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On the advantage of free beta carotene over its liposomal form in restraining the growth of radiation-exposed breast carcinoma cell line (MCF-7)

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ABSTRACT

This work aimed to provide an in-depth study of the interaction between β-carotene and lecithin liposomes. The study additionally investigated the toxicity of beta-carotene, in both its unencapsulated and liposome-bound forms, on MCF-7 breast cancer cells that had been subjected to radiation and those that had not. The interactions between β-carotene and lecithin liposomes, serving as model membranes, were analyzed. Liposomes exhibited a predominantly well dispersed, spherical morphology with minimal aggregation. Reduced zeta potential values were observed after β-carotene was incorporated into the liposomal membranes, indicating a change in surface charge. Furthermore, incorporating β-carotene caused a decrease in the temperatures observed, in contrast to empty liposomes, suggesting a conformational change within the phospholipids. This was confirmed by FTIR studies, which demonstrated interactions between β-carotene and the liposomal components. In cytotoxic assays with MCF-7 cells, if external gamma irradiation was withheld, the IC50 value for free β-carotene was 3.45 µg/ml. In contrast, liposomal β-carotene exhibited a higher IC50 of 52.30 µg/ml. When combined with gamma irradiation at 5 Gy followed by 10 Gy, the IC50 values for free β-carotene and liposomal β-carotene increased slightly, from 3.14 to 4.05 µg/ml and from 50.21 to 53.92 µg/ml, respectively. The data suggests free β-carotene's cytotoxic effect is more powerful than that of liposomal β-carotene. The study emphasizes the potential of free β-carotene as an effective anticancer agent against the MCF-7 cell line. These results advocate for considering the natural product, free β-carotene, as a promising alternative to pharmacological interventions in breast carcinoma treatment.

1. INTRODUCTION

Cancer is a general name for a group of diseases in which normal cells begin to spread in an uncontrolled manner. When left untreated, death is the usual outcome. Cancer is now the second most frequent cause of death in the world, after only heart disease. Present treatments include surgical resection, radiation, chemotherapy, hormonal therapy, immunotherapy, and targeted therapy. [1]

Among different cancers, breast cancer ranks second in terms of mortality rates. The World Health Organization (WHO) projects a staggering increase in new breast cancer cases, with an estimated 27 million cases and 17.5 million deaths by 2050. [2]

In the field of cancer therapy, impressive progress has been achieved by the employment of nanocarriers, particularly lipid-based nanocarriers. Different lipid systems, such as lipid nanoparticles (LN) and nanostructured lipid carriers (NLC) have been developed. The lipid vehicles are biocompatible and biodegradable and are thus less toxic than other drug delivery systems, such as polymeric nanoparticles.[3].

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Liposomes, specifically, are artificial spherical vesicles composed of a lipid bilayer surrounding a hollow core. They can be loaded with chemotherapeutic drugs and targeted to tumor sites. Given their effectiveness, biocompatibility, lack of immunogenicity, improved drug solubility, and capacity to encapsulate diverse agents, liposomes have gained widespread use in drug delivery systems. The administration of anticancer drugs via liposomal encapsulation enhances their efficacy by directing the medication away from healthy tissues and potentially reducing the dose required to achieve cytotoxic effects within tumor cells. [4,5].

Lecithin is a complex mixture comprising glycolipids, triglycerides, and various phospholipids such as phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol. However, in the field of biochemistry, Commonly, lecithin denotes phosphatidylcholine in its pure form, which is an important phospholipid largely present in the phosphate portion of egg yolks. This substance is recognized as a non-toxic surfactant that organisms readily accept. It's an essential part of cell membranes and undergoes complete metabolic breakdown within the body. Its safety profile and accessibility make lecithin an ideal choice for generating liposomal structures. [6].

In radiation therapy, radiosensitizers are vital because they boost the amount of radiation a tumor absorbs by making cancer cells more susceptible to radiation. This method allows doctors to use lower radiation doses, which helps reduce harm to healthy tissues [7].

Radiation therapy, a widely employed cancer treatment, targets tumors confined to specific regions of the body. By disrupting the DNA or genetic material responsible for cell replication and growth, radiation therapy effectively combats cancer. Radiation can directly damage DNA, leading to cell death, or induce the generation of free radicals by ionizing intracellular water molecules, particularly within the DNA vicinity. [8].

Combining radiation therapy with anticancer drugs is a common strategy for treating various cancers, capitalizing on their synergistic effects. However, the precise mechanisms underlying this synergy remain largely elusive. Research into the mechanism of sensitization to radiotherapy with cisplatin, particularly in breast cancer has been undertaken. Given that many tumors are localized upon diagnosis, radiotherapy stands as a primary and effective treatment modality.

Carotenoids are plant-based, fat-soluble pigments with a basic polyisoprenoid backbone. Population studies find that women with more α-carotene, β-carotene, lycopene, or lutein-zeaxanthin circulating in their blood tend to develop breast cancer less often. [9]

Population studies repeatedly find that people who load their plates with green and yellow fruits and vegetables tend to develop cancer less often. Since these foods are packed with -carotene, scientists have focused on this compound as a possible protective agent. A large body of research now points to the everyday -carotene in the current research diets as playing a role in lowering cancer risk.[10, 11]

Furthermore, previous studies have illuminated the diverse biological properties of β-carotene, encompassing anti-inflammatory, antioxidant, and anticancer effects. Tests in several cancer cell lines and animal models show that the compound slows growth and kills tumors, strengthening the case that it could play a real role in preventing and treating the disease.[12]

Exploring natural treatments could offer a promising alternative for cancer management, circumventing the diverse physical side effects commonly associated with chemotherapy and radiotherapy. Such treatments hold potential for effectively sparing normal, healthy cells in the vicinity of cancerous ones, minimizing collateral damage [13].

Currently, no existing research has investigated how β-carotene interacts with phospholipids. Specifically, so far, no research has looked at how the lipids actually shift their melting points or at any changes in the way their tails curl up or in the telltale PO^{2-} signals near the head. equally, nobody has tested how free β-carotene or β-carotene trapped in liposomes-if given alone or alongside gamma rays, stops MCF-7 breast cancer cells from growing.

One aim of this study was to investigate the impact of β-carotene on the physical properties of lecithin, serving as a model lipid membrane. Various analytical techniques, including A range of analytical techniques—including FTIR, TEM, DLS, polydispersity measurements, zeta potential analysis, and DSC—were utilized to thoroughly investigate structural changes in the lipid bilayer.

Additionally, this research evaluated the cytotoxic power of β-carotene, both when free and when enclosed in liposomes. The goal was to clarify how these agents might affect the survival of MCF-7 breast cancer cells in a lab setting, particularly when exposed to different levels of gamma radiation.

2. MATERIALS USED AND METHODS

2.1. Chemicals

Beta-carotene was isolated from natural sources and subsequently purified. Its identity and purity were verified using thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). Structural confirmation relied on spectral data, as depicted in (**Figure1**). The compound's molecular weight was determined to be 536.9.

Ethanol of absolute purity (99.9%) was sourced from DaeJung Chemicals (Seohaean-ro, Gyeonggi-do, Korea). L-α-phosphatidylcholine (Soy Lecithin), in powder form, was obtained from Carl Roth (Karlsruhe, Germany), with a molecular weight of 760 and purity ≥97%. Its chemical structure is illustrated in **Figure 2**. Tris base, in powder form, with a molecular weight of 121.1, was sourced from CDH, New Delhi, India.

The MCF7 human breast cancer cell line was stored at -180°C (in liquid nitrogen) according to "The American Form Culture Array" guidelines. It was then kept alive by repeated sub-culturing at the "Vacsera Vaccination Center, Cairo, Egypt."

Several reagents were sourced from Sigma Chemical Co., St. Louis, MO, USA, including:

Dimethylsulphoxide (DMSO), DMEM medium, Sodium bicarbonate, Trypan blue (0.05% isotonic solution in normal saline), Penicillin/Streptomycin, Trypsin, Acetic acid, Fetal bovine serum (FBS), Sulphorhodamine-B (SRB), Trichloroacetic acid (TCA)

For the experiments, 0.4% SRB (dissolved in 1% acetic acid) and 100% isopropanol were also obtained. All solutions were made with ultrapure distilled water, and all solvents and reagents met HPLC grade standards.

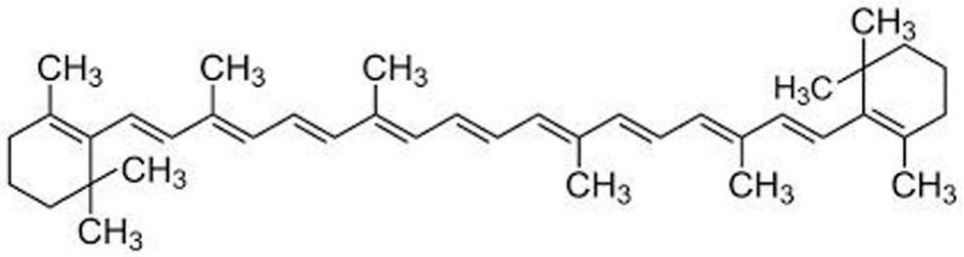

Fig. (1): The chemical structure of β-carotene.

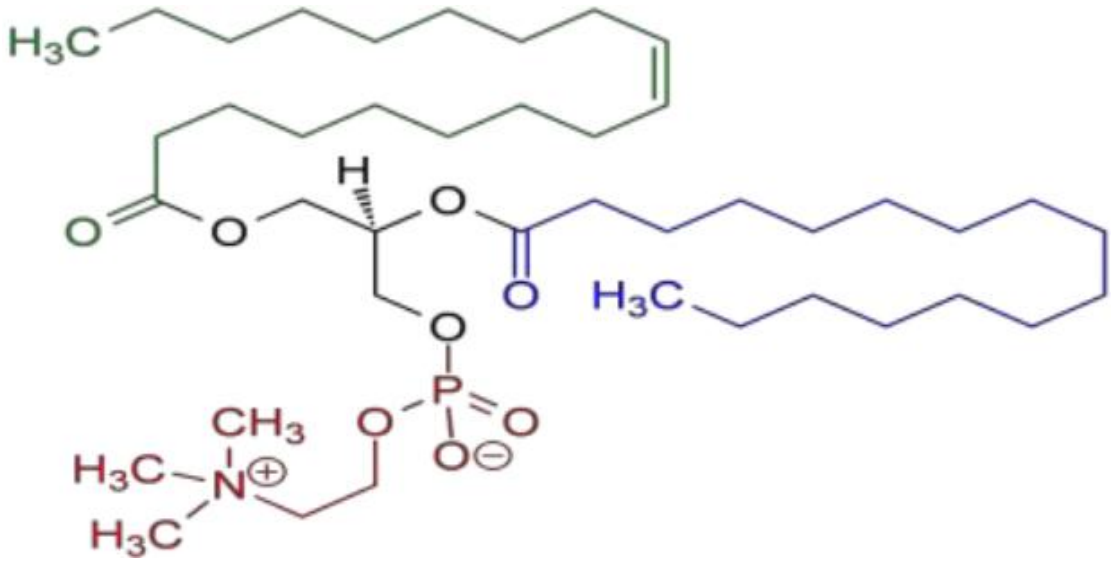

Fig. (2): Schematic chemical structure of L-α-phosphatidyl choline (Soy Lecithin).

2.2. Preparation of liposomes

Neutral multilamellar vesicles (MLVs) were produced by combining lecithin and β-carotene at a 7:2 molar ratio, following the Bangham thin-film hydration method [14]. The procedure began by placing 30 mg of lecithin and 6 mg of β-carotene into a 100 ml round-bottom flask, then adding 20 ml of ethanol (EtOH) and agitating until fully dissolved.

A vacuum rotary evaporator was used with a 50°C warm water bath to gradually evaporate the organic solvent, resulting in a uniform thin lipid film on the flask's inner surface. Subsequently, this lipid film was rehydrated with Tris buffer (0.2 M, pH 7.2 at 37°C) in a 50°C water bath for 15 minutes to facilitate MLV formation. The flask was then mechanically agitated at 50°C for 1 hour, immediately followed by nitrogen flushing and sealing. For a comparative control, empty liposomes were prepared using the identical method, but only with the corresponding amount of lecithin from the initial preparation.

2.3. Encapsulation efficiency measurement

To figure out how much β-carotene was successfully encapsulated in the liposomes, researchers performed an extraction as described in protocols [15, 16]. They began by vigorously vortexing 100 µl samples of β-carotene-loaded liposomes with 2 mL of ethanol for three minutes at room temperature. This process aimed to extract any free β-carotene from the suspension. Afterward, the mixture was centrifuged at 6000 rpm for 20 minutes to separate the liquid (supernatant) from the remaining sample. Finally, a spectrophotometer was used to measure the β-carotene's absorbance at 446 nm, allowing for quantification.

The quantification of β-carotene was achieved using a specific formula, which relied on measuring precise volumes of the β-carotene solution to determine its weight. To enhance the accuracy and reliability of the data, each experiment was performed in triplicate.

$$W(mg) = \frac{A * V * 10^3}{A_{1cm}^{1\%} * 100} * DF$$

Where W represents the carotenoid weight, while A refers to the optical density—ideally maintained between 0.2 and 0.8 for reliable results. The volume of the lutein-containing solution is denoted by V. The term $A_{1cm}^{1\%}$ indicates the specific extinction coefficient for each carotenoid in the solvent used—for instance, β-carotene in ethanol has a value of 2620. DF denotes the dilution factor, which accounts for any sample dilution during preparation. [17]

2.3 Determination of Entrapment efficiency percentage

The entrapment efficiency (EE%) was calculated through the following relationship:

$$EE\% = \frac{Total\ drug\ imput(\text{mg}) - Drug\ in\ supernatant(\text{mg})}{Total\ drug\ input(\text{mg})} \times 100 \quad \textbf{(1)}$$

2.4. Liposome morphology by transmission electron microscopy

Researchers used a negative stain transmission electron microscope (JEOL, JEM-2100, Japan), set at 200 kV, to examine the size and structure of both empty liposomes and those containing β-carotene. A 1% (w/v) solution of phosphotungstic acid in water was the negative staining agent.

The process involved first diluting liposome samples (1:10) in Tris buffer (pH 7.4 at 37°C). Then, a 20 µl drop of the diluted solution was placed on a carbon-coated copper TEM grid. After letting it sit for one minute, any extra liquid was carefully blotted away from the grid. Finally, TEM images were taken and thoroughly analyzed to understand the liposomes' size and shape.

2.5. Dynamic light scattering and Zeta potential

The zeta potential, mean particle size, and size distribution of both β-carotene-loaded liposomes and empty liposomes were assessed using Dynamic Light Scattering (DLS), with instrumentation from PSS (Santa Barbara, CA, USA). All measurements were carried out in Tris buffer (pH 7.4) at 25°C. Each experiment was performed in triplicate to ensure reproducibility. Results are reported as mean values ± standard deviation.

2.6. DSC measurements

Differential Scanning Calorimetry (DSC) was performed using a Setaram Labsys instrument (France), which had been calibrated with indium. Lyophilized samples—both empty liposomes and those containing β-carotene—were each sealed in standard aluminum pans, with a sample mass of 5 mg. Thermal scans were carried out at a heating rate of 3 °C per minute, over a temperature range from 25°C to 200°C, in order to characterize the thermograms for each sample.

2.7. FTIR Spectroscopy

FTIR spectra of lyophilized samples of liposomes doped with β-carotene or blank liposomes deposited on

KBr discs was recorded by "Alpha 11, Bruker spectrometer (Switzerland)". At room temperature, the scanning was carried out with a speed of 2 mm /s within the range of 4000-400 cm^{-1}.

2.8. In-Vitro Cytotoxicity by MTT Assay

The MCF-7 breast cancer cells were first rinsed with PBS to remove residual medium or debris. After that, they were treated with trypsin and then resuspended in RPMI-1640 complete culture medium. Cell viability and counts were determined using a hemocytometer with the Trypan blue exclusion method. This standard method is commonly used for cell preparation. for prepping cells before experiments. [18].

For the chemosensitivity assays, approximately 10,000 cells were seeded into each well of a 96-well plate and allowed to adhere for 24 hours. After this attachment period, the medium was replaced with fresh culture medium containing the test compounds at a range of concentrations, prepared by two-fold serial dilution. Both blank liposomes and β-carotene-loaded liposomes were evaluated independently for cytotoxicity, with each concentration tested in triplicate to ensure reproducibility.

Cells were then exposed to the drug-containing medium for a further 48 hours at 37°C with 5% CO_2. Following incubation, the medium was carefully aspirated, and the cells were washed twice using PBS to remove residual compounds. Subsequently, 50 µl of MTT solution (0.5 mg/ml) was added to each well, and the plates were incubated for an additional 4 hours at 37°C. After this, the supernatant was removed, and 50 µl of dimethyl sulfoxide (DMSO) was used to dissolve the formazan crystals formed by metabolically active cells. This procedure facilitated the subsequent quantification of cell viability.

To assess cell viability, absorbance was measured at 490 nm using an ELISA reader (Boster Immunoleader, USA). The percentage of surviving cells was determined by comparing the absorbance values of treated wells to the control wells—basically, dividing one by the other and multiplying by 100 for the percent.

For the MCF-7 breast cancer cell line, a survival curve was constructed by plotting cell viability against various concentrations of each drug. This allowed us to determine the IC50 value, which represents the drug concentration required to inhibit 50% of cell growth.

2.9. *In Vitro* Irradiation of MCF-7 breast cancer cell line

MCF-7 breast cancer cells were cultured in 25 cm^2 flasks at a density of 1×10^4 cells per well and incubated for 24 hours. After this initial period, the cells were exposed to the IC_{50} concentration of the respective chemotherapeutic agents, as determined by an MTT cytotoxicity assay. Subsequently, the cells underwent gamma irradiation at doses of either 5 or 10 Gy, delivered using a ^{137}Cs unit (Gamma-cell 40, Canada) operating at a dose rate of 0.653 rad/sec.

3. RESULTS AND DISCUSSION

When β-carotene was pre-mixed with the lipid powder prior to ethanol dissolution, the entrapment efficiency consistently exceeded 90% across all liposomal suspensions. Transmission electron microscopy (Figure 3) demonstrated that the liposomes produced in this study predominantly exhibited a spherical morphology, were well-dispersed, and showed minimal aggregation, regardless of whether they were empty or encapsulated β-carotene.

In terms of size, empty liposomes measured approximately 160 ± 20 nm, while β-carotene-loaded liposomes were notably larger, averaging around 380 ± 60 nm, as illustrated in Figures 3A and 3 B.

Transmission Electron Microscopy (TEM) analysis demonstrated that β-carotene directly influences membrane organization in liposomes (Figure 3B). Incorporation of β-carotene resulted in increased spacing between adjacent bilayers, producing larger liposomes compared to the control. This enlargement likely reflects stronger interactions—possibly hydrogen bonding—between β-carotene and the lipid bilayer. It is suggested that β-carotene becomes embedded within the hydrophobic core of the bilayer. Notably, these observations are in agreement with the results obtained from Differential Scanning Calorimetry (DSC).

Nano drug delivery systems play a crucial role in enhancing the bioavailability of entrapped drugs, with their size tailored to match with cell dimensions. The polydispersity index (PDI) serves as a reliable indicator of particle homogeneity in colloidal suspensions. Values exceeding 0.7 indicate a wide range of sizes, which can lead to instability, particularly in dynamic light scattering techniques [19].

Figure 4A displays the size distribution for a pure sample of L-α-phosphatidylcholine (soy lecithin), with the mean particle diameter measured at approximately 154.4 ± 88.88 nm and a polydispersity index (PDI) of 0.187. Following the incorporation of β-carotene, the average diameter of the resulting liposomes increased notably to 194 ± 64.74 nm, accompanied by a higher PDI of 0.382. This enlargement can be attributed to enhanced interactions between β-carotene and the polar head groups of the phospholipids, likely through hydrogen bonding near the

PO_2^- moiety (as shown in Figure 4B). The random distribution of the nonpolar β-carotene within the lipid bilayer, lacking any preferred orientation, appears to increase the motional freedom of both the alkyl hydrocarbon chains and the polar head groups, resulting in a more fluid lipid bilayer.[20].

These observations suggest that β-carotene physically associates with liposomes, embedding itself within the core and interacting extensively with the bilayer, thereby inducing structural perturbations. Notably, these conclusions are consistent with findings from differential scanning calorimetry (DSC) and Fourier-transform infrared spectroscopy (FTIR) analyses.

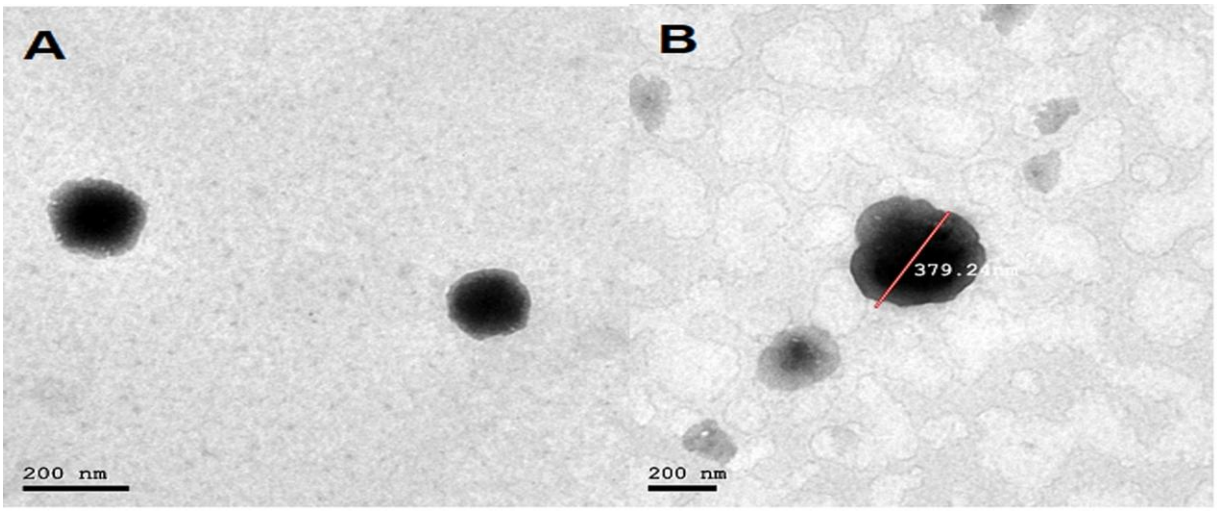

Fig. (3.): TEM images for empty liposomes (A), β-carotene -loaded liposomes (B).

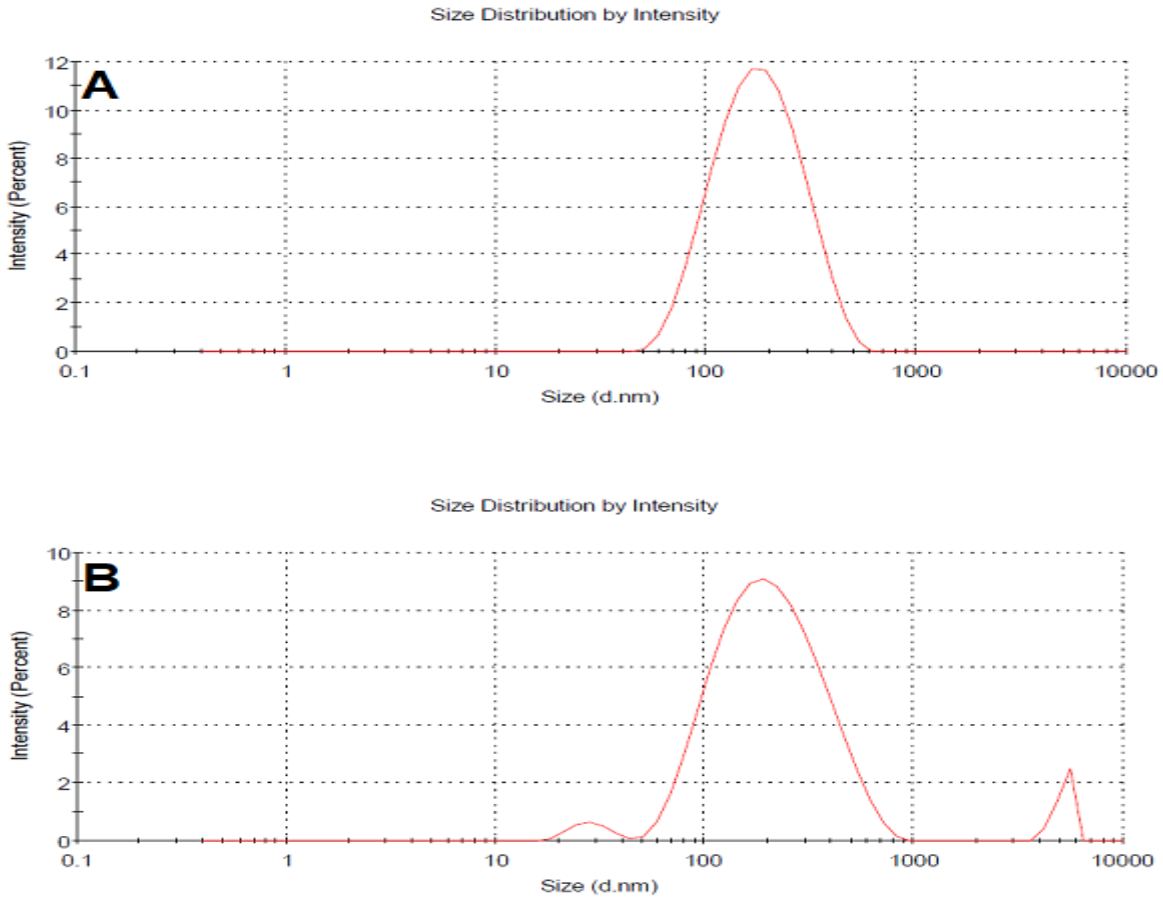

Fig. (4): Liposomes size distribution measured by dynamic light scattering (DLS) for (A) empty liposomal sample, (B) β-carotene-encapsulated liposomes.

A colloidal system's potential stability is directly reflected by the value of its zeta potential. As the zeta potential rises, the repulsive forces among individual particles grow stronger, promoting a more stable dispersion. Consequently, particles with a considerable negative or positive zeta potential actively repel each other, effectively stopping them from combining or forming clumps.[21].

Other studies have reported a negative zeta potential of blank liposomes (-29.7±7.06 mV) [22-25]. β-carotene-loaded liposomes exhibited a slightly lower negative zeta potential (-25.8±5.88 mV) compared to empty liposomes, attributed to the incorporation of β-carotene into the liposomal membranes. Generally, nanoparticles with zeta potential values exceeding +30 mV or falling below -30 mV are considered more stable. The presence of β-carotene in the liposomal membranes appears to reduce the density of negative charge, resulting in weaker repulsion between particles and consequently a less stable colloidal dispersion (**Figure 5**).

Table 1 presents a summary of the particle size and zeta potential measurements for liposomal β-carotene.

Table (1): summary of the DLS and Zeta potential data for liposomes, both prior to and after encapsulation with β-carotene.

Sample name	Mean diameter (nm) ± SD (nm)	PDI average	Mean zeta potential ± SD (mV)
Blank Liposomes	154.4 ± 88.88	0.187	-29.7 ± 7.06
Liposomal β-carotene	194 ± 64.74	0.382	-25.8 ± 5.88

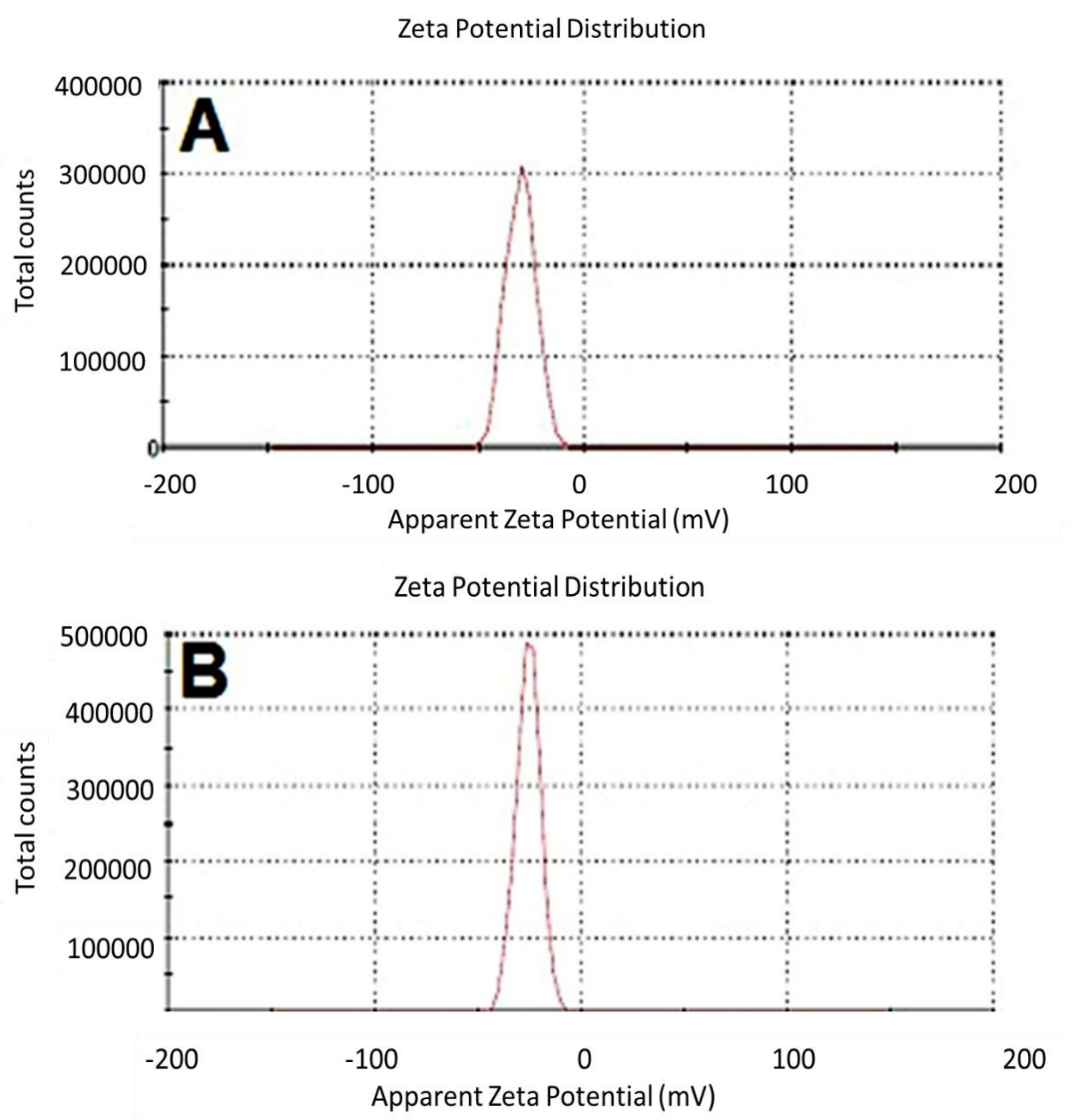

Fig. (5): Zeta potential for (A) empty lecithin liposomal sample and (B) β-carotene-encapsulated liposomes.

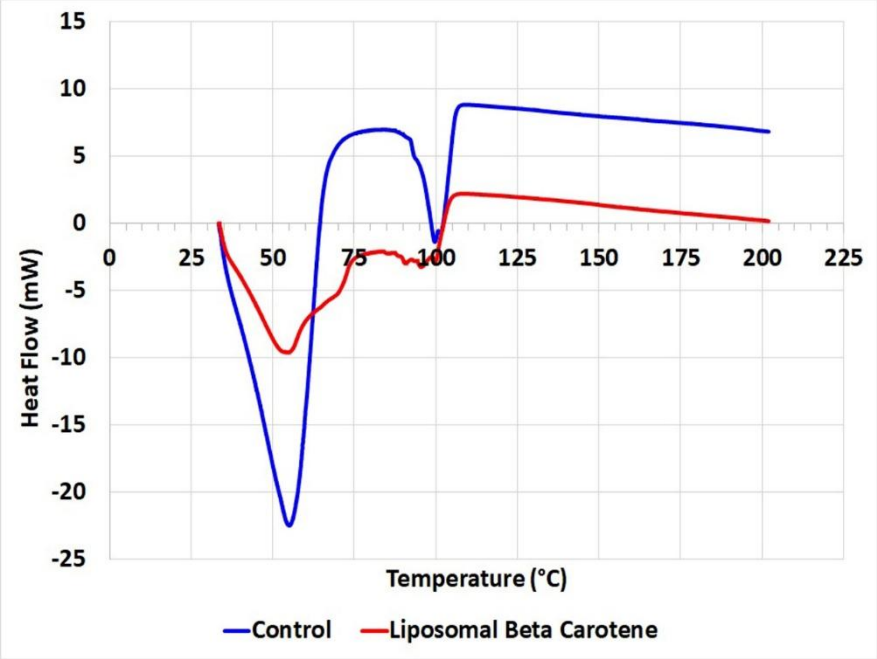

Fig. (6): DSC diagrams of liposomes doped with β-carotene versus liposomes composed entirely of lecithin.

Differential scanning calorimetry (DSC) was employed to investigate how drug incorporation altered the phase transition behavior of the lipid bilayer in liposomes. This approach allowed for the assessment of changes in bilayer properties resulting from drug–liposome interactions. [26, 27]

Lecithin vesicles serve as effective model membranes, largely due to their capacity to replicate several key features of biological membranes. Following dehydration and differential scanning calorimetry analysis, these pure lecithin vesicles exhibited a distinct main endothermic transition (Tm) at 101.37 °C. This observation aligns closely with previously reported data in the literature.[28-30]. A pre-transition peak was also observed in pure lecithin liposomes. (T_p =-55.08 °C).

When a compound is present within lecithin liposome membranes, it can alter the thermotropic parameters of the vesicles' transitions. The incorporation of β-carotene into lecithin liposomes caused the main endothermic peak (Tm) to shift to a lower temperature of 79.38 °C, a notable change from the 101.37 °C observed for empty lecithin liposomes. This shift suggests that β-carotene substantially impacts the acyl chains of the lecithin bilayer, leading to conformational disorder in the phospholipids and enhancing the transition cooperativity of the lipid acyl chains [31-32]. The reduction in the main endothermic peak (Tm) temperature in β-carotene-containing liposomal lecithin indicates that β-carotene promotes the formation of disordered and loosely packed acyl chains.

The pre-transition temperature (Tp) peak for β-carotene liposomes is noticeably broader and occurs at a lower temperature (around 40.58°C), indicating a clear interaction between β-carotene and the polar head groups of the phospholipids (see Figure 6). This interaction likely involves β-carotene inserting itself between the polar head groups of lecithin, which disrupts the regular packing and promotes the formation of a less ordered liquid crystalline phase, as opposed to the more organized gel phase. Differential scanning calorimetry (DSC) analysis supports this, revealing a slight reduction in the gel-to-liquid crystal transition temperature. [33].

The DSC results indicated that mixing lecithin with β-carotene produced a single peak—suggesting the two components are indeed miscible. In other words, they appear to integrate rather than remain as distinct phases. [34].

FTIR Analysis of Liposomal Membrane Structure

FTIR analysis was employed to investigate changes in the structure of the liposomal membrane. By examining the wavenumbers associated with specific vibrational modes, this study were able to

identify and confirm several structural alterations, many of which were also indicated by the DSC results.

Structural Characterization of Lecithin via FTIR

This study utilized Fourier Transform Infrared (FTIR) spectroscopy to probe potential structural modifications in lecithin. This technique allowed for the assessment of wavenumbers corresponding to various functional groups, particularly within the acyl chains and the head group region of the lipid molecule. These analyses were conducted both in the presence and absence of additional compounds.

Comparative FTIR Spectra of Liposomal Samples

Figure 7 presents the FTIR spectra for both empty, lyophilized lecithin liposomes and β-carotene/lecithin liposomal formulations, measured across the 4000–400 cm^{-1} range. This comparison highlights the spectral distinctions arising from the incorporation of β-carotene.

The primary FTIR absorption peaks for the liposome vesicles were consistent with those presented in [35]. When β-carotene was incorporated into the lecithin liposomes, there was a distinct shift in the wavenumber of the antisymmetric CH2 stretching bands within the acyl chain (see Figure 7). This alteration indicates that β-carotene disrupts the typical order of the phospholipid acyl chains, introducing a degree of conformational disorder to the membrane structure. More specifically, the peak observed for pure lecithin at 2923.11 cm^{-1} shifted to 2927.41 cm^{-1} in the presence of β-carotene. This increase in wavenumber is commonly associated with a higher prevalence of gauche conformers, suggesting enhanced disorder within the lipid bilayer.[36].

The CH_2 symmetric and antisymmetric stretching peaks act as reliable markers for alkyl chain organization. Notably, shifts in the CH_2 stretching band around 2923.11 cm^{-1} suggest that β-carotene promotes an increase in gauche conformers, which, in turn, points to greater conformational disorder within the bilayer structure. Essentially, the presence of β-carotene disrupts the ordered arrangement of the membrane, resulting in a less structured environment.[37, 38].

Analysis of the C=O stretching band provides insight into the interaction between β-carotene and the glycerol backbone near the head group of phospholipids in the interfacial zone [39]. As depicted in Figure 7, the wavenumber value of the C=O group for pure lecithin at 1740.44 cm^{-1} decreases in liposomal samples containing β-carotene (1734.65 cm^{-1}), The observed shifts in the shape of the ester C=O stretching contours within the glycerol backbone region of the lecithin molecule suggest the modulation of hydrogen bond formation. Consequently, alterations in the spectrum of this particular area could stem from interactions between β-carotene (or other compounds) and the apolar/polar interfacial region of the membrane. [40].

Researchers examined how β-carotene interacts with the head group of lecithin liposomes by looking at the PO_2^- symmetric stretching band at 1045.22 cm^{-1}. As **Figure 7** illustrates, adding β-carotene to lecithin liposomes caused this wavenumber to shift to a higher value (1050.05 cm^{-1}). This upward shift indicates that hydrogen bonding is absent between the liposome head group and β-carotene. Conversely, a decrease in the wavenumber value would suggest either stronger existing hydrogen bonds or the formation of new ones between the components. [36].

Because β-carotene has no polar atoms in its structure, it's unable to interact with the liposome's head groups. This leads to a less fixed orientation within the membrane and increases the disorder in the normally well-organized fatty acid chains when the membrane is in its gel state. When non-polar β-carotene is incorporated into the membrane, it might create free space in the polar region of the bilayer, potentially allowing the polar head groups more freedom to move. Ultimately, this would make the membrane's polar section less compact [41].

The inclusion of β-carotene in the lecithin liposomal preparation did not change the CH_2 scissoring vibration mode, which is found at 1463.70 cm^{-1}. This indicates that β-carotene doesn't function as a small spacer for the polar head group. Instead, it causes considerable disorder within the hydrocarbon chains. **Table 2** displays the chemical shifts observed for β-carotene after its incorporation into lecithin liposomes.

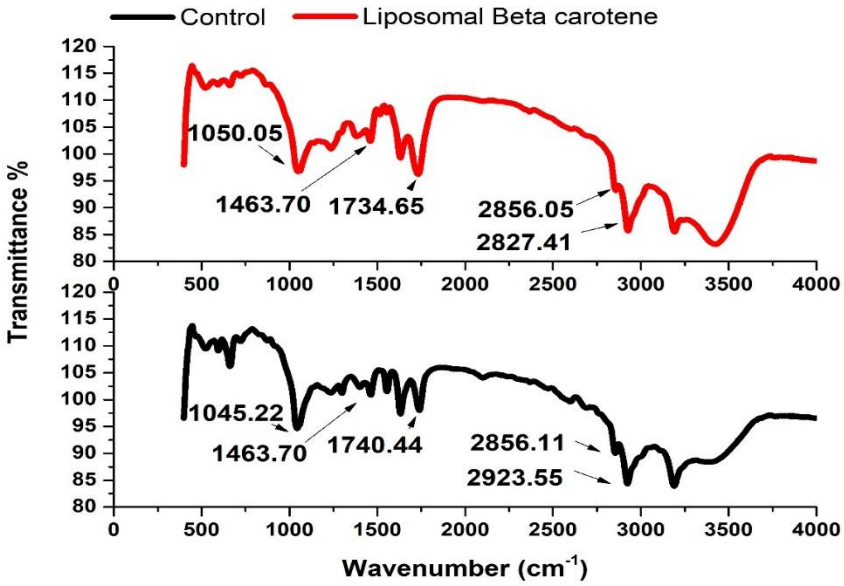

Fig. (7): The full FTIR spectra of empty liposomal lecithin and liposomes doped with β-carotene.

Table (2): The chemical shifts observed for β-carotene after the incorporation into liposomes.

Feature of Band assignment	Wavenumber (cm^{-1})	Blank Liposomes	Liposomal β-carotene
acyl chain's symmetric stretching vibration of CH2	(2800–2860)	2856.11	2856.05
acyl chain's antisymmetric stretching vibration of CH2	(2900–2930)	2923.55	2927.41
Stretching vibration of carbonyl C=O	(1730–1740)	1740.44	1734.65
CH_2 scissoring vibration	(1456–1470)	1463.70	1463.70
Symmetric PO_2^- stretching vibrations	(1000–1090)	1045.22	1050.05

To evaluate how effective the drug delivery system is—both with and without external gamma irradiation—researchers conducted in vitro cytotoxicity tests on the MCF-7 breast carcinoma cell line, utilizing the MTT assay.(18) They examined a range of concentrations for both free β-carotene and its liposomal formulation. Untreated cells acted as the control group at zero drug concentration. The MCF-7 cells were exposed to drug concentrations ranging from 100 to 1200 µg/ml and incubated for 48 hours, as illustrated in **Figure 8**. After incubation, cell viability was assessed to determine the cytotoxic effects of the various treatments.

In the absence of external gamma irradiation, free β-carotene demonstrated the most pronounced cytotoxic effect on the MCF-7 cell line when compared to other tested concentrations. At the highest concentration examined (1200 µg/ml), cell viability dropped to approximately 14.95% for free β-carotene and 15.45% for β-carotene encapsulated in liposomes after 48 hours of incubation. The diminished efficacy observed with the liposomal formulation is likely due to the drug becoming sequestered within the vesicle's multiple lipoidal domains, limiting its bioavailability. At a lower concentration (700 µg/ml), treatment with free β-carotene still resulted in low cell viability (15.96%), whereas the liposomal form maintained substantially higher viability at around 79.60%. These findings **(see Figure 8)** underscore the enhanced cytotoxic potential of free β-carotene relative to its liposomal counterpart, particularly at reduced concentrations.

at both 700 and 1200 µg/ml, free β-carotene demonstrated greater cytotoxic activity compared to the liposomal form. In other words, the free β-carotene was consistently more effective at these concentrations. The liposomal formulation, while active, didn't match the potency seen with the free compound.as shown in **Figure 8**.

In the absence of external gamma-irradiation, the IC50 value for free β-carotene in the cytotoxic assay with MCF-7 treated cells was 3.45 µg/ml, while liposomal β-carotene for MCF-7 treated cells was 52.30 µg/ml. These results indicate that depending on the cancer cell type, free β-carotene exhibited the highest therapeutic efficacy against the MCF-7 cell line (**Figure 9**).

According to Mahrous et al. (**42**), the carotenoid β-carotene demonstrates selective inhibition of breast cancer cell development. Their findings suggest a notable impact of both free β-carotene and its nanoliposomal form in eradicating breast cancer cells. These results introduce a novel treatment approach where β-carotene or its liposomal form could potentially replace cyclophosphamide to enhance its anticancer activity against the MCF-7 cancer cell line.

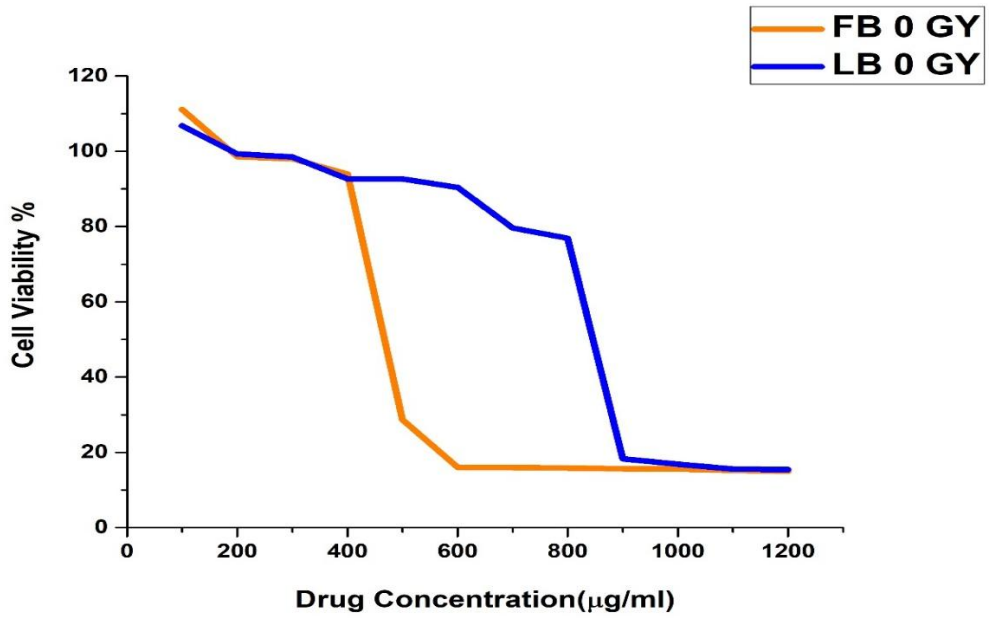

Fig. (8): The cytotoxic effects of both free β-carotene (FB) and its liposomal form (LB) were assessed in vitro using the MCF-7 human breast carcinoma cell line, without the application of external gamma-irradiation. Cells were treated with a range of concentrations (100–1200 µg/ml) and incubated for 48 hours. Cell viability was quantified via the MTT assay. All data are presented as mean ± standard error, based on three independent replicate experiments.

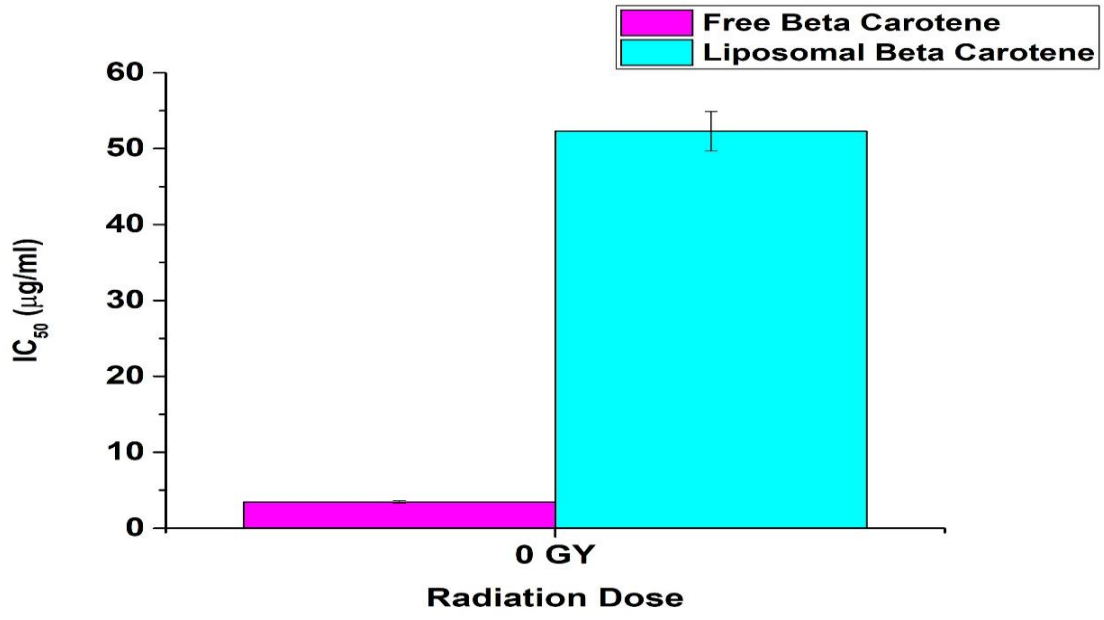

Fig. (9): IC_{50} values of free β-carotene and its liposomal form in the absence of external gamma-irradiation against breast carcinoma (MCF-7) cell line using MTT assay, 48 h post-treatment.

The proliferation/viability of MCF-7 cells was assessed by MTT assay after the incubation with the tested chemotherapeutic agents in the presence of external irradiation of cells with radiation doses of 5 and 10 gray using a ^{137}Cs, respectively (**Figure 10**). The cytotoxic effect of the tested chemotherapeutic agents (free β-carotene and its liposomal form) did not increase significantly with an increase in radiation dose from 5 to 10 gray. The toxicity of all tested chemotherapeutic agents increased as the drug concentration increased in a concentration-dependent manner.

At the maximum concentration (1200 $\mu g.ml^{-1}$) combined with irradiation of cells at 5 Gy (**Figure 10**), the toxic effect of liposomal β-carotene followed by free β-carotene markedly decreased the cell viability to 11.85% and 14.23%, respectively. In this cytotoxicity test, the lipo-state of β-carotene caused more death of viable cells than free β-carotene, where the cell viability was 14.23%. This increased toxicity may be due to the preferential uptake of nanoparticles than that of the free drug. It is obvious that the combination therapy regimen (free β-carotene or its liposomal form + radiation) has no significant anti-cancer effect than the treatment regimen without radiation. This suggests that the single therapy regimen (β-carotene or its liposomal form without radiation) might be more successful than the combination of formulated β-carotene and radiation therapy. At the lower concentration of 700 $\mu g.ml^{-1}$ combined with irradiation of cells at 5 Gy, MCF-7 treated cells with free β-carotene displayed cell viability of 26.83 %, while 100 % of the cell remained viable for liposomal β-carotene, **Figure 10**. Cytotoxic activity among β-carotene formulations at the lower concentration (700 $\mu g.ml^{-1}$) combined with irradiation of cells at 5 Gy displayed the order of free β-carotene > liposomal β-carotene according to **Figure 10.**

At the maximum concentration (1200 $\mu g.ml^{-1}$) combined with irradiation of cells at 10 Gy, the cytotoxic activity among β-carotene formulations displayed equal activity between free β-carotene (11.11%) and liposomal β-carotene (11.23%) according to **Figure 10.** Consequently, at the lower concentration of 700 $\mu g.ml^{-1}$ combined with irradiation of cells at 10 Gy, the cytotoxic activity among β-carotene formulations displayed the order of free β-carotene (23.20 % viable) > liposomal β-carotene (100% viable)**.**

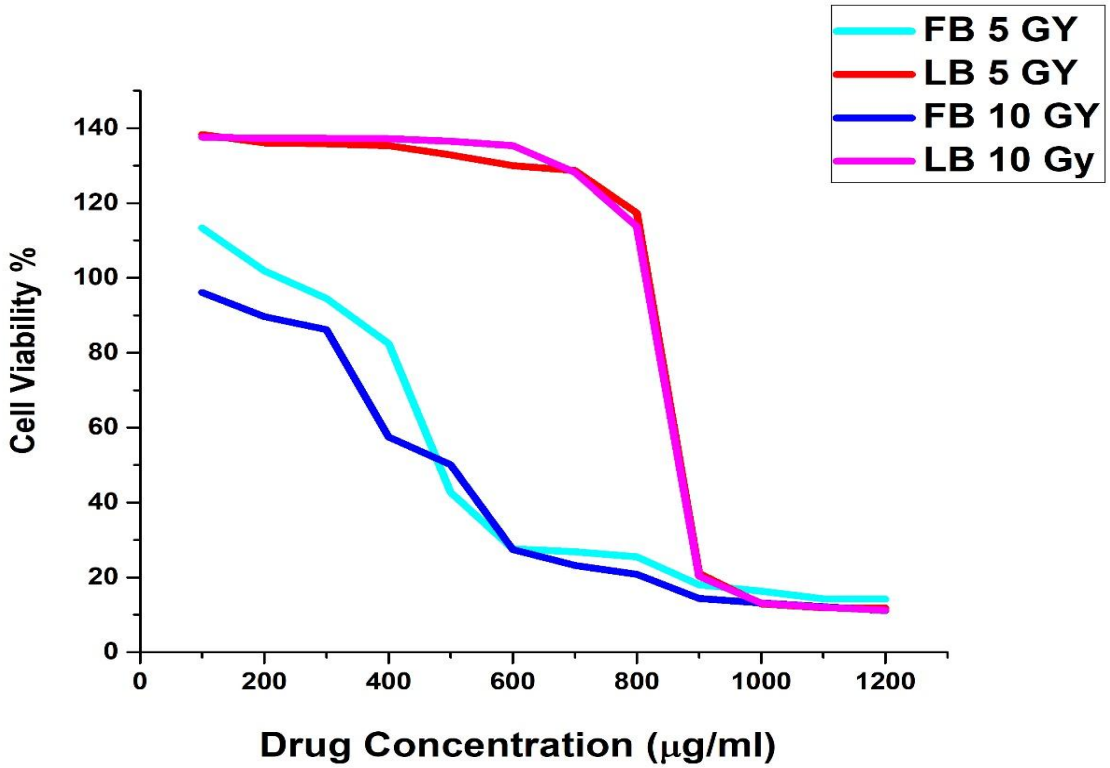

Fig. (10): In vitro cytotoxicity of free β-carotene (FB) and its liposomal form (LB) in the presence of external gamma-irradiation at doses of 5 and 10 Gy against breast carcinoma (MCF-7) cell line; incubated for 48 h with different drug concentrations starting from 100 to 1200 $\mu g.ml^{-1}$. The MTT assay was used to measure cell viability. The results are the average standard error of three replicate studies.

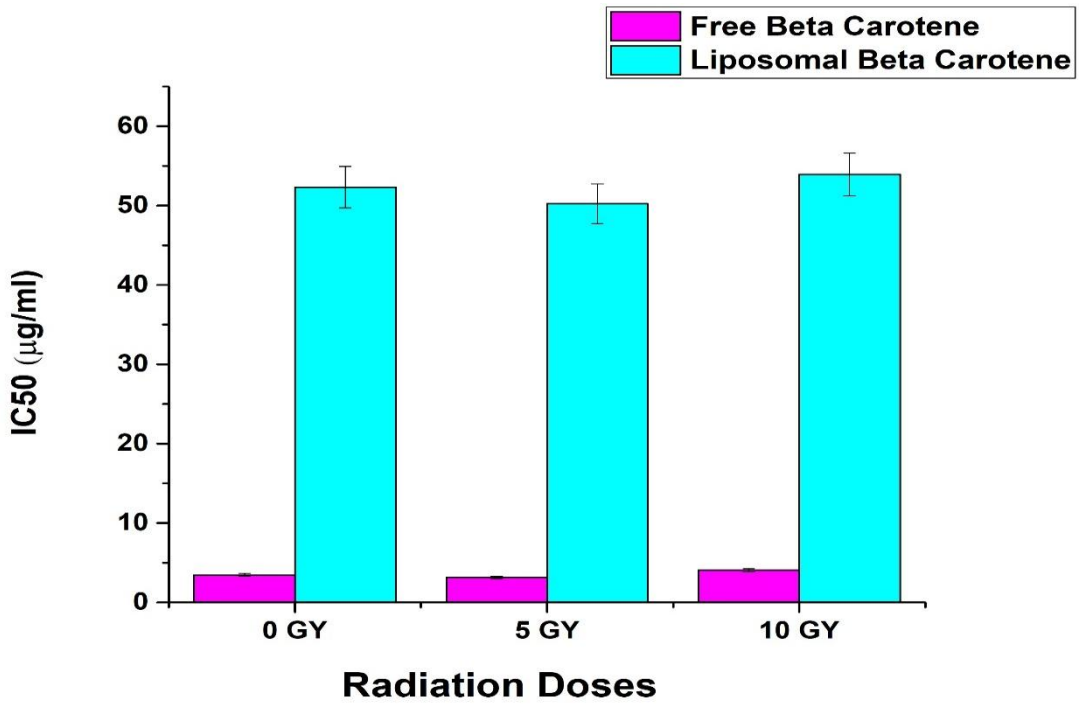

Fig. (11): Summarized IC_{50} values obtained for free β-carotene and its liposomal form in the absence or presence of gamma-irradiation at different doses against breast carcinoma (MCF-7) cell line by using MTT assay, 48 h post-treatment.

Contrary to the general assumption that β-carotene, being hydrophobic, would be sequestered within the hydrophobic core of the liposomal bilayer, observations revealed that it associates closely with the boundary surface of the liposomes and induces noticeable morphological changes (see Figure 3B). This behavior suggests that the liposomes become physically more rigid, with β-carotene localizing at the surface and disrupting the typical membrane packing. As a result, the leakage rate of β-carotene is notably reduced, which likely contributes to the observed increase in MCF-7 cell viability.

Under gamma irradiation at a dose of 5 Gy, free β-carotene demonstrated a notably lower IC50 value (3.14 μg/ml) in MCF-7 cytotoxicity assays compared to the liposomal formulation, which required a much higher concentration (50.21 μg/ml) to achieve similar effects. This clearly highlights the greater therapeutic efficacy of free β-carotene against the MCF-7 cell line under these experimental conditions (refer to Figure 11 for data visualization).

The trend persisted at a higher irradiation dose of 10 Gy: free β-carotene maintained a substantially lower IC50 (4.05 μg/ml) than its liposomal counterpart (53.92 μg/ml). Across all tested conditions, the IC50 values for each β-carotene formulation remained consistent, indicating reproducibility in the cytotoxic response. Figure 11 comprehensively summarizes the IC50 values for both free and liposomal β-carotene, with and without gamma irradiation, as determined by the MTT assay against the breast carcinoma (MCF-7) cell line.

Radiosensitizers play a crucial role in radiation therapy by enhancing the radiation's effectiveness in targeting tumor cells while minimizing damage to healthy tissues [10]. However, there exists a notable disparity in the cytotoxicity between free β-carotene and its liposomal counterpart. The current research hypothesis suggests that the encapsulation of β-carotene into lecithin liposomes could promote nanoparticle aggregation, particularly at higher concentrations, thereby increasing their size (refer to Table 1). Studies by Xiao-Dong et al. have emphasized the significance of nanoparticle size in radio sensitization [43]. Consequently, at elevated concentrations, the drug-loaded nanoparticles exhibit a notably reduced radio sensitization effect compared to the empty ones. To counteract this, decrease, chemotherapy assumes a pivotal role. Combining chemotherapy with radiation therapy is essential as it, not only hampers metastasis and impedes cell repair, but also complements localized radiation therapy. Additionally, the diminished cytotoxic efficacy of lutein in its liposomal form may be attributed to its entrapment within various lipoidal domains of the nanoparticles.

Recent evidence from this work suggests a promising treatment approach involving free β-carotene, which demonstrated enhanced anticancer activity against the MCF-7 cancer cell line, particularly in the destruction of breast cancer cells. This underscores the potential of this natural treatment as a possible viable alternative to conventional cancer therapies like chemotherapy and radiation. Notably, natural treatments offer the advantage of minimizing the physical side effects associated with conventional therapies while effectively preserving the integrity of normal, healthy cells surrounding cancerous tissues [11].

4. CONCLUSION

Combining β-carotene (a chemotherapy agent) with radiation doesn't produce synergistic growth inhibition in MCF-7 breast cancer cell lines. Despite this, the current findings underscore β-carotene's promise as a new anticancer agent. By allowing cytotoxic drugs to be used at lower doses, β-carotene could enable breast cancer treatment that's both more targeted and less toxic for human patients. This research points to a hopeful treatment approach where free β-carotene shows improved anticancer activity against MCF-7 cells. The results suggest that β-carotene, as a natural product, could be a feasible alternative to traditional pharmacological treatments for breast carcinoma.

5. CONFLICT OF INTEREST:

No conflicts of interest were identified in relation to this article.

6. REFERENCES

[1] Tarver T. Cancer facts & figures 2012. American cancer society (ACS) Atlanta, GA: American Cancer Society, 2012. 66 p., pdf. Available from.

[2] Talluri S V, Kuppusamy G, Karri, V V S R, Tummala, S, Madhunapantula, S V. Lipid-based nanocarriers for breast cancer treatment–comprehensive review. *Drug delivery* 2016*;* **23** (4), 1291-1305.

[3] Leucuta, SE. Nanotechnology for delivery of drugs and biomedical applications. *Cur. Clin Pharmaco.* 2010; **5**, 257–280.

[4] El-Ghaffar OA, Mohamed NO, El-Nagdy MS, Shafaa MW. Laser biospeckle contrast measurements stimulated from liposomal nanocarriers incubated in vitro model cancer cells: A proven promising tool in clinical therapy. Journal of Laser Applications. 2024 Feb 1;36(1).

[5] Liu P, Chen G, Zhang J. A Review of Liposomes as a Drug Delivery System: Current Status of Approved Products, Regulatory Environments, and Future Perspectives. Molecules. 2022 Feb 17;27(4):1372.

[6] Rashidinejad A, Birch EJ, Sun-Waterhouse D, Everett DW. Delivery of green tea catechin and epigallocatechin gallate in liposomes incorporated into low-fat hard cheese. Food Chem.2014; **156**,176–183.

[7] Sisin N, Abdul Razak K, Zainal Abidin S, Che Mat, N F, Abdullah R, Ab Rashid R., Khairil Anuar M A, Mohd Zainudin N H, Tagiling, N, Mat Nawi N, Rahman W N. Radiosensitization Effects by Bismuth Oxide Nanoparticles in Combination with Cisplatin for High Dose Rate Brachytherapy. *International journal of nanomedicine 2019*; ***14***, 9941–9954.

[8] Baskar R, Lee KA, Yeo R, Yeoh K-W. Cancer and radiation therapy: current advances and future directions. *Int J Med Sci.* 2012; **9**(3):193–199.

[9] Yan, B, Lu MS, Wang L, Mo XF, Luo WP, Du YF, Zhang CX. Specific serum carotenoids are inversely associated with breast cancer risk among Chinese women: A case-control study. *Br J Nutr* 2016; **115**, 129–137.

[10] Kris-Etherton, PM, Hecker KD., Bonanome A, Coval, SM., Binkowski AE, Hilpert KF, Griel AE, Etherton, T.D. Bioactive compounds in food: their role in the prevention of cardiovascular disease and cancer. *American Journal of Med*icine 2002; **113**, 71S-88S.

[11] Fiedor J, Burda, K. Potential role of carotenoids as antioxidants in human health and disease. *Nutrients 2014;* ***6***(2), 466-488.

[12] Tanaka T, Shnimizu, M, Moriwaki H. Cancer chemoprevention by carotenoids. Molecules 2012; **17**, 3202–3242.

[13] Baskar, R., Lee, K. A., Yeo, R., Yeoh, K. W. Cancer and radiation therapy: current advances and future directions. *International journal of medical sciences 2012*, ***9*** (3), 193.

[14] Bangham AD, Hill MW, and Miller NGA. Preparation and use of liposomes as models of biological membranes. In: Methods in Membrane Biology. (ed. Karn ED) Vol. 1, Plenum, New York, 1974; 1–68.

[15] Shafaa M.W, Diehl HA, Socaciu C. The solubilisation pattern of lutein, zeaxanthin, canthaxanthin and beta-carotene differ characteristically in liposomes, liver microsomes and retinal epithelial cells. *Biophys Chem* 2007; **129**, 111–119.

[16] Elkholy NS, Shafaa MW, Mohammed HS. Biophysical characterization of lutein or beta carotene-loaded cationic liposomes. RSC advances 2020; **10**:32409-32422.

[17] Rodriguez-Amaya D.B., A guide to carotenoid analysis in foods (Vol. 71), Washington: ILSI press (**2001**).

[18] Bellamakondi PK, Godavarthi A, Ibrahim M, Kulkarni S, Ramachandra Naik M, Sunitha M. *In vitro* cytotoxicity of caralluma species by MTT and Trypan blue dye exclusion. *Asian J Pharm Clin Res* 2014;**7**(2):17-19.

[19] Kätzel U. (2007). Dynamic light scattering for the characterization of polydisperse fractal systems by the example of pyrogenic silica. PhD Thesis Technische Universitat Dresden. http://nbn-resolving.de/urn:nbn:de:swb:14-1197634640783-66357.

[20] Gabrielska, J, Gruszecki W I. Zeaxanthin (dihydroxy-β-carotene) but not β-carotene rigidifies lipid membranes: a 1H-NMR study of carotenoid-egg phosphatidylcholine liposomes. *Biochimica et Biophysica Acta (BBA)-Biomembranes* 1996; ***1285*** (2), 167-174.

[21] Paolino D, Fresta M, Sinha P, Ferrari M. Drug delivery systems. In: Webster JG (ed) Encyclopedia of medical devices and instrumentation, 2nd edn. Wiley, New York, 2006, pp 437–495

[22] Plank , Dahl C E, Ware B R. Effect of sterol incorporation on head group separation in liposomes. *Chemistry and physics of lipids* 1985; **36**(4), 319-328.

[23] Klein J W, Ware B R, Barclay G, Petty H R. Phospholipid dependence of calcium ion effects on electrophoretic mobilities of liposomes. *Chemistry and physics of lipids* 1987*;* **43**(1), 13-23.

[24] Law S, Lo W, Pai S, Teh G. The electrokinetic behavior of liposomes adsorbed with bovine serum albumin. *International journal of pharmaceutics 1988;* **43**(3), 257-260.

[25] Makino K, Yamada T, Kimura, M, Oka T, Ohshima H, Kondo T. Temperature-and ionic strength-induced conformational changes in the lipid head group region of liposomes as suggested by zeta potential data. *Biophysical chemistry 1991;* **41**(2), 175-183.

[26] Kolman, I, Pippa N, Meristoudi, A, Pispas, S, Demetzos C. A dual-stimuli-responsive polymer into phospholipid membranes. Journal of Thermal *Analysis and Calorimetry* 2016; **123**(3), 2257-2271.

[27] Riske K A, Barroso R P, Vequi-Suplicy CC, Germano R., Henriques V B, Lamy M T. Lipid bilayer pre-transition as the beginning of the melting process. *Biochimica et Biophysica Acta (BBA)-Biomembranes* 2009; **1788**(5), 954-963.

[28] Koynova R, Caffrey M. Phases and phase transitions of the phosphatidylcholines. *Biochimica et Biophysica Acta (BBA)-Reviews on Biomembranes* 1998; **1376**(1), 91-145.

[29] Spink C H. Differential scanning calorimetry. *Methods in cell biology* 2008; **84**, 115-141.

[30] Shafaa M W, Sabra N M, Fouad R A. The extended ocular hypotensive effect of positive liposomal cholesterol bound timolol maleate in glaucomatous rabbits. *Biopharmaceutics & drug disposition 2011*, **32**(9), 507-517.

[31] Pedersen T B, Kaasgaard T, Jensen M Ø, Frokjaer S, Mouritsen O G, Jørgensen K. Phase behavior and nanoscale structure of phospholipid membranes incorporated with acylated C14-peptides. *Biophysical journal* 2005; **89** (4), 2494-2503.

[32] Popova A V, Hincha D K. Effects of cholesterol on dry bilayers: interactions between phosphatidylcholine unsaturation and glycolipid or free sugar. *Biophysical journal 2007*; **93**(4), 1204-1214.

[33] Fa N, Ronkart S, Schanck A, Deleu M, Gaigneaux A, Goormaghtigh E, Mingeot-Leclercq M P. Effect of the antibiotic azithromycin on thermotropic behavior of DOPC or DPPC bilayers. *Chemistry and physics of lipids 2006*; **144**(1), 108-116.

[34] Bafna S S, Sun T, Baird D G. The role of partial miscibility on the properties of blends of a polyetherimide and two liquid crystalline polymers. *Polymer* 1993; **34**(4), 708-715.

[35] Blume A. Properties of lipid vesicles: FT-IR spectroscopy and fluorescence probe studies. *Current Opinion Colloid Interface Science* 1996; **1**:64–77.

[36] Severcan F, Sahin I, Kazancı N. Melatonin strongly interacts with zwitterionic model membranes—evidence from Fourier transform infrared spectroscopy and differential scanning calorimetry. *Biochimica et Biophysica Acta (BBA)-Biomembranes 2005*; **1668**(2), 215-222.

[37] Mady M M, Shafaa,M W, Abbase E R, Fahium A H. Interaction of doxorubicin and dipalmitoylphosphatidylcholine liposomes. *Cell biochemistry and biophysics 2012*; **62**(3), 481-486.

[38] Kushwaha K, Saxena J, Tripathi B K, Agarwal M K. Detection of carotenoids in psychrotrophic bacteria by spectroscopic approach. *Journal of BioScience & Biotechnology* 2014; **3**(3), 253-260.

[39] Llansola-Portoles, M J, Pascal A A, Robert B. Electronic and vibrational properties of carotenoids: from in vitro to in vivo. *Journal of The Royal Society Interface* 2017; **14**(135), 20170504.

[40] Blume A, Hubner W, Messner G. Fourier transform infrared spectroscopy of 13C: O labeled phospholipids. Hydrogen bonding to carbonyl groups. Biochem 1988; **27**: 8239-8249.

[41] Socaciu C, Jessel R, Diehl H A. Competitive carotenoid and cholesterol incorporation into liposomes: effects on membrane phase transition, fluidity, polarity and anisotropy. *Chemistry and physics of lipids 2000;* **106** (1), 79-88.

[42] Mahrous GR, Elkholy NS, Safwat G, and Shafaa MW. Enhanced cytotoxic activity of beta carotene conjugated liposomes towards breast cancer cell line: comparative studies with cyclophosphamide. *Anti-Cancer Drugs 2022;* vol. **33**, no. 1, pp. e462–e476.

[43] Zhang XD, Wu D, Shen X, Chen J, Sun YM, Liu PX, et al. Size-dependent radiosensitization of PEG-coated gold nanoparticles for cancer radiation therapy. *Biomaterials* 2012; **33** (27):6408–19.