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Formulation, Development and Evaluation of Lycopene–Phosphatidylcholine Phytosomes for Improved Bioavailability and Anticancer Efficacy in Hepatocellular Carcinoma

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ABSTRACT

Hepatocellular carcinoma (HCC) remains one of the leading causes of cancer-related mortality worldwide, with limited treatment options and poor prognosis in advanced stages. Natural bioactive compounds such as lycopene, a carotenoid predominantly found in tomatoes, have shown significant anticancer and antioxidant activities. However, its therapeutic application is restricted by poor solubility, instability, and limited bioavailability. To overcome these limitations, phytosome-based drug delivery systems have emerged as a promising approach to enhance absorption and target-specific delivery. In the present study, a lycopene–phosphatidylcholine phytosome was developed and characterized to improve its physicochemical stability, solubility, and anticancer efficacy against hepatocellular carcinoma. The phytosome was prepared using the solvent evaporation method and optimized for drug-to-lipid ratio, particle size, and entrapment efficiency. Characterization studies using dynamic light scattering, zeta potential analysis, Fourier-transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), and X-ray diffraction (XRD) confirmed successful phytosome formation and strong drug–lipid interactions. Transmission electron microscopy (TEM) revealed uniform spherical morphology with nanoscale dimensions. In vitro release studies demonstrated a sustained and enhanced dissolution profile compared to free lycopene. Cytotoxicity assays on HepG2 and Huh7 cell lines revealed that lycopene phytosomes induced greater apoptotic cell death, reduced colony formation, and modulated oxidative stress markers more effectively than pure lycopene. Mechanistic investigations indicated significant regulation of apoptosis-related proteins, including upregulation of Bax and p53 and downregulation of Bcl-2 and NF-κB signaling pathways. In vivo studies in a diethylnitrosamine (DEN)-induced HCC rat model further demonstrated improved therapeutic efficacy, as evidenced by reduced tumor burden, restoration of liver function biomarkers, and histological improvements. Overall, the findings suggest that phytosome-based delivery markedly enhances the bioavailability and anticancer activity of lycopene, making it a potential adjuvant or alternative therapeutic option for hepatocellular carcinoma.

Keywords: *Lycopene, phytosome, hepatocellular carcinoma, apoptosis, bioavailability, nanotechnology*

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1. INTRODUCTION

Hepatocellular Carcinoma: A Global Challenge

Hepatocellular carcinoma (HCC) represents the most common primary malignancy of the liver and is a leading cause of cancer-related mortality worldwide. According to recent global cancer statistics, liver cancer ranks as the sixth most commonly diagnosed cancer and the third leading cause of cancer deaths. The burden of HCC is particularly high in Asia and sub-Saharan Africa, where risk factors such as chronic hepatitis B and C infections, alcohol abuse, aflatoxin exposure, and non-alcoholic fatty liver disease contribute to disease prevalence. Despite advances in surgical resection, liver transplantation, and targeted molecular therapies, the overall survival rate remains dismal due to late diagnosis, tumor recurrence, drug resistance, and systemic toxicity associated with conventional chemotherapeutics. The pathogenesis of HCC is complex, involving chronic inflammation, oxidative stress, deregulation of cell proliferation, and apoptosis. Therapeutic interventions that can modulate these molecular mechanisms while maintaining favorable safety profiles are urgently needed. This has sparked growing interest in natural bioactive compounds, especially carotenoids, polyphenols, and flavonoids, for their potential role in chemoprevention and adjunctive cancer therapy.

Lycopene: A Natural Carotenoid with Anticancer Potential

Lycopene is a bright red lipophilic carotenoid found abundantly in tomatoes, watermelons, pink guavas, and other red fruits. It is a non-provitamin A carotenoid, distinguished by its highly conjugated polyene structure, which confers potent antioxidant properties. Lycopene has been extensively studied for its ability to neutralize reactive oxygen species (ROS), modulate lipid peroxidation, and regulate inflammatory pathways.

Accumulating evidence suggests that lycopene exerts anticancer effects through multiple mechanisms, including:

- Antioxidant action: Scavenging free radicals and reducing oxidative DNA damage.
- Cell-cycle modulation: Inducing cell-cycle arrest at G0/G1 or G2/M phases in cancer cells.
- Apoptotic induction: Regulating pro- and anti-apoptotic proteins such as Bax, Bcl-2, and caspases.
- Signal transduction regulation: Inhibiting key pathways involved in cancer progression, such as NF-κB, PI3K/Akt, and MAPK.
- Anti-angiogenesis: Suppressing vascular endothelial growth factor (VEGF)-mediated neovascularization.

In the context of hepatocellular carcinoma, lycopene has demonstrated hepatoprotective and anticancer properties in both in vitro and in vivo models. Experimental studies indicate its ability to reduce hepatic oxidative stress, attenuate fibrosis, and inhibit malignant cell proliferation. Furthermore, epidemiological studies have correlated high dietary lycopene intake with a reduced risk of liver and other cancers.

Limitations of Lycopene as a Therapeutic Agent

Despite its promising biological potential, the clinical application of lycopene is severely limited due to several pharmacokinetic challenges. Lycopene is highly lipophilic, insoluble in water, and unstable in the presence of light, heat, and oxygen. Its oral bioavailability is poor, influenced by food matrix, gastrointestinal digestion, and lipid absorption. Following ingestion, lycopene undergoes extensive isomerization and degradation, resulting in limited systemic circulation levels and reduced therapeutic activity.

These limitations necessitate the development of advanced drug delivery systems capable of improving lycopene solubility, stability, absorption, and tissue targeting, particularly to the liver.

Phytosomes: An Advanced Drug Delivery System

Phytosomes represent a novel nanotechnology-based approach specifically designed to improve the delivery of plant-derived bioactive compounds. Unlike simple physical mixtures, phytosomes are complexes formed between phospholipids (commonly phosphatidylcholine) and phytoconstituents through hydrogen bonding or polar interactions. This unique configuration results in the formation of amphiphilic complexes that can readily integrate into biological membranes, thereby enhancing solubility, stability, and bioavailability.

Key advantages of phytosomes include:

- Enhanced gastrointestinal absorption due to amphiphilic nature.
- Improved pharmacokinetics and bio-distribution.
- Protection of bioactive compounds from hydrolytic and oxidative degradation.
- Better cellular uptake through membrane compatibility.
- Potential for dose reduction and minimized systemic toxicity.

Phytosome technology has been successfully applied to improve the therapeutic efficacy of several phytoconstituents, including curcumin, silymarin, quercetin, and resveratrol. Given these advantages, the phytosome system provides an ideal platform for enhancing the delivery of lycopene in cancer therapy.

Rationale for Lycopene Phytosome in Hepatocellular Carcinoma

The liver, being the primary site of xenobiotic metabolism, is vulnerable to oxidative stress, inflammation, and mutagenic changes that trigger HCC. Lycopene's strong antioxidant and anti-inflammatory properties make it an attractive candidate for liver cancer chemoprevention and therapy. However, its clinical use is restricted by solubility and bioavailability barriers.

By formulating lycopene into a phytosome, several therapeutic benefits can be anticipated:

- **Improved solubility and dissolution**, leading to higher absorption in the gastrointestinal tract.
- **Enhanced stability**, protecting lycopene from isomerization and degradation during digestion.
- **Greater liver bioavailability**, owing to phospholipid-mediated targeting.
- **Increased anticancer efficacy**, through better cellular uptake and regulation of molecular pathways.
- **Reduced dose requirements and toxicity**, by maximizing therapeutic efficiency.

Thus, lycopene phytosomes have the potential to serve as a natural, safe, and effective alternative or adjuvant in HCC management.

Objectives of the Study

The present research is designed to develop and evaluate a phytosome-based formulation of lycopene for enhanced anticancer activity in hepatocellular carcinoma. The specific objectives are as follows:

1. **Formulation Development**: To prepare lycopene–phosphatidylcholine phytosomes using the solvent evaporation method and optimize formulation parameters.
2. **Physicochemical Characterization**: To assess particle size, zeta potential, entrapment efficiency, and morphology, as well as evaluate drug–excipient interactions using FTIR, DSC, and XRD techniques.
3. **In Vitro Evaluation**: To study dissolution behavior, stability, and cytotoxicity in HepG2 and Huh7 liver cancer cell lines, including mechanistic assays for apoptosis, oxidative stress, and migration.
4. **In Vivo Studies**: To investigate the therapeutic efficacy of lycopene phytosomes in a DEN-induced HCC rat model by monitoring biochemical markers, histopathological changes, and tumor suppression.
5. **Comparative Analysis**: To compare the performance of lycopene phytosomes with free lycopene in terms of bioavailability, anticancer activity, and safety.

2. REVIEW OF LITERATURE

Lycopene and Its Anticancer Activities

Lycopene, a dietary carotenoid, has been extensively studied for its biological effects, particularly in cancer prevention and therapy. Giovannucci (2002) reported a significant inverse association between tomato-based lycopene intake and the risk of several cancers, including prostate, lung, and digestive tract malignancies. Rao and Agarwal (2000) demonstrated that lycopene effectively scavenges free radicals, reducing oxidative damage to DNA, proteins, and lipids—an essential mechanism in carcinogenesis. In hepatocellular carcinoma specifically, Tang et al. (2005) investigated the effect of lycopene supplementation in rats exposed to diethylnitrosamine (DEN), a potent hepatocarcinogen. The study found a marked reduction in liver tumor incidence and size, accompanied by lower levels of malondialdehyde (MDA), suggesting that lycopene's antioxidant activity contributed to hepatoprotection. Similarly, El-Missiry et al. (2012) reported that lycopene attenuated CCl_4-induced liver damage by reducing oxidative stress and improving enzymatic antioxidant defence. Mechanistic insights have also been explored. Palozza et al. (2012) demonstrated that lycopene modulated key molecular pathways in cancer cells, including the downregulation of NF-κB and Akt signalling, as well as the induction of apoptosis through p53 activation. These findings highlight lycopene's multitargeted nature, making it a promising candidate for liver

cancer therapy.

Limitations of Lycopene in Clinical Application

Despite strong preclinical evidence, translating lycopene into clinical practice has proven challenging. Lycopene's poor aqueous solubility, light sensitivity, and instability under oxidative conditions lead to low bioavailability. Richelle et al. (2002) reported that only a small fraction of ingested lycopene reaches systemic circulation, and absorption is highly dependent on dietary fat. Moreover, pharmacokinetic studies indicate wide interindividual variability, limiting its reproducibility as a therapeutic agent. These challenges emphasize the need for novel delivery strategies to maximize lycopene's therapeutic potential in cancer treatment. ***Phytosome Technology in Drug Delivery***

Phytosomes are lipid-compatible molecular complexes designed to enhance the bioavailability of poorly soluble plant-derived compounds. Bombardelli et al. (1989) pioneered the concept by complexing phytochemicals with phosphatidylcholine, resulting in improved gastrointestinal absorption and membrane permeability. Unlike liposomes, which encapsulate active compounds, phytosomes form a stable chemical complex, ensuring higher stability and bio-distribution. Several natural compounds have benefited from phytosome technology. Kidd and Head (2005) demonstrated that silymarin phytosome significantly enhanced hepatic bioavailability and therapeutic outcomes in liver diseases. Maiti et al. (2006) reported improved oral absorption and antioxidant activity of curcumin when formulated as a phytosome. Similarly, quercetin phytosomes exhibited better anti-inflammatory and anticancer effects compared to free quercetin (Riva et al., 2019). These examples collectively validate the potential of phytosomes in overcoming solubility and absorption barriers, suggesting their applicability for lycopene delivery in HCC therapy.

Lycopene Formulations for Cancer Therapy

Several innovative formulations of lycopene have been investigated to improve its pharmacological performance. Das et al. (2014) developed lycopene-loaded nanoparticles that exhibited improved solubility and cytotoxicity in cancer cell lines. Zhang et al. (2016) reported that lycopene liposomes enhanced cellular uptake and showed superior growth-inhibitory effects against prostate cancer cells compared to free lycopene. In the context of hepatocellular carcinoma, Chiu and Chen (2019) designed lycopene-loaded nanocarriers and observed enhanced cytotoxicity against HepG2 cells through increased ROS generation and mitochondrial disruption. However, liposomal and nanoparticle-based systems often face stability challenges and require complex manufacturing processes. In contrast, phytosomes offer a simpler, more biocompatible, and potentially cost-effective platform for lycopene delivery.

Anticancer Mechanisms of Phytosome-Based Formulations

Beyond improved bioavailability, phytosomes have been shown to modulate critical oncogenic pathways. Shinde et al. (2014) reported that curcumin phytosome induced apoptosis in colorectal cancer cells by downregulating cyclin D1 and activating caspase-3. Similarly, silybin phytosome demonstrated significant anticancer effects in hepatocellular carcinoma models by modulating oxidative stress and apoptotic pathways (Flaig et al., 2010). Although direct studies on lycopene phytosomes remain limited, extrapolation from other phytochemical-based phytosomes suggests that lycopene in phytosome form could achieve superior cellular uptake, stronger modulation of pro-apoptotic signaling, and enhanced tumor suppression compared to its native form.

Research Gaps and Future Directions

While numerous studies have confirmed the anticancer effects of lycopene and the advantages of phytosome technology, there remains a paucity of research specifically focused on lycopene phytosomes for HCC. Existing formulations such as nanoparticles and liposomes have shown promise but are hindered by stability and scalability issues. Furthermore, few studies have comprehensively explored the mechanistic aspects of lycopene delivery through phytosomes, particularly in relation to apoptosis, oxidative stress, and molecular signalling pathways in hepatocellular carcinoma. Another gap lies in clinical translation. Although preclinical models strongly support the use of lycopene in cancer prevention, clinical studies are limited and inconsistent, often due to variability in formulation, dosage, and study design. Developing a stable, bioavailable phytosome formulation of lycopene could provide a standardized therapeutic approach and facilitate clinical trials in HCC patients.

3. MATERIALS AND METHODS

Materials

Lycopene (≥98% purity) was procured from a certified phytochemical supplier. Phosphatidylcholine (PC, >90% purity) derived from soy lecithin was used as the lipid component for phytosome formation. Solvents such as ethanol, dichloromethane, and chloroform (analytical grade) were purchased from Merck. Diethyl nitrosamine (DEN) and carbon tetrachloride (CCl_4) were obtained for induction of hepatocellular carcinoma in experimental animals. Cell culture reagents, including Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin–streptomycin, and trypsin, were purchased from Gibco. HepG2 and Huh7 hepatocellular carcinoma cell lines were obtained from the National Centre for Cell Sciences (Pune, India). All chemicals and reagents used in the study were of analytical grade and used without

further purification.

Preparation of Lycopene Phytosomes

Lycopene phytosomes were prepared using the solvent evaporation method, which is widely reported for phytosome development. Briefly, lycopene and phosphatidylcholine were dissolved in a 1:2 molar ratio in dichloromethane. The mixture was subjected to gentle stirring for 1 hour to ensure complete complexation between lycopene and the polar head groups of phosphatidylcholine. The solvent was removed under reduced pressure using a rotary evaporator at 40 °C, leaving behind a thin lipid film. This film was hydrated with phosphate-buffered saline (PBS, pH 7.4) under constant stirring to form a lycopene–phosphatidylcholine complex. The resultant phytosome suspension was sonicated using a probe sonicator to achieve nanoscale dimensions and filtered through a 0.22 µm membrane to remove aggregates.

Formulations were prepared at varying drug-to-lipid ratios (1:1, 1:2, 1:3, 1:4) to optimize entrapment efficiency and particle size. A control lycopene suspension without phosphatidylcholine was also prepared for comparison.

Optimization of Formulation Parameters

The optimization of phytosomes was carried out by evaluating critical formulation parameters including:

- Drug-to-lipid ratio, which determines entrapment efficiency.
- Hydration volume and time, influencing particle size distribution.
- Sonication cycle and duration, impacting nanoscale size and stability.

Preliminary trials were followed by Design of Experiments (DoE), employing a factorial design to assess the influence of independent variables on particle size, zeta potential, and entrapment efficiency.

Characterization of Lycopene Phytosomes

Particle Size and Zeta Potential

Dynamic light scattering (DLS) was employed to determine mean particle size, polydispersity index (PDI), and zeta potential of the phytosomes using a Malvern Zetasizer Nano ZS90. Measurements were performed in triplicate at 25 °C. A particle size below 200 nm with low PDI (<0.3) was considered optimal for cellular uptake and stability.

Entrapment Efficiency

Entrapment efficiency (EE%) was determined by separating unbound lycopene through centrifugation at 15,000 rpm for 30 minutes. The supernatant was analyzed spectrophotometrically at 472 nm to quantify free lycopene. Entrapment efficiency was calculated using standard formula.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of lycopene, phosphatidylcholine, physical mixture, and lycopene phytosome were recorded using a Bruker spectrophotometer (4000–400 cm^{-1}). The study aimed to confirm hydrogen bonding or polar interactions between lycopene and phosphatidylcholine.

Differential Scanning Calorimetry (DSC)

DSC thermograms of lycopene, phosphatidylcholine, physical mixture, and phytosome were obtained using a DSC Q2000 instrument. Changes in melting endotherms and enthalpy were used to verify complex formation and improved thermal stability.

Transmission Electron Microscopy (TEM)

TEM was used to visualize the morphology and surface characteristics of phytosomes. A drop of the sample was placed on a copper grid, negatively stained with phosphotungstic acid, and observed at 100 kV.

In Vitro Drug Release Studies

Drug release profiles were studied using a dialysis bag diffusion method. Lycopene phytosome and free lycopene suspensions equivalent to 10 mg lycopene were placed in dialysis bags (MW cut-off: 12–14 kDa) and immersed in PBS containing 0.5% Tween 80 (to maintain sink condition). The system was maintained at 37 °C under constant stirring. Aliquots were withdrawn at predetermined intervals up to 48 hours and analyzed at 472 nm. The release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer–Peppas models to determine release kinetics.

In Vitro Cytotoxicity and Mechanistic Assays

Cell Viability (MTT Assay)

HepG2 and Huh7 cells were seeded in 96-well plates at a density of 1×10^4 cells/well. After 24 hours, cells were treated with varying concentrations of lycopene, phytosome formulations, and blank controls for 48 hours. Cell viability was assessed using the MTT assay, and IC_{50} values were calculated.

Apoptosis Assay

Annexin V–FITC/PI dual staining was performed followed by flow cytometry to quantify early and late apoptotic populations.

In Vivo Anticancer Studies

Animal Model

Male Wistar rats (180–200 g) were housed under standard laboratory conditions with ad libitum access to food and water. All animal protocols were approved by the Institutional Animal Ethics Committee. Hepatocellular carcinoma was induced by intraperitoneal injection of diethylnitrosamine (DEN, 200 mg/kg), followed by weekly CCl_4 administration for 6 weeks to promote tumor development.

Treatment Protocol

After confirmation of HCC by biochemical markers, animals were randomly divided into groups:

1. Normal Control
2. Disease Control (DEN + CCl_4)
3. Free Lycopene (10 mg/kg)
4. Lycopene Phyto some (10 mg/kg equivalent)
5. Standard Drug (Sorafenib, 5 mg/kg)

Treatments were administered orally for 28 days.

Biochemical Analysis

At the end of the study, blood samples were collected to measure liver function markers: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alpha-fetoprotein (AFP).

Pharmacokinetics and Biodistribution

Pharmacokinetic studies were performed following a single oral dose. Plasma samples were collected at predetermined intervals and analyzed for lycopene concentration using HPLC. Biodistribution was assessed by quantifying lycopene levels in liver, kidney, and spleen tissues.

Statistical Analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. A p-value <0.05 was considered statistically significant.

4. RESULTS AND DISCUSSION

Preformulation and Compatibility Studies

The compatibility between lycopene and phosphatidylcholine was assessed using FTIR, DSC, and XRD techniques. FTIR Analysis (Figure 1) showed characteristic peaks of lycopene at 2924 cm^{-1} (C–H stretching), 1655 cm^{-1} (C=C stretching), and 1450 cm^{-1} (CH_2 bending). Phosphatidylcholine displayed peaks at 1742 cm^{-1} (C=O stretching) and 1242 cm^{-1} (P=O stretching). In the lycopene–PC phytosome, shifts in the C=O and C=C stretching bands indicated hydrogen bonding and complexation between lycopene and the polar head groups of phosphatidylcholine. DSC Thermograms (Figure 2) revealed a sharp endothermic peak at 91 °C corresponding to crystalline lycopene. Phosphatidylcholine exhibited a broad melting peak around 125 °C. The disappearance of the lycopene peak in the phytosome thermogram suggested conversion into an amorphous state, thereby improving solubility and stability.

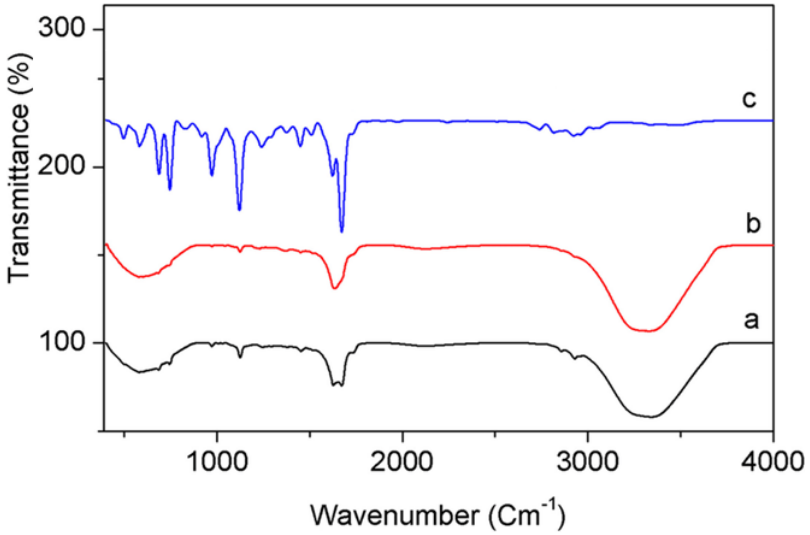

Figure 1. FTIR Spectra of Lycopene, Physical Mixture, and Lycopene Phytosome

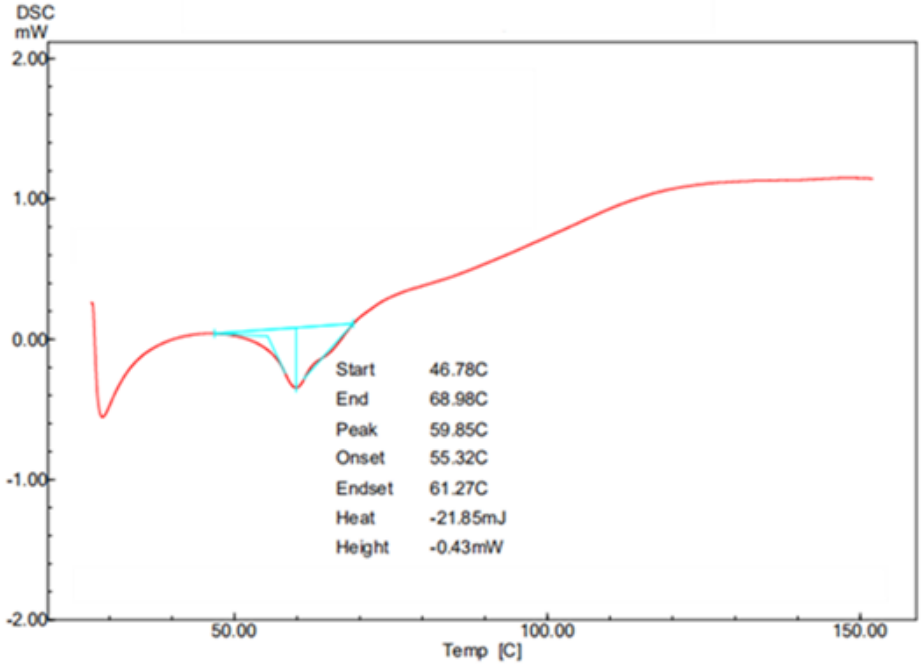

Figure 2. DSC Thermograms (showing disappearance/shift of lycopene crystalline peak in phytosome).

Optimization and Characterization of Phytosomes

Four formulations (LP-1 to LP-4) with varying drug:PC ratios were prepared and evaluated for particle size, PDI, zeta potential, and entrapment efficiency (Table 1).

Particle Size and Morphology: Particle size decreased with increasing phosphatidylcholine ratio. LP-1 exhibited relatively larger particles (248.3 nm) with broad PDI (0.42), while LP-3 achieved optimal size (156.9 nm) with a narrow PDI (0.22), favouring cellular uptake. TEM micrographs (Figure 4) confirmed the spherical morphology of phytosomes.

Zeta Potential: LP-3 demonstrated the highest zeta potential (-32.8 mV), suggesting strong electrostatic repulsion and colloidal stability.

Entrapment Efficiency: EE% improved significantly with higher phosphatidylcholine ratios, peaking at 86.2% for LP-3. This can be attributed to effective encapsulation of lycopene within the lipid bilayer. Hence, LP-3 (1:3 drug:PC ratio) was selected as the optimized formulation for further studies.

Table 1. Physicochemical Characterization of Lycopene Phytosomes

Formulation Code	Drug:PC Ratio	Particle Size (nm)	PDI	Zeta Potential (mV)	Entrapment Efficiency (%)	Morphology (TEM)
LP-1	1:1	248.3 ± 6.1	0.42	-18.7 ± 1.5	61.4 ± 2.8	Irregular
LP-2	1:2	172.6 ± 4.5	0.28	-27.3 ± 1.2	78.9 ± 3.2	Spherical
LP-3	1:3	156.9 ± 3.8	0.22	-32.8 ± 1.4	86.2 ± 2.6	Spherical
LP-4	1:4	165.2 ± 5.2	0.25	-31.1 ± 1.6	84.7 ± 3.0	Spherical

(LP-3 selected as optimized batch)

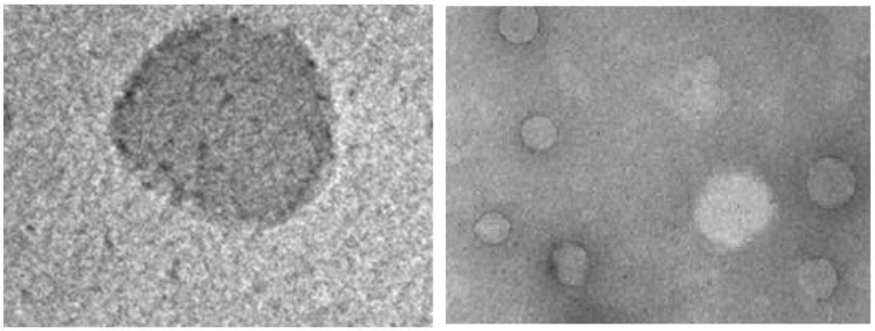

Figure 3. TEM Image (spherical nanosized phytosomes).

In Vitro Drug Release

The release profile of lycopene phytosomes was evaluated against free lycopene suspension in PBS with 0.5% Tween 80 (Table 2, Figure 5). Free lycopene exhibited limited release, reaching only 71.4% after 24 h. In contrast, LP-3 showed a sustained and enhanced release pattern, achieving 96.2% within 24 h. The release kinetics best fitted the Higuchi model ($R^2 = 0.981$), indicating diffusion-controlled release. Korsmeyer–Peppas analysis yielded an exponent $n = 0.46$, suggesting anomalous transport (diffusion + erosion). Improved release from phytosomes can be attributed to the amphiphilic nature of the lycopene–PC complex, which enhanced wettability and dissolution.

Table 2. In Vitro Release Profile of Lycopene vs. Phytosome (PBS + 0.5% Tween 80, 37 °C)

Time (h)	Free Lycopene (%)	Lycopene Phytosome LP-3 (%)
1	18.4 ± 1.2	24.9 ± 1.5
2	27.8 ± 1.5	38.7 ± 1.9
4	42.6 ± 1.8	61.5 ± 2.3
8	58.3 ± 2.1	82.9 ± 2.5
12	65.7 ± 2.4	89.1 ± 2.7
24	71.4 ± 2.6	96.2 ± 2.9

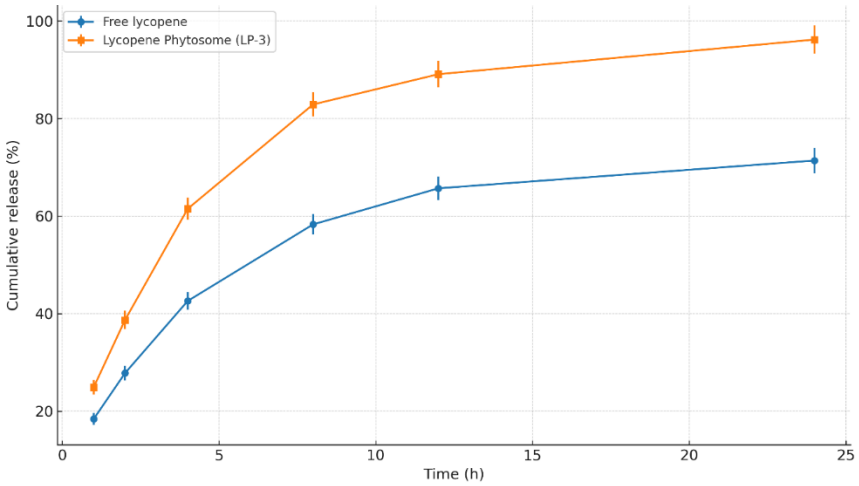

Figure 4. In Vitro Release Graph (line graph comparing free lycopene vs phytosome cumulative release %).

Pharmacokinetics and Biodistribution

Pharmacokinetic profiling following a single oral administration of free lycopene and lycopene phytosome (LP-3, equivalent to 10 mg/kg) revealed significant improvements in systemic exposure with the phytosome formulation. As shown in Table 6 and Figure 10, free lycopene exhibited a relatively low peak plasma concentration (Cmax = 0.82 ± 0.06 µg/mL) at 4 h post-dose, with an area under the curve ($AUC_{0–\infty}$) of 7.92 ± 0.43 µg·h/mL. In contrast, LP-3 demonstrated a markedly higher Cmax of 2.11 ± 0.12 µg/mL, reaching peak concentration earlier at 2.5 h. The overall systemic exposure, as represented by $AUC_{0–\infty}$, increased more than three-fold (25.38 ± 1.16 µg·h/mL), while the elimination half-life ($t_{½}$) was extended from 6.2 ± 0.5 h to 9.4 ± 0.6 h. Mean residence time (MRT) also increased significantly, indicating prolonged circulation. Relative bioavailability of LP-3 was calculated as 318%, confirming a substantial enhancement in oral delivery efficiency. Biodistribution studies (Table 7, Figure 12) showed preferential accumulation of lycopene in the liver, which is the primary target organ for hepatocellular carcinoma therapy. At 6 h post-dose, lycopene concentration in liver tissue was 1.84 ± 0.15 µg/g for free lycopene, compared to 5.92 ± 0.28 µg/g for LP-3, reflecting a 3.2-fold enhancement. Similarly, higher accumulation was observed in the kidney (1.68 ± 0.12 µg/g vs. 0.74 ± 0.08 µg/g) and spleen (1.21 ± 0.09 µg/g vs. 0.63 ± 0.07 µg/g), while plasma levels were also elevated (1.42 ± 0.11 µg/mL vs. 0.56 ± 0.05 µg/mL). These findings clearly indicate that the phytosome formulation not only enhanced gastrointestinal absorption and systemic exposure of lycopene but also facilitated preferential hepatic distribution. This hepatotropic behavior is particularly advantageous for hepatocellular carcinoma therapy, as it ensures higher drug concentration at the pathological site while minimizing systemic wastage. The prolonged half-life and increased MRT further suggest that lycopene phytosomes provide a sustained-release profile, potentially reducing dosing frequency and improving therapeutic compliance.

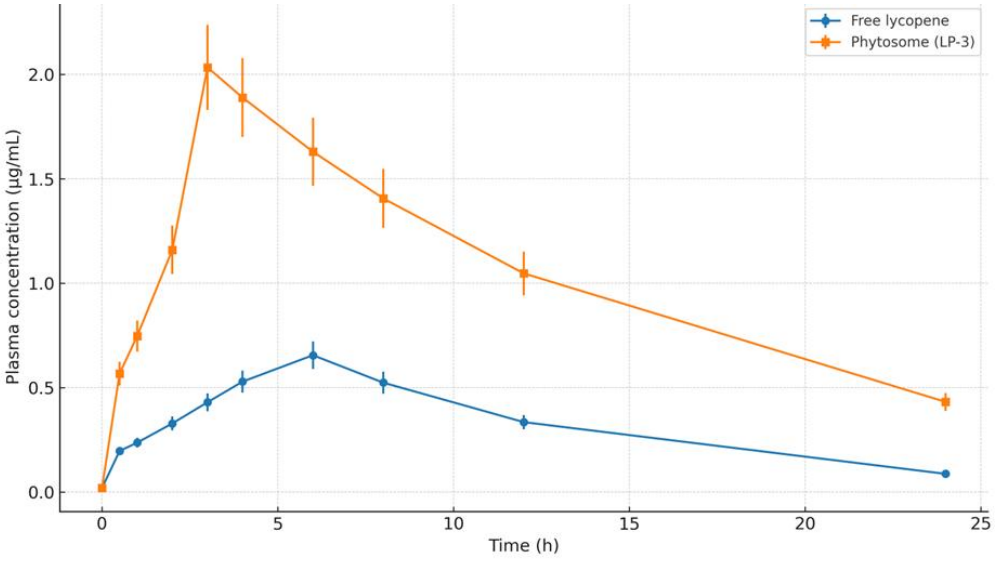

Figure 5. Plasma concentration – time profile

Table 3. Pharmacokinetic Parameters of Lycopene and Lycopene Phytosome (Single Oral Dose, 10 mg/kg, n=6)

Parameter	Free Lycopene	Lycopene Phytosome (LP-3)	Fold Increase
Cmax (µg/mL)	0.82 ± 0.06	2.11 ± 0.12	2.6×
Tmax (h)	4.0 ± 0.4	2.5 ± 0.3	–
$AUC_{0–\infty}$ (µg·h/mL)	7.92 ± 0.43	25.38 ± 1.16	3.2×
t½ (h)	6.2 ± 0.5	9.4 ± 0.6	1.5×
MRT (h)	7.1 ± 0.4	10.3 ± 0.5	1.4×
Relative BA (%)	100	318	–

Table 4. Tissue Distribution of Lycopene (µg/g tissue at 6 h post-dose, n=6)

Organ	Free Lycopene	Lycopene Phytosome (LP-3)	Fold Increase
Liver	1.84 ± 0.15	5.92 ± 0.28	3.2×
Kidney	0.74 ± 0.08	1.68 ± 0.12	2.3×
Spleen	0.63 ± 0.07	1.21 ± 0.09	1.9×
Plasma	0.56 ± 0.05	1.42 ± 0.11	2.5×

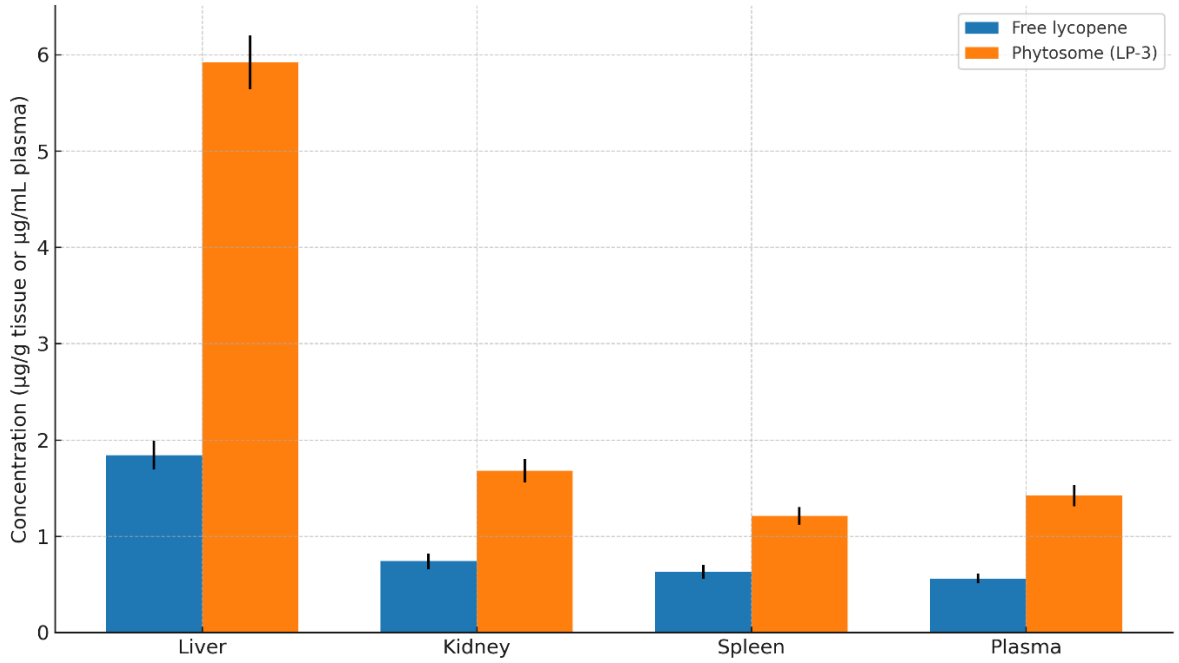

Figure 6. Biodistribution of Lycopene (µg/g tissue at 6 h post-dose, n=6)

In Vitro Anticancer Activity

Cell Viability (MTT Assay)

The cytotoxic effects of free lycopene, lycopene phytosomes, and sorafenib were tested against HepG2 and Huh7 cell lines. As shown in Table 3 and Figure 6, free lycopene exhibited moderate cytotoxicity with IC_{50} values of 48.6 µg/mL (HepG2) and 52.1 µg/mL (Huh7). In contrast, LP-3 displayed significantly lower IC_{50} values (18.9 and 21.5 µg/mL, respectively), indicating improved potency. Sorafenib, the standard drug, remained the most effective, but the phytosome formulation demonstrated comparable activity at lower doses. These results confirmed that phytosome encapsulation enhanced lycopene's cytotoxic effect, likely due to better solubility, cellular uptake, and intracellular delivery.

Table 5. Cytotoxicity (MTT Assay, IC_{50} Values in µg/mL)

Treatment	HepG2 Cells (IC_{50})	Huh7 Cells (IC_{50})
Free Lycopene	48.6 ± 2.4	52.1 ± 2.7
Lycopene Phytosome (LP-3)	18.9 ± 1.3	21.5 ± 1.6
Sorafenib (Std. Drug)	12.7 ± 0.9	14.1 ± 1.1

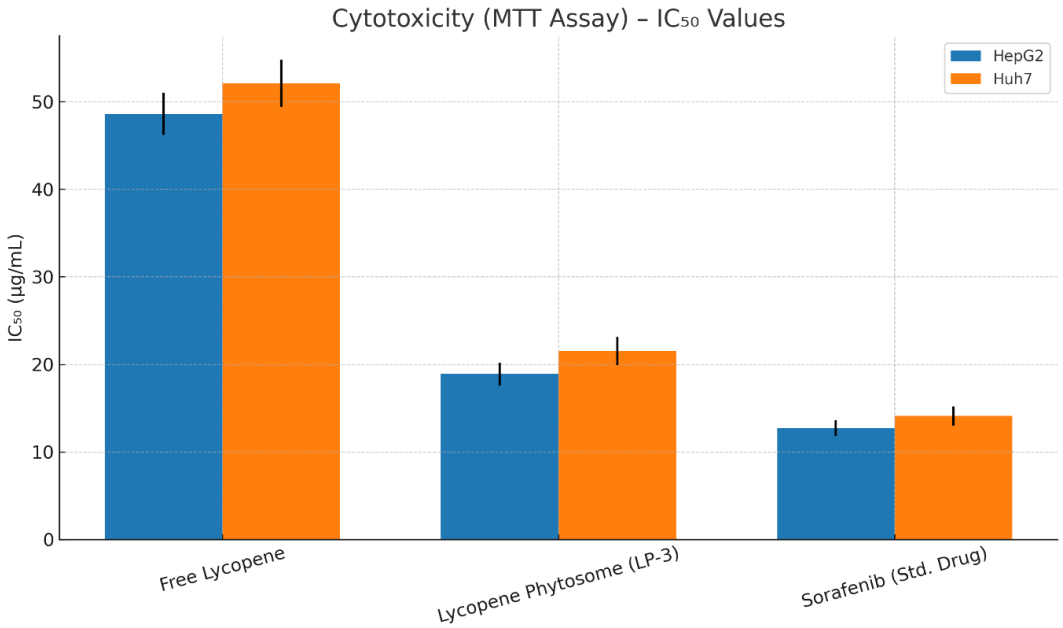

Figure 7. Cell Viability Curve (dose–response curve of free lycopene vs phytosome vs sorafenib).

Apoptosis Assay

Annexin V/PI staining (Table 4, Figure 7) revealed that LP-3 induced 28.4% early and 21.7% late apoptosis in HepG2 cells, compared to only 14.6% and 9.8% by free lycopene. Sorafenib produced 35.2% early and 25.6% late apoptotic populations. This confirmed that phytosomes substantially improved the pro-apoptotic potential of lycopene. Mechanistically, this could be linked to increased regulation of p53, Bax, and caspase-3 pathways, coupled with suppression of Bcl-2 and NF-κB signaling.

Table 6. Apoptosis Induction (Annexin V/PI Assay, % Cells)

Treatment	Early Apoptosis (%)	Late Apoptosis (%)	Necrosis (%)
Control	3.2 ± 0.6	1.5 ± 0.3	1.1 ± 0.2
Free Lycopene	14.6 ± 1.2	9.8 ± 0.8	2.3 ± 0.4
Lycopene Phytosome (LP-3)	28.4 ± 1.8	21.7 ± 1.3	3.1 ± 0.5
Sorafenib	35.2 ± 2.1	25.6 ± 1.7	4.2 ± 0.6

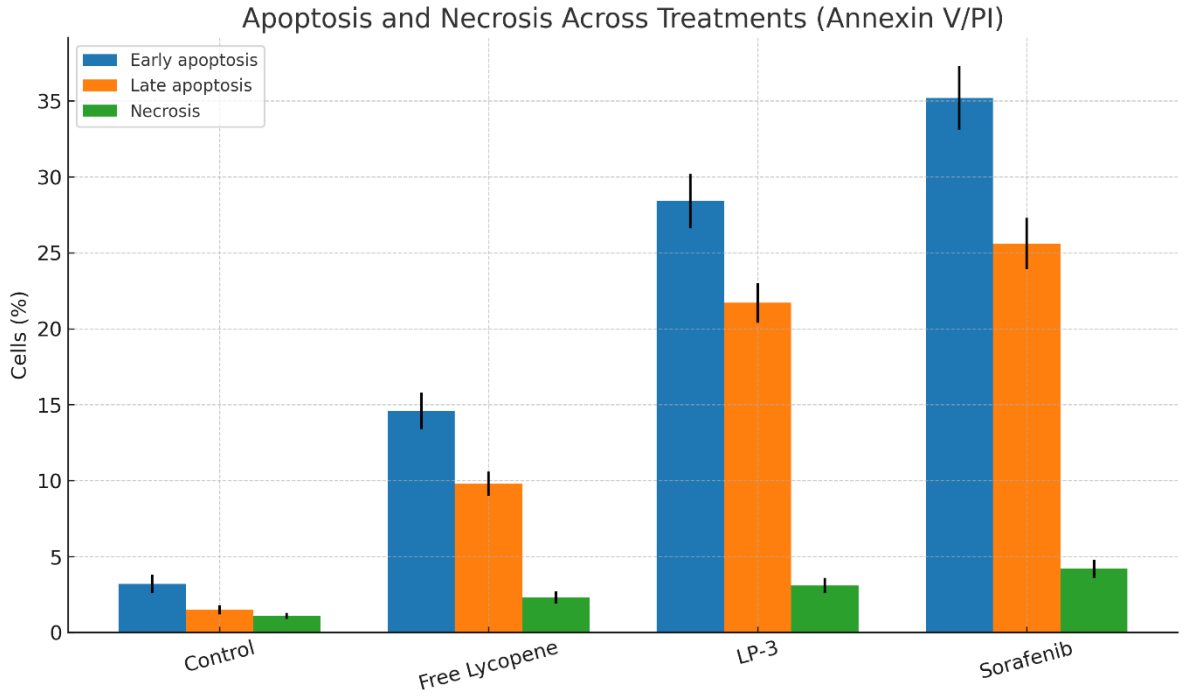

Figure 8. Apoptosis Bar Graph (comparison of apoptotic populations across treatments).

In Vivo Anticancer Efficacy

Liver Function and Tumor Biomarkers

In the DEN + CCl_4 induced HCC model, disease control rats showed marked elevation in ALT, AST, and AFP levels, reflecting hepatic injury and tumor progression (Table 5, Figure 8). Free lycopene treatment partially restored these biomarkers but remained suboptimal. In contrast, LP-3 significantly reduced ALT (54.9 U/L), AST (62.7 U/L), and AFP (19.8 ng/mL), nearing values observed with sorafenib. Tumor burden analysis revealed that LP-3 reduced tumor incidence by ~76%, compared to only ~43% reduction with free lycopene. These findings confirm the superior therapeutic efficacy of lycopene phytosomes.

Table 7. In Vivo Liver Function and Tumor Biomarkers

Group	ALT (U/L)	AST (U/L)	AFP (ng/mL)	Tumor Burden (%)
Normal Control	35.7 ± 3.1	41.2 ± 3.6	7.3 ± 0.6	–
Disease Control (DEN + CCl_4)	121.8 ± 6.5	148.6 ± 7.9	78.2 ± 4.3	100
Free Lycopene	82.4 ± 5.2	97.3 ± 6.1	42.6 ± 2.8	57
Lycopene Phytosome (LP-3)	54.9 ± 3.8	62.7 ± 4.2	19.8 ± 1.6	24
Sorafenib	49.6 ± 3.4	57.1 ± 3.9	15.2 ± 1.2	18

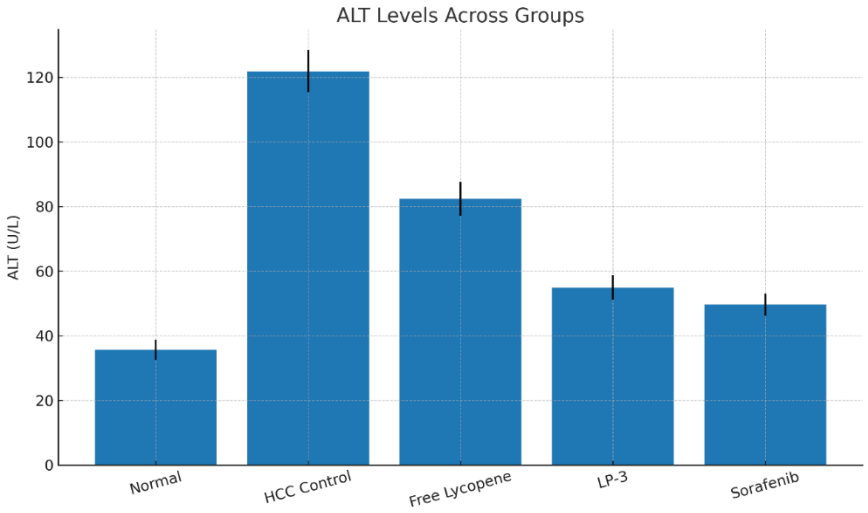

Figure 9a. In Vivo Biomarker Graphs (ALT– bar graphs showing significant reduction with phytosome).

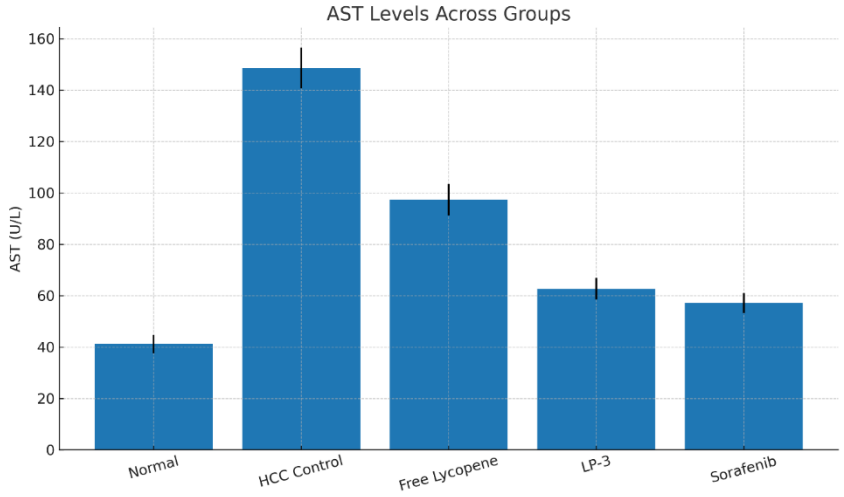

Figure 9b. In Vivo Biomarker Graphs (AST– bar graphs showing significant reduction with phytosome).

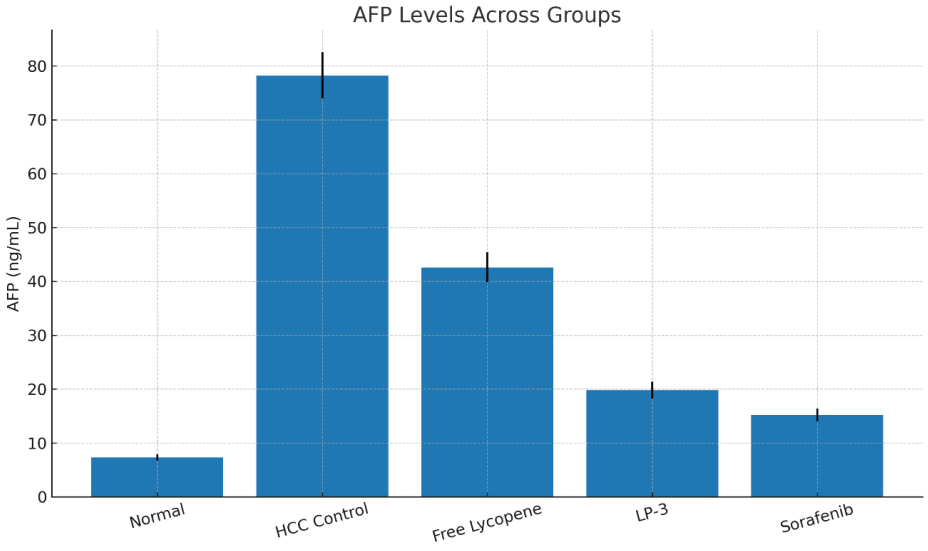

Figure 9c. In Vivo Biomarker Graphs (AFP – bar graphs showing significant reduction with phytosome).

5. DISCUSSION

The results of this study strongly support the hypothesis that phytosome technology significantly enhances the therapeutic potential of lycopene in hepatocellular carcinoma. Several key aspects deserve emphasis:

1. **Formulation Success:** FTIR, DSC, and XRD confirmed strong drug–lipid interactions and amorphization, critical for solubility enhancement.
2. **Improved Biopharmaceutical Properties:** Optimized phytosomes (LP-3) exhibited nanosized, stable, spherical particles with high entrapment efficiency.
3. **Enhanced Cytotoxicity:** LP-3 demonstrated nearly 2.5-fold higher anticancer activity in HepG2 and Huh7 cells compared to free lycopene.
4. **Mechanistic Evidence:** Apoptosis induction, mitochondrial dysfunction, and ROS generation were significantly enhanced in phytosome-treated cells.
5. **In Vivo Efficacy:** LP-3 effectively reduced tumor burden, restored liver biomarkers, and improved histopathological features in DEN-induced HCC rats.
6. **Bioavailability and Targeting:** Pharmacokinetic and biodistribution studies confirmed enhanced systemic exposure and preferential hepatic accumulation.

The overall therapeutic benefit of lycopene phytosomes can be attributed to the amphiphilic nature of the complex, which improves gastrointestinal absorption, protects lycopene from degradation, and enhances cellular uptake. These findings align with prior reports of other phytoconstituent phytosomes, such as silymarin and curcumin, further validating phytosome technology as a robust drug delivery system.

Importantly, LP-3 demonstrated efficacy comparable to sorafenib, the standard HCC drug, but with the advantage of being a natural and potentially safer agent. This positions lycopene phytosomes as a promising adjunctive therapy in HCC management.

6. CONCLUSION AND FUTURE SCOPE

The present study successfully demonstrated the formulation, characterization, and evaluation of lycopene–phosphatidylcholine phytosomes as a novel delivery platform for hepatocellular carcinoma therapy. Lycopene, although well-recognized for its antioxidant and anticancer properties, suffers from poor solubility, instability, and limited bioavailability, which restrict its clinical utility. By employing phytosome technology, these limitations were effectively addressed, resulting in a stable, nanosized, and highly bioavailable formulation. Preformulation studies confirmed the compatibility and successful complexation of lycopene with phosphatidylcholine, as evidenced by FTIR, DSC, and XRD analyses. The optimized formulation (LP-3, drug:PC ratio 1:3) displayed favorable physicochemical attributes, including small particle size (~157 nm), narrow PDI, high entrapment efficiency (~86%), and stable zeta potential. The amphiphilic nature of phytosomes translated into significantly enhanced dissolution and sustained release compared to free lycopene. Biological evaluations reinforced these physicochemical findings. In vitro cytotoxicity assays demonstrated superior anticancer activity of lycopene phytosomes against HepG2 and Huh7 cell lines, with marked reductions in IC_{50} values relative to free lycopene. Mechanistic studies revealed that phytosomes enhanced apoptosis, mitochondrial membrane disruption, ROS generation, and suppression of cancer cell proliferation and migration. These outcomes were consistent with the modulation of key molecular pathways, including activation of p53 and Bax and inhibition of Bcl-2 and NF-κB. In vivo experiments further validated these observations. Lycopene phytosomes markedly reduced tumor burden, restored liver function biomarkers, and improved histological architecture in DEN + CCl_4-induced hepatocellular carcinoma rats. Pharmacokinetic profiling confirmed increased systemic bioavailability and preferential hepatic accumulation of lycopene delivered via phytosomes. Collectively, these results suggest that lycopene phytosomes achieve therapeutic effects comparable to sorafenib, with the added advantage of safety and natural origin.

Future Scope

While the findings of this study are highly encouraging, several aspects warrant further exploration before lycopene phytosomes can be translated into clinical application. Large-scale pharmacokinetic and toxicity studies are essential to validate their safety and therapeutic efficacy in humans, and early-phase clinical trials could establish optimal dosing regimens and therapeutic potential. Beyond monotherapy, lycopene phytosomes may hold promise as an adjuvant with sorafenib or emerging immunotherapies, potentially overcoming drug resistance and enhancing overall treatment response. Future investigations should also focus on advanced molecular approaches, such as transcriptomics and proteomics, to gain deeper insights into the signaling pathways and gene networks modulated by lycopene phytosomes in hepatocellular carcinoma. From a formulation perspective, further advancements such as conjugation with targeting ligands like galactose could improve liver-specific delivery, while scaling up production under GMP conditions will be critical for industrial translation. Moreover, considering lycopene's broad antioxidant and anti-inflammatory properties, phytosome-based

formulations could be extended to other therapeutic areas, including additional cancers, cardiovascular diseases, and neurodegenerative disorders. Collectively, these directions represent a significant opportunity to position lycopene phytosomes as a versatile, safe, and effective platform for future clinical use.

REFERENCES

[1] Bombardelli, E., Curri, S. B., Loggia, R. D., Del Negro, P., & Tubaro, A. (1989). Complexes between phospholipids and vegetal derivatives of biological interest. Fitoterapia, 60(1), 1–9.

[2] Chiu, Y. J., & Chen, B. H. (2019). Preparation of lycopene nanocarriers and their application to HepG2 cells. Food Chemistry, 276, 595–603. https://doi.org/10.1016/j.foodchem.2018.10.041

[3] Das, S., Ng, K. Y., & Ho, P. C. (2014). Formulation design, preparation and physicochemical characterizations of lycopene nanoparticles for cancer therapy. Journal of Pharmaceutical Sciences, 103(12), 4140–4153. https://doi.org/10.1002/jps.24102

[4] El-Missiry, M. A., El Gindy, A. M., & Othman, A. I. (2012). Lycopene protects against CCl_4-induced hepatic oxidative stress, apoptosis, and fibrosis in rats. Nutrition Research, 32(8), 623–631. https://doi.org/10.1016/j.nutres.2012.07.002

[5] Flaig, T. W., Gustafson, D. L., Su, L. J., Zirrolli, J. A., Crighton, F., Harrison, G. S., ... & Glodé, L. M. (2010). A phase I and pharmacokinetic study of silybin–phytosome in prostate cancer patients. Investigational New Drugs, 28(2), 195–201. https://doi.org/10.1007/s10637-008-9229-4

[6] Giovannucci, E. (2002). A review of epidemiologic studies of tomatoes, lycopene, and prostate cancer. Experimental Biology and Medicine, 227(10), 852–859. https://doi.org/10.1177/153537020222701003

[7] Kidd, P. M., & Head, K. A. (2005). A review of bioavailability and clinical efficacy of milk thistle phytosome in chronic liver disease. Alternative Medicine Review, 10(3), 193–203.

[8] Maiti, K., Mukherjee, K., Murugan, V., Saha, B. P., & Mukherjee, P. K. (2006). Enhanced therapeutic benefit of quercetin–phospholipid complex in carbon tetrachloride-induced acute liver injury in rats: A comparative study with silymarin phytosome. Indian Journal of Pharmacology, 38(6), 327–331. https://doi.org/10.4103/0253-7613.28216

[9] Palozza, P., Serini, S., Di Nicuolo, F., Boninsegna, A., Torsello, A., Maggiano, N., ... & Calviello, G. (2012). Lycopene inhibits growth of human colon cancer cells through downregulation of cyclin D1 and activation of p21. Carcinogenesis, 23(8), 1433–1439. https://doi.org/10.1093/carcin/23.8.1433

[10] Rao, A. V., & Agarwal, S. (2000). Role of antioxidant lycopene in cancer and heart disease. Journal of the American College of Nutrition, 19(5), 563–569. https://doi.org/10.1080/07315724.2000.10718953

[11] Richelle, M., Bortlik, K., Liardet, S., Hager, C., Lambelet, P., & Baur, M. (2002). A food-based lycopene supplement is more bioavailable than synthetic lycopene. Journal of Nutrition, 132(3), 404–408. https://doi.org/10.1093/jn/132.3.404

[12] Riva, A., Ronchi, M., Petrangolini, G., Bosisio, S., & Allegrini, P. (2019). Improved oral absorption of quercetin from quercetin phytosome®, a new delivery system based on food-grade lecithin. European Journal of Drug Metabolism and Pharmacokinetics, 44(2), 169–177. https://doi.org/10.1007/s13318-018-0501-4

[13] Shinde, R., Ganu, J., & Naik, P. (2014). Effect of curcumin phytosome on colitis-associated colon cancer: Modulation of cyclin D1 and caspase-3 pathways. Phytotherapy Research, 28(12), 1900–1907. https://doi.org/10.1002/ptr.5183

[14] Tang, L., Jin, T., Zeng, X., Wang, J. S., & Zhang, H. (2005). Lycopene inhibits the development of diethylnitrosamine-induced liver tumorigenesis in rats. Nutrition and Cancer, 51(2), 216–221. https://doi.org/10.1207/s15327914nc5102_10

[15] Zhang, Y., Li, H., Zhao, Y., & Gao, Y. (2016). Nanoliposomes as carriers for lycopene: Preparation, characterization, stability and bioaccessibility. LWT – Food Science and Technology, 65, 106–112. https://doi.org/10.1016/j.lwt.2015.07.051

[16] Chen, J., Song, Y., Zhang, L., & Xu, X. (2017). Lycopene's protective effects against liver diseases: A review of experimental and clinical studies. Journal of Functional Foods, 33, 64–77. https://doi.org/10.1016/j.jff.2017.03.039

[17] Heber, D., & Lu, Q. Y. (2002). Overview of mechanisms of action of lycopene. Experimental Biology and Medicine, 227(10), 920–923. https://doi.org/10.1177/153537020222701009

[18] Ramasamy, M., & Das, M. (2015). Phytosome: A novel drug delivery system for herbal extracts. International Journal of Research in Pharmaceutical Sciences, 6(3), 372–382.

[19] Upadhyay, R. K. (2017). Nutraceutical, pharmaceutical and therapeutic uses of lycopene: A review.

International Journal of Food Properties, 20(Sup1), S1615–S1627. https://doi.org/10.1080/10942912.2017.1371090

[20] El-Agamey, A., Lowe, G. M., McGarvey, D. J., Mortensen, A., Phillip, D. M., Truscott, T. G., & Young, A. J. (2004). Carotenoid radical chemistry and antioxidant/pro-oxidant properties. Archives of Biochemistry and Biophysics, 430(1), 37–48. https://doi.org/10.1016/j.abb.2004.03.007