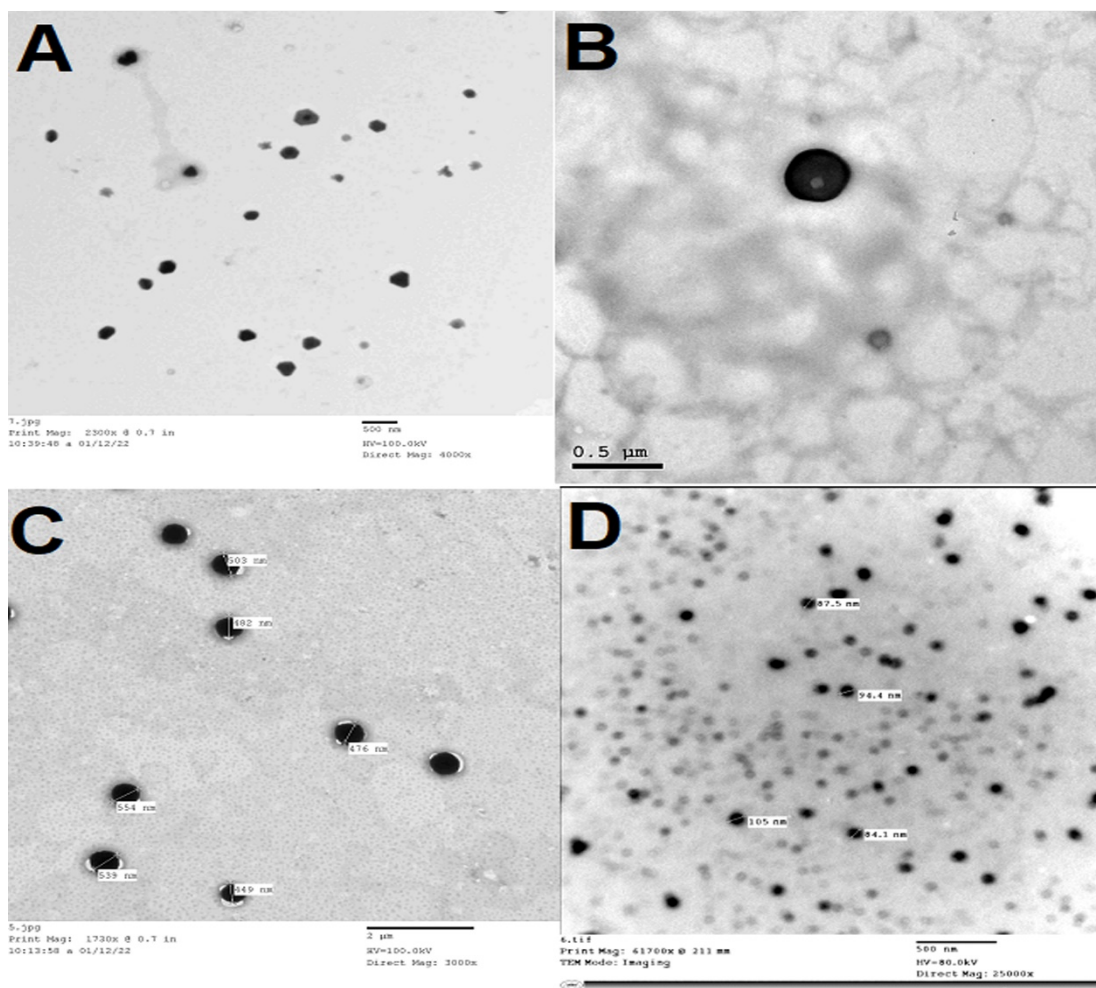
Fig. 3. TEM scan for blank liposomes (A), liposomes loaded with curcumin (B), liposomes loaded with gallic acid (C) and Curcumin combined with Gallic acid into liposomes (D).

Colloidal suspension particle homogeneity is effectively accounted for by the polydispersity index (PDI). Values greater than 0.7 indicate that the sample's size range is extremely broad and that the dynamic light scattering method is consequently unstable. .

Figure (4A) indicates that a pure soy lecithin lipid sample's size distribution occurred at 345.6±109.5 nanometer mean size diameter and 0.409 PDI. **Figure (4B)** indicates a decrease in the average diameter of pure soy lecithin to **142.6±78.87** nm with **0.376** PDI upon the encapsulation of curcumin into pure soy lecithin lipid and this probably could be explained by the electrostatic attraction between the positive charge of lecithin $N(CH_3)_3^+$ molecule and the negative charge of OH^- group of Curcumin Whereas the medicine curcumin is primarily thought of as a lipophilic substance and may become stuck in the hydrophobic core of the bilayer.

When curcumin was added to liposomes, the distance between adjacent bilayers shrunk, causing the liposomes to form smaller than they would have otherwise. The calculated mean size diameter of blank liposomes decreased to 276.9±74.56 nanometer with 0.311 PDI as a result of the addition of gallic acid into soy lecithin lipid **Figure (4C)**. In comparison to liposomal curcumin, larger liposomes were formed when gallic acid was added to the liposomes because it increased the distance between adjacent bilayers. A decrease in gallic acid particle size as compared to empty liposomes may be brought on by more potent drugs interactions with the polar head group of phospholipids due to the electrostatic attraction between the positive charge of soy lecithin $N(CH3)3+$ and the negative charge of gallic acid's $OH-$ group. Those results indicate that curcumin or gallic acid may be physically linked to the liposomes at the core and their molecules frequently interacts with the lipid bilayer and disturbs it. **Figure (5D)** showed that after incorporation of Curcumin associated with gallic acid together into soy lecithin liposomes, the calculated mean size of blank PE was increased to **905.8±146.2** nm with **0.609** PDI. The results of these studies suggest that the center of the liposomes may be physically linked to Curcumin or Gallic acid and their

molecules tends to be interacted to large extent with the lipid bilayer and perturbed them. The results of the particle size shown by TEM are shown in Figure (4)

are in agreement with the results of DLS measurements (**Figure 5**).
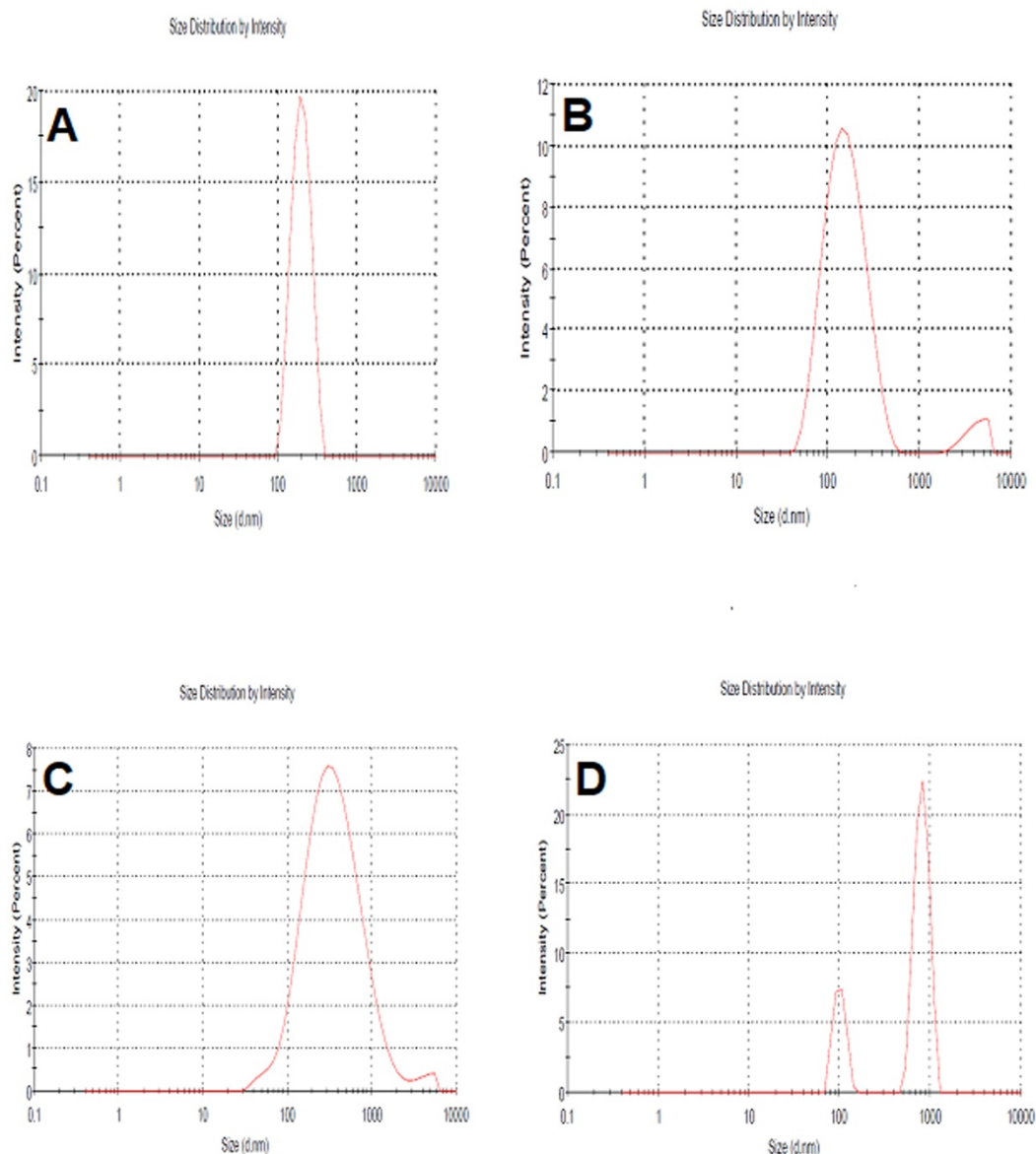

Fig.4. Liposome size distribution was determined using dynamic light scattering (DLS) for (A) Blank Liposomal soy lecithin sample, (B) Curcumin doped with liposomes, (C) Gallic acid doped with liposomes and (D) Liposomes carrying curcumin and gallic acid.

The magnitude of zeta potential signifies the possible stability of a colloidal system. The repulsion between particles would be greater as the zeta potential increases, causing a more stable colloidal suspension. If all particles in suspension have a significant negative or positive zeta potential, they appear to resist one other and do not tend to come together. **(17)**.
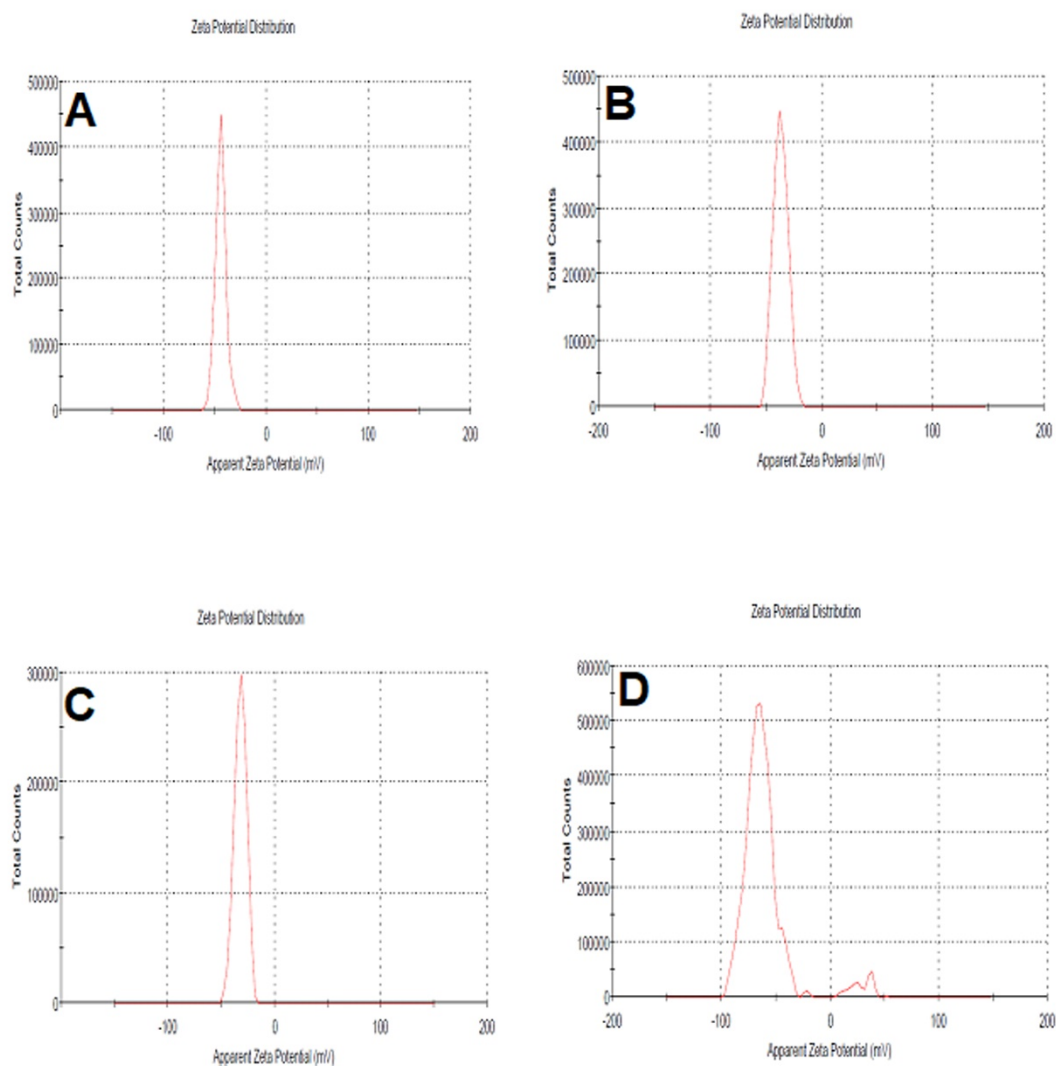
Fig.5. Zeta potential for (A) Blank Liposomal soy lecithin sample, (B) Curcumin doped with liposomes, (C) Gallic acid doped with liposomes and (D) Liposomes carrying curcumin and gallic acid.

According to the observation of others, empty liposomes displayed negative zeta potential (**-44 ±5.35** mV) **(18),** curcumin or curcumin combined with gallic acid-loaded liposomes had lower negative zeta potential (**-32±5.69** mV and **-36.6±6.56** mV) in comparison to blank liposomes due to their incorporation into the soy lecithin liposomal vesicles. Particles with zeta potentials more positive than +30 mV or more negative than -30 mV are normally considered stable. The incorporation of curcumin or gallic acid turns to reduce the total amount of negative charge, resulting in a less negative zeta potential. The liposomal inclusion of gallic acid was recorded the highest zeta potential value among drug formulations (**-51.5±6.08** mV) as the repulsion between particles becomes stronger, resulting in a more stable colloidal suspension (**Fig.5**). **Table (1)** shows the results of particle size and zeta potential measures of several liposomal formulations.

Table 1 shows the results obtained for dynamic light scattering (DLS) and Zeta potential of liposomes before and after incorporation with curcumin, gallic acid, or their combination.

Sample	DLS		Zeta Potential
	Mean size ± SD (nm)	PDI	Mean Zeta Potential ± SD (mV)
Empty liposomes	**345.6±109.5**	**0.409**	**-44 ±5.35**
Liposomal Curcumin +gallic acid	**905.8±146.2**	**0.609**	**-36.6±6.56**
Liposomal Curcumin	**142.6±78.87**	**0.376**	**-32±5.69**
Liposomal gallic acid	**276.9±74.56**	**0.311**	**-51.5±6.08**

The analysis of DSC was utilized to analyze variations in the lipid bilayer phase transition due to altering interactions among the loaded drugs and liposomes **(19)**.

Soy lecithin vesicles were employed as a model membrane because this phospholipid can mimic many characteristics of biological membranes. After dehydration, pure soy lecithin vesicles showed a large main endothermic peak (T_m) at 146 °C (**Figure 5**) when submitted for DSC examination, in accordance with **(21)**. For pure soy lecithin liposomes, the pre-transition temperature (T_p) was disappeared.

The soy lecithin membranes may be affected by the drug, which could change the thermotropic parameters of the vesicles transition. The main endothermic peak (T_m) of empty soy lecithin presents at 146 °C, whereas the addition of curcumin to soy lecithin liposomes showed a slight shift to a higher temperature at 147.6 °C, indicating that the lipid membrane's phase-transition temperature directly influences its liquidity. which in turn affects the release of curcumin from liposomes.

As a result of the drug molecules' potential to break specific structural arrangements of polar head group regions, the melting point of liposomes may be further lowered. Hydrogen bonds spanning adjacent head-groups may be broken by the drug molecules. Curcumin significantly affected the conformational order within the phospholipids and increased the cooperative transition of lipid acyl chains in the soy lecithin bilayer. **(22)**.

the addition of gallic acid to soy lecithin liposomes showed a shift to a lower temperature at 129 °C compared to the empty soy lecithin's primary endothermic peak (T_m), which presents at 146 °C , which indicates that the soy lecithin bilayer of acyl chains was significantly impacted by gallic acid, resulting in a structural disorder within the phospholipids and a decrease in the cooperative transition of lipid acyl chains. Gallic acid inclusion is more conducive to the production of acyl chains in a disordered and loose condition, as evidenced by the lower temperature of the primary endothermic peak (T_m) of empty soy lecithin.

Interestingly, upon the inclusion of Curcumin combined with gallic acid into soy lecithin liposomes exhibited a significant rise to a higher temperature at 149.5 °C compared the major characteristic endothermic peak of pure soy lecithin that exists at 146 °C. The inclusion of the drug between the polar heads of the soy lecithin will make it easier to produce a less crystalline liquid phase than the gel phase and, as shown by DSC, it will also slightly decrease the transition temperature from the gel to liquid phase **(23)**.

Combinations of soy lecithin with curcumin or gallic acid have been discovered to have a single peak when studied using dynamic light scattering, indicating their miscibility **(23)**.
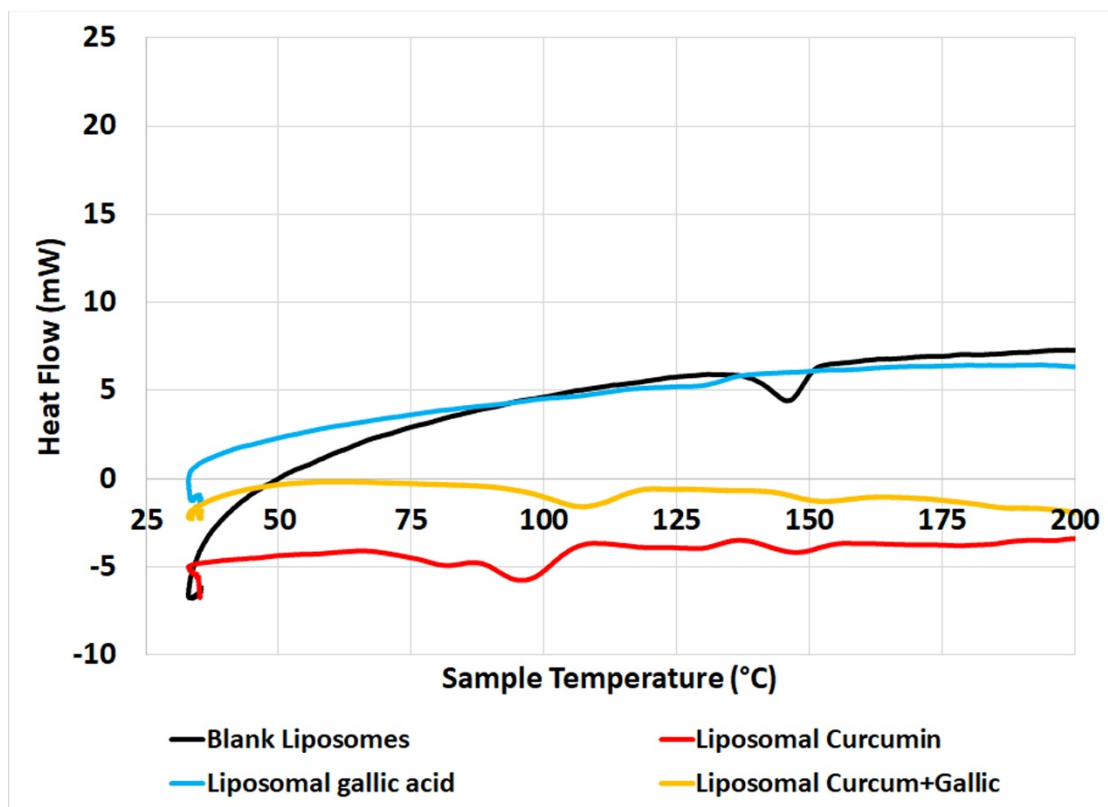
Figure.5 dynamic light scattering diagrams of liposomes made of empty soy lecithin, liposomes doped with either curcumin or gallic acid or both.

FTIR spectroscopy was used to examine the wavenumber of various functional groups taking into account the acyl chains and the lipid molecule head group area in the presence or absence of foreign molecules in order to examine potential structural changes in pure soy lecithin.

FTIR spectra of empty lyophilized soy lecithin liposomes compared with liposomal curcumin or liposomal gallic acid or liposomal preparation of their mix samples in the 4000-400 cm^{-1} range as shown in (**Figure 6**). The soy lecithin liposomes' spectrum shows the maximum absorption FTIR distinctive peaks., the symmetric and antisymmetric PO$_2^{-}$ stretching vibrations at about 1090 and 1220 cm^{-1}, respectively; the CH$_2$ bending vibration at about 1470 cm^{-1}, the carbonyl stretching vibration C=O at about 1734 cm^{-1}, the OH stretching and bending vibrations at about 3470 and 1640 cm^{-1}, and the symmetric and antisymmetric stretching vibrations of the CH$_2$ in the acyl chain at about 2850 and 2920 cm^{-1}. These results are fairly consistent with the information given in the literature**.** In the distinct spectral region, namely 400-4000 cm^{-1} (**Figure 6**), FTIR spectra are analyzed.
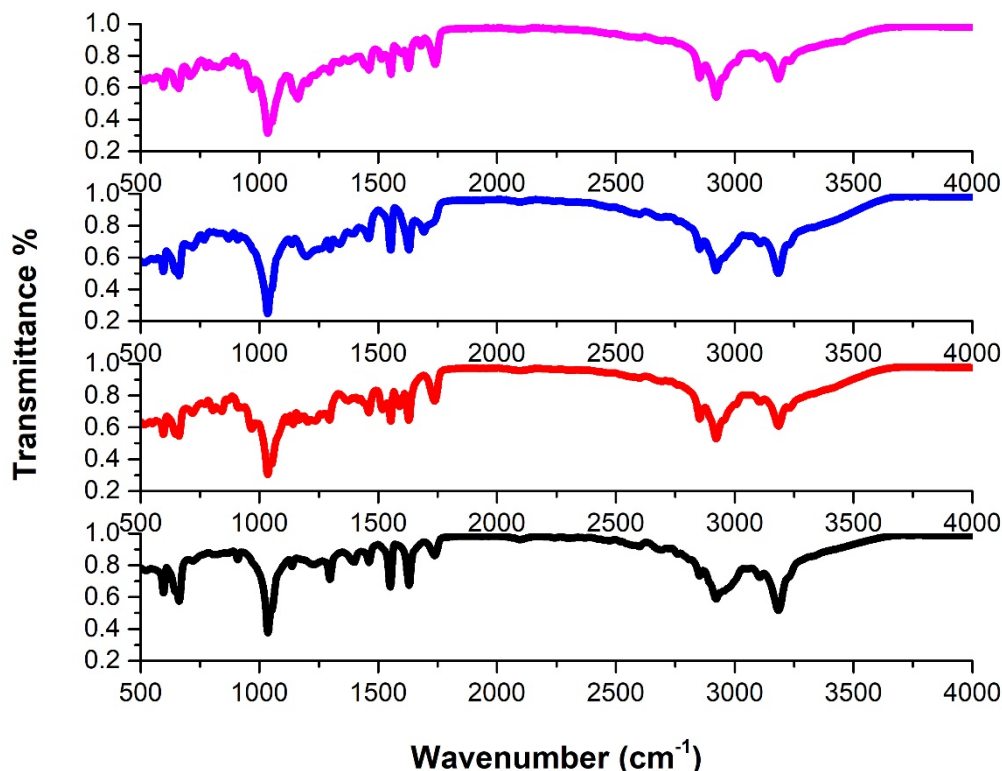
Fig. 6. The full FTIR spectra of empty soy lecithin and soy lecithin/ curcumin or gallic acid or combination of both liposomal samples.

Entrapment of curcumin or gallic acid or both into the soybean lecithin liposomes induced a shift in the frequency of the acyl chain's antisymmetric CH_2 stretching bands appeared in **Figure 6**, suggesting that both curcumin or gallic acid or both give the acyl chains of phospholipids a conformational order. In other words, they affected the lipid bilayer's order. The frequency peak of the acyl chain's antisymmetric CH_2 stretching bands, which is centered at 2931.51 cm^{-1} for the pure soy lecithin is shifted towards lower wave number 2923.27 cm^{-1} for both curcumin or gallic acid or their mix doped-liposomes. This would signify a decline in the amount of gauche conformers, which would suggest an increase in the rigidity of the membrane or bilayer and, consequently, a stabilization of the system in the gel phase. **(24)**.

The frequency values can be used to track the average trans/gauche isomerization in the systems since the frequencies of the CH_2 stretching bands of acyl chains depend on the level of conformational instability. Increases in the quantity of gauche conformers are correlated with shifts to higher wavenumbers. **(25,26)**.

Peaks of the symmetric and asymmetric stretching vibrations of CH_2 have been employed as a sensitive diagnostic of the ordering of the alkyl chains. The frequency of the CH_2 stretching bands has changed significantly, showing that the quantity of gauche conformers was reduced by either curcumin or gallic acid embedded in liposomes and this implies an increase in the conformational order (trans-gauche isomerization) of the bilayer.

The C=O stretching band was used to study how curcumin and gallic acid interacted with the glycerol backbone close to the interfacial area. For the liposomes sample containing curcumin, the frequency value of C=O group was changed to lower degrees (from 1740.48 cm^{-1} to 1738.72 cm^{-1}), providing evidence of the establishment of hydrogen bonds. The frequency value of C=O group remained stable without change for the liposomes sample containing gallic acid or its mix with curcumin. So, any changes in the spectra of this region can be attributed to an interaction between the interfacial region of the membrane and either gallic acid or curcumin (**Figure 6) (27,28)**. By observing variations in the shapes of the ester C=O stretching, the degree of hydrogen-bond formation in the glycerol backbone region of the soy lecithin molecule was determined. According to the empirical guidelines, lowering frequency values shows an increase in the production of new hydrogen bonds between the components or the strengthening of existing hydrogen bonds. **(24,29)**.

The hydrogen bonding and other interactions affect the ester C=O's absorption bands, which are sensitive to changes in the polarity of their local surroundings. As a result, modifications in the shape of the ester C=O absorption band can frequently be explained by modifications in the hydration or structural makeup of the bilayer polar/apolar interface.

Not all compounds examined in soy lecithin liposomal sample preparations had an impact on the CH_2 scissoring vibration modes, which are located at 1458.54 cm^{-1}. The wavenumber was not shifted after the integration of these molecules into soy lecithin liposomes. This might assume that the molecules of both curcumin or gallic acid or both were not act as small spacer of the polar head group (**Figure 6**).

The interaction between the head group of the soybean lecithin liposomes and the molecules of either curcumin or gallic acid was investigated at 1244.53 cm^{-1} utilizing the PO_2^- antisymmetric stretching band. **Figure 6 shows** the PO_2^- antisymmetric stretching band for soybean lecithin was moved to lower values (1236.29 cm^{-1}) after the introduction of both curcumin or gallic acid into soybean lecithin liposomes. This suggested that the investigated compounds and the liposome head group had formed hydrogen bonds. Wavenumber values that have decreased could be a sign that the components are forming new hydrogen bonds or that existing hydrogen bonds are being strengthened. **Table 2** summarized the chemical changes noticed for liposomal curcumin or liposomal gallic acid after the incorporation into pure soybean lecithin vesicles liposomes.

Table 2. the chemical changes noticed for Liposomal Curcumin or Liposomal Gallic acid or both of them after the incorporation into pure soy lecithin vesicles liposomes.

Peak assignment	Wavenumber (cm^{-1})	Wavenumber (cm^{-1})			
		Control Liposomes	Liposomal Curcumin	Liposomal Gallic acid	Liposomal Curcumin mixed with Gallic acid
Antisymmetric stretching vibration of CH_2 in acyl chain	(2916–2925)	2931.51	2923.27	2923.27	2923.27
Carbonyl stretching vibrations (C=O)	(1724–1745)	1740.48	1738.72	1740.48	1740.48
CH_2 scissoring vibration	(1457-1466)	1458.54	1460.60	1460.60	1460.60
Anti-symmetric PO_2 stretching vibration	(1215-1245)	1244.53	1236.29	1236.29	1236.29

The potential cytotoxic activity of the drug delivery system has been investigated at various drug concentrations of free curcumin or curcumin nanoliposomes and free gallic acid or encapsulated into liposomes or their combination together against breast carcinoma (MCF-7) or hepatocellular carcinoma cell line (HEPG-2) using cell viability (In Vitro Cytotoxicity sulforhodamine B) assay **(36,37).**

Untreated cells act as controls when the concentration of each drug was zero. Cell viability measurements were taken once the test was stopped after 72 hours. Cancer cell lines MCF-7 or HEPG-2 were treated independently for 72 hours with various drug concentrations in the same order, ranging from 0 to 1500 μg/ml. (**Figure 7**).
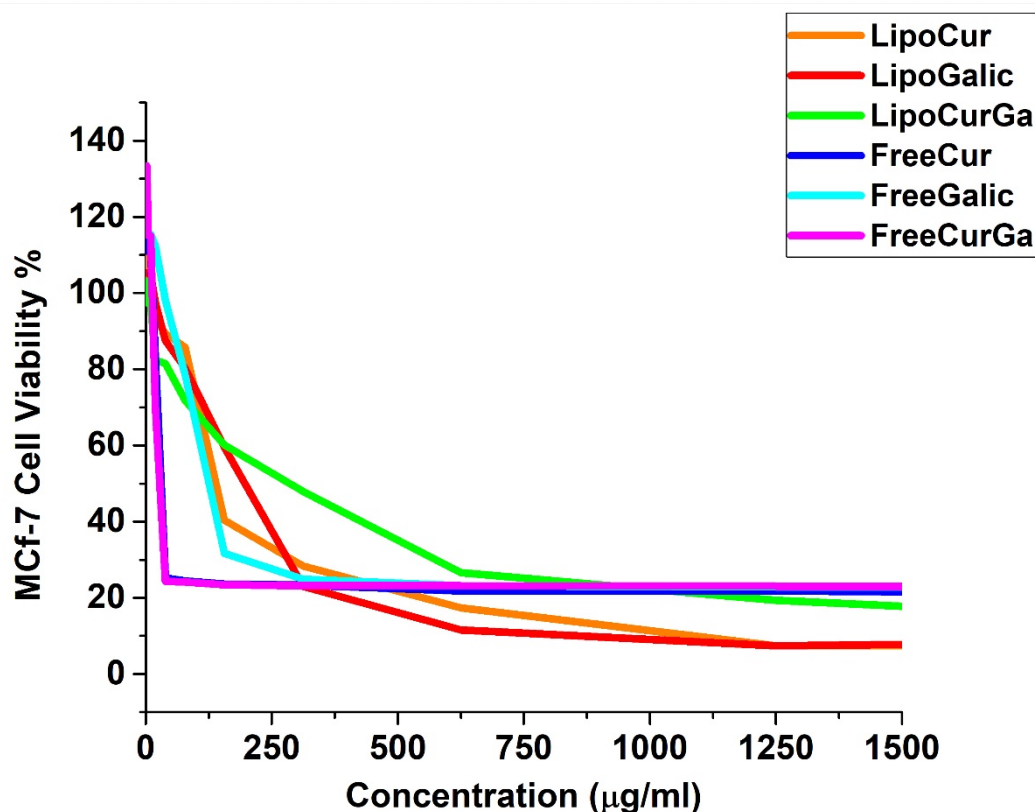
Figure.7 Cytotoxicity of free curcumin, free gallic acid, free mix of them and curcumin or gallic acid or both bound liposomes as opposed to untreated control breast cancer cell line (MCF-7); incubated for 72 h with different concentrations starting from 0 to 1500 µg/ml. The cell viability was determined using the (sulforhodamine B) SRB assay. The data represent the mean ± standard error of triplicate experiments

According to cell viability studies, liposomal curcumin or gallic acid displayed the highest rate of cytotoxicity against MCF-7 cell line treated with the same sequence of different concentrations exposed to other drugs. MCF-7 treated cells had a cell survival of roughly 7% at the maximum liposomal curcumin or gallic acid concentration (1500 µg/ml) 72 hours post-incubation. While free curcumin treated cells had a cell vitality of around 21.8%, free gallic acid had a cell viability of around 22.9%, compared to 23% for free curcumin mixed with gallic acid and 19.3% for its liposomal form at the same dose (1500 µg/ml). The lower in cell viability of liposomal form in comparison with free drug (curcumin or gallic acid) may be due to the sustained release of curcumin or gallic acid from liposomes in cells treated with liposomes with curcumin or gallic acid. So, the quantity of the released drug grows as the concentration of the encapsulated drug increases, and as a result, the viability of the cell diminishes as the concentration of the encapsulated drug increases.

MCF-7 cells treated with free curcumin or curcumin combined with gallic acid at a lower dose of roughly 80 µg /ml demonstrated cell survival of 24% compared to liposomal forms of around 85% and 72%, respectively. While the liposomal form of gallic acid remained alive in 80% of the cells. **Figure (7).**

Liposomal curcumin displayed the highest rate of cytotoxic against HEPG-2 cell line treated with the same sequence of different concentrations available for other drugs, according to cell viability assays. HEPG-2 treated cells demonstrated cell viability at the maximum curcumin concentration or its liposomal form (1500 µg/ml) of about 17% and 13%, respectively 72 hours after incubation. While free gallic acid treated cells, Cell viability was around 18.5%. and for liposomes with gallic acid loaded, Cell viability was around 18.5%. 48.48 % at the same concentration (1500 µg/ml). While for free curcumin combined together with gallic acid treated cells, the cell viability was 18% compared to 16% relative to its liposomal form.

At a lower concentration of around 80 µg /ml, HEPG-2 treated cells with free curcumin or curcumin mixed with gallic acid together demonstrated cell survival of 19% compared to liposomal forms of about 86 % and 76%, respectively. While 80 % of the cell remained viable for liposomal form of gallic acid, **Figure (7).**
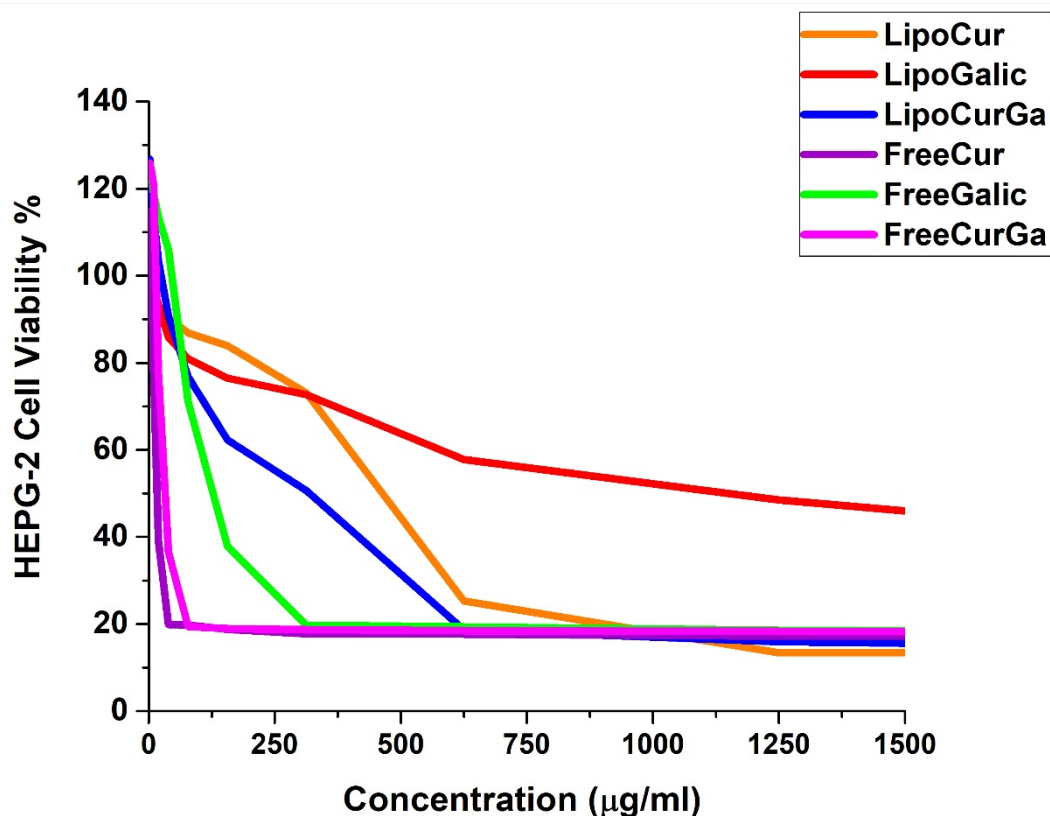
Fig.8. Cytotoxicity of free curcumin, free gallic acid, free mix of them and curcumin or gallic acid or both bound liposomes as opposed to untreated control liver cancer cell line (HEPG-2); incubated for 72 h with different concentrations starting from 0 to 1500 μg/ml. The SRB (sulforhodamine B) assay was used to determine the cell viability. The figure show the mean ± standard error of experiments conducted in triplicate.

The concentration of a drug needed to inhibit a biological process or response by 50% (IC_{50}) value for free curcumin in cytotoxic assay with MCF-7 treated cells has counted as 27.94 μg/ml, while MCF-7 treated cells with liposomal curcumin had a higher value of 141.16 μg/ml. The IC50 for MCF-7 treated cells with free gallic acid was 50.71 μg/ml and 167.88 μg/ml for its liposomal version. **Figure 9**.

This low efficacy can be attributed to the drug's lipo-solubilized form as a result of its entrapment within several lipoidal domains of vesicles. IC_{50} value for MCF-7 treated cells with free curcumin combined together with gallic acid was 47.2 μg/ml, while its liposomal form was 54 μg/ml.
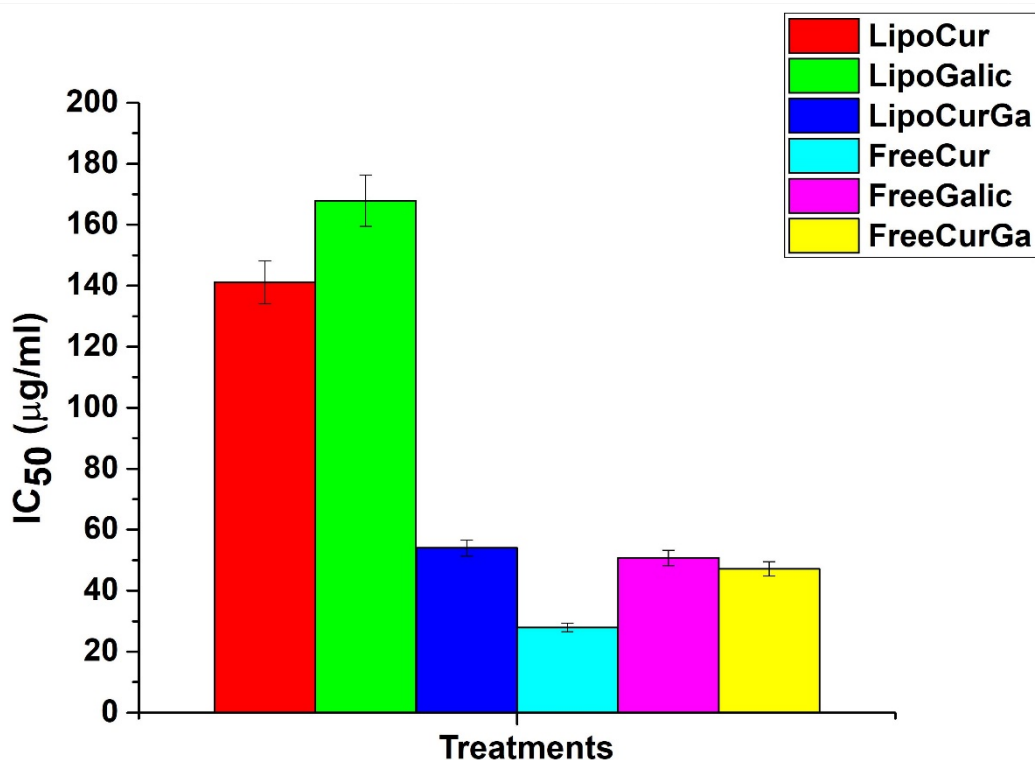
Fig.9. IC$_{50}$ curve with significant values for free curcumin, free gallic acid, free mix of them and curcumin or gallic acid or both bound liposomes against MCF-7 cell line by using SRB (sulforhodamine B) assay, after 72-hour post-treatment.

The concentration of a drug needed to inhibit a biological process or response by 50% (IC$_{50}$) for HEPG-2 treated cells with free curcumin was 13.39 μg/ml, while liposomal curcumin was 467.7 μg/ml. Based on the results, encapsulating free curcumin into liposomes shown a reduced therapeutic efficacy against the HEPG-2 cell line, depending on the cancer cell type (Figure **10).** The IC50 value for free gallic acid in cytotoxic assay with HEPG-2 treated cells has counted as 47.84 μg/ml, while the lipo-state of gallic acid treated cells for Hep-2 treated cells was counted as 497 μg/ml. IC$_{50}$ value for HEPG-2 treated cells with free curcumin combined together with gallic acid was 57 μg/ml, while its liposomal form was 34.6 μg/ml.

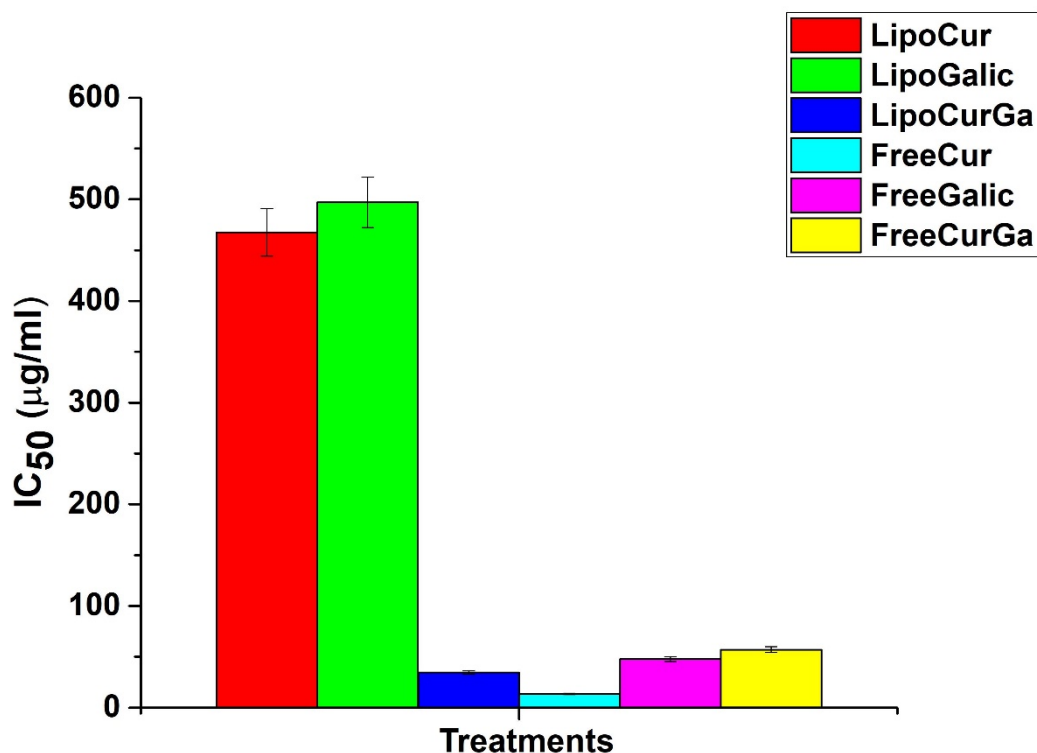**Fig.10. IC$_{50}$ curve with significant values for free curcumin, free gallic acid, free mix of them and curcumin or gallic acid or both bound liposomes against HEPG-2 cell line by using SRB (sulforhodamine B) assay, after 72-hour post-treatment.**

Conclusion

When curcumin or gallic acid is incorporated into lipid bilayers, it actively interacts with the lipids, altering their physico-chemical properties. The data obtained allow proposing a possible antitumor treatment regimen where free curcumin is administered to increase the effectiveness of breast or liver cancer therapy. These findings could help in the creation and design of curcumin for the treatment of breast and hepatocellular cancers. Future research should look into different combinations of liposomes and other cancer treatments.

Conflict of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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