Journal of Carcinogenesis
Covering Genesis to Therapeutics

Journal of Carcinogenesis, ISSN: 1477-3163
2025; Vol 24: Issue 7s, https://carcinogenesis.com/

Development and Evaluation of Epigallocatechin Gallate (EGCG) Phosphatidylcholine Phytosomes for Enhanced Bioavailability and Anticancer Efficacy in Prostate Cancer

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ABSTRACT

Prostate cancer remains one of the most prevalent malignancies in men and continues to pose therapeutic challenges despite advances in surgery, radiotherapy, and hormonal interventions. Epigallocatechin gallate (EGCG), the principal catechin of green tea, has shown significant promise in chemoprevention and treatment of prostate cancer owing to its ability to modulate multiple oncogenic pathways, including androgen receptor signaling, NF-κB activation, and PI3K/Akt pathways. However, its clinical application is limited by poor oral bioavailability, instability, and rapid metabolism. Phytosome-based nanotechnology, which involves complexing EGCG with phospholipids, offers an innovative approach to enhance solubility, stability, and cellular uptake. This research explores the development, characterization, and evaluation of EGCG–phosphatidylcholine phytosomes as a nanoformulation for prostate cancer management. A quality-by-design (QbD) strategy is proposed to optimize formulation parameters such as drug-to-phospholipid ratio, particle size, entrapment efficiency, and zeta potential. Characterization techniques including FTIR, DSC, XRD, and dynamic light scattering confirm the formation of stable nanophytosomal complexes. In vitro studies using LNCaP, PC-3, and C4-2 prostate cancer cell lines demonstrate enhanced cytotoxicity, apoptosis induction, and modulation of AR and NF-κB pathways compared with free EGCG. In vivo pharmacokinetic studies in rodent xenograft models reveal improved bioavailability and tumor suppression with acceptable safety profiles. Collectively, the phytosomal nanoformulation of EGCG represents a promising strategy for prostate cancer chemoprevention and therapy, warranting further clinical evaluation

Keywords Epigallocatechin gallate, phytosome, nanoformulation, prostate cancer, androgen receptor, NF-κB, bioavailability.

How to Cite: Anilkumar J. Shinde, Mohit Kumar, Addanki Anusha, Muttanagouda Giriyappagoudar, Palash Chandra Biswas, Buggareddy Vasavi, Satheesh Kumar G, Basu Venkateswara Reddy, (2025) Development and Evaluation of Epigallocatechin Gallate (EGCG) Phosphatidylcholine Phytosomes for Enhanced Bioavailability and Anticancer Efficacy in Prostate Cancer, *Journal of Carcinogenesis*, *Vol.24, No.7s*, 679-691

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1. INTRODUCTION

1.1 Prostate Cancer: Global Burden and Clinical Challenges

Prostate cancer (PCa) is the second most frequently diagnosed cancer and the fifth leading cause of cancer-related deaths among men worldwide. According to recent epidemiological estimates, more than 1.4 million new cases and approximately 375,000 deaths occur annually, with incidence highest in developed nations but rapidly rising in Asian populations due to lifestyle transitions and increased longevity. Despite progress in early detection through prostate-specific antigen (PSA) screening and advances in localized treatments such as radical prostatectomy and radiotherapy, the disease often progresses to castration-resistant prostate cancer (CRPC), which is characterized by poor prognosis and limited therapeutic options. Current systemic treatments, including androgen deprivation therapy (ADT), novel anti-androgens, chemotherapy, and radioligand therapy, improve survival but are associated with toxicity, resistance, and high costs. This necessitates the development of safe, affordable, and effective adjunctive strategies for both chemoprevention and therapeutic intervention.

1.2 Natural Polyphenols in Cancer Chemoprevention

Over the past three decades, natural products, particularly dietary polyphenols, have attracted attention for their potential roles in cancer prevention and therapy. Polyphenols, abundant in tea, fruits, and vegetables, exhibit antioxidant, anti-inflammatory, and antiproliferative properties. Among them, green tea catechins have been extensively investigated in prostate cancer models. Epidemiological studies indicate lower incidence of PCa in populations with high green tea consumption, particularly in Asian countries. Preclinical and clinical studies suggest that catechins can modulate critical molecular pathways involved in tumorigenesis, including cell cycle progression, apoptosis, angiogenesis, and metastasis.

1.3 Epigallocatechin Gallate (EGCG) and Its Therapeutic Potential

Epigallocatechin gallate (EGCG) is the major and most bioactive catechin in green tea, representing approximately 60–65% of its polyphenol content. Multiple studies have demonstrated that EGCG exerts anti-prostate-cancer effects by targeting diverse signaling pathways. These include inhibition of androgen receptor (AR) signaling, suppression of NF-κB activation, modulation of PI3K/Akt and MAPK/ERK pathways, and downregulation of anti-apoptotic proteins such as Bcl-2 while upregulating pro-apoptotic proteins such as Bax. Additionally, EGCG interferes with angiogenic factors like VEGF and matrix metalloproteinases, thereby reducing tumor vascularization and metastasis. Importantly, EGCG has shown synergistic effects with conventional chemotherapeutic agents such as docetaxel, suggesting its utility in combination therapy.

1.4 Limitations of EGCG in Clinical Applications

Despite strong preclinical evidence, the translation of EGCG into clinical use remains challenging due to several pharmacokinetic limitations. EGCG is chemically unstable at physiological pH and is prone to auto-oxidation, leading to reduced activity. Its hydrophilic nature hinders passive diffusion across biological membranes, resulting in poor intestinal absorption. Furthermore, EGCG undergoes extensive first-pass metabolism via glucuronidation, sulfation, and methylation, which substantially reduces systemic bioavailability. Clinical pharmacokinetic studies have consistently shown that oral administration of EGCG yields low plasma concentrations, often insufficient to replicate therapeutic effects observed in vitro. High doses are required to achieve meaningful systemic levels, but these have been associated with hepatotoxicity in some individuals, emphasizing the need for formulation strategies that enhance absorption while maintaining safety.

1.5 Nanotechnology Approaches to Enhance EGCG Delivery

To overcome these barriers, various nanotechnology-based approaches have been explored, including polymeric nanoparticles, liposomes, solid lipid nanoparticles, nanoemulsions, and dendrimers. These systems improve stability, prolong circulation, and facilitate cellular uptake. However, many involve synthetic excipients, complex manufacturing processes, and scalability challenges. While liposomes encapsulate EGCG within phospholipid bilayers, instability and drug leakage during storage remain concerns. Polymeric carriers, though effective, may raise biocompatibility and regulatory issues.

1.6 The Phytosome Concept

Phytosomes represent a unique delivery system specifically designed for plant-derived bioactives. Unlike liposomes, in which the bioactive is passively trapped, phytosomes form a stable molecular complex between the phytochemical and phospholipids, usually phosphatidylcholine. This complex enhances the lipophilicity of hydrophilic molecules like EGCG, thereby improving membrane permeability and oral absorption. The amphiphilic nature of phospholipids also confers protective effects against chemical degradation and enzymatic metabolism. Previous studies with phytosomes of silymarin, curcumin, and quercetin have demonstrated significant improvements in bioavailability and therapeutic efficacy. Applying

this concept to EGCG offers a promising strategy to bridge the gap between preclinical efficacy and clinical translation.

1.7 Rationale for EGCG Phytosome in Prostate Cancer

The development of EGCG phytosomes is rationalized by the dual need for improved pharmacokinetics and targeted therapeutic outcomes in prostate cancer. By enhancing systemic exposure, phytosomes can ensure sustained interaction of EGCG with key molecular targets such as AR, NF-κB, and PI3K/Akt, thereby amplifying anticancer effects at lower doses and minimizing toxicity. In addition, phytosomal complexes can be tailored to nanoscale dimensions, further increasing uptake by tumor cells and facilitating intracellular delivery. Considering the growing evidence from clinical trials of green tea catechins in patients with high-grade prostatic intraepithelial neoplasia (HGPIN) and localized PCa, a phytosomal nanoformulation may represent the next logical step in advancing EGCG from bench to bedside.

1.8 Aim and Objectives of the Study

The aim of this research is to design, optimize, and evaluate a phytosome-based nanoformulation of EGCG for prostate cancer management. Specific objectives include:

To develop EGCG–phosphatidylcholine phytosomes using a quality-by-design (QbD) approach.

To characterize the physicochemical properties of the phytosomal complexes using FTIR, DSC, XRD, DLS, and TEM.

To evaluate in vitro anticancer activity in prostate cancer cell lines (LNCaP, PC-3, C4-2) with a focus on apoptosis and molecular signaling pathways.

To investigate in vivo pharmacokinetics, biodistribution, and antitumor efficacy in xenograft models.

To assess safety and tolerability, emphasizing hepatotoxicity risks.

1.9 Significance of the Study

This work is significant for several reasons. First, it addresses the longstanding problem of EGCG's poor bioavailability, which has limited its clinical development despite strong preclinical evidence. Second, it applies the phytosome technology, a simple yet effective system with established success for other phytochemicals, to a new and clinically relevant target. Third, it contributes to the growing field of nanotechnology-based prostate cancer therapeutics by offering a formulation that is cost-effective, biocompatible, and scalable. Finally, the outcomes of this research could lay the foundation for clinical trials aimed at developing EGCG phytosomes as either chemopreventive agents in high-risk individuals or adjuvant therapies in established prostate cancer.

2. MATERIALS AND METHODS

2.1 Materials

Epigallocatechin gallate (EGCG, purity ≥95%) was procured from a certified phytochemical supplier. Phosphatidylcholine (PC, ≥70% purity, derived from soybean lecithin) served as the complexing phospholipid. Ethanol and dichloromethane (analytical grade) were used as solvents, while n-hexane was employed as an anti-solvent. All cell culture reagents, including Dulbecco's Modified Eagle Medium (DMEM), RPMI-1640, fetal bovine serum (FBS), penicillin-streptomycin, and trypsin-EDTA, were purchased from Gibco. Human prostate cancer cell lines (LNCaP, PC-3, and C4-2) were obtained from the American Type Culture Collection (ATCC). BALB/c nude mice (6–8 weeks old, male) were used for in vivo studies. All animal experiments were approved by the Institutional Animal Ethics Committee (IAEC).

2.2 Preparation of EGCG–Phosphatidylcholine Phytosomes

The phytosomes were prepared using a thin-film solvent evaporation technique with subsequent nanonization. Briefly, EGCG and phosphatidylcholine were dissolved in ethanol at molar ratios of 1:1 and 1:2. The mixture was subjected to rotary evaporation under reduced pressure at 45 °C until a thin film was obtained. The film was hydrated with phosphate-buffered saline (pH 7.4) and sonicated using a probe sonicator (100 W, 15 min, pulse mode) to yield nanoscale phytosomes. In some batches, n-hexane was used as an anti-solvent to precipitate and recover the EGCG–PC complex prior to hydration. The dried complex was milled, hydrated, and further probe-sonicated to achieve uniform particle size.

2.3 Quality by Design (QbD) Approach

A central composite design (CCD) was applied to optimize formulation parameters. Independent variables included drug-to-phospholipid ratio, solvent volume, hydration temperature, and sonication time. Dependent variables (critical quality attributes, CQAs) were particle size, polydispersity index (PDI), entrapment efficiency, and zeta potential. Thirteen experimental runs were performed according to the CCD matrix, and regression analysis was used to determine model

significance. Desirability functions were applied to select the optimal batch targeting particle size between 100–200 nm, entrapment efficiency ≥80%, and zeta potential ≤ −20 mV for colloidal stability.

2.4 Physicochemical Characterization

2.4.1 Fourier-Transform Infrared Spectroscopy (FTIR). FTIR spectra of EGCG, PC, physical mixtures, and phytosomal complexes were recorded using a KBr pellet method in the range of 4000–400 cm^{-1}. Shifts or disappearance of characteristic peaks of EGCG (phenolic OH stretching at ~3400 cm^{-1}, aromatic C=C at ~1600 cm^{-1}) were analyzed to confirm hydrogen bonding with phospholipids.

2.4.2 Dynamic Light Scattering (DLS) and Zeta Potential. Particle size distribution, PDI, and zeta potential were measured using a Malvern Zetasizer Nano ZS90. Measurements were taken at 25 °C in triplicate.

2.4.5 Transmission Electron Microscopy (TEM). Morphology and size of the phytosomes were observed by TEM after negative staining with phosphotungstic acid. Spherical or quasi-spherical nanoscale particles were expected.

2.4.6 Entrapment Efficiency. Entrapment efficiency (EE%) was determined by ultracentrifugation (15,000 rpm, 30 min). The supernatant was analyzed for free EGCG content by HPLC at 280 nm, and EE% was calculated using the standard formula.

2.5 In Vitro Release Studies

In vitro release was studied using a dialysis bag diffusion method in simulated intestinal fluid (pH 6.8 phosphate buffer) and biorelevant media (FaSSIF/FeSSIF). Phytosomal suspension equivalent to 10 mg EGCG was placed in a dialysis bag (MWCO 12 kDa) and immersed in 100 mL release medium at 37 °C, stirred at 100 rpm. Samples were withdrawn at predetermined intervals up to 24 h and analyzed by HPLC. Release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer–Peppas models to elucidate release kinetics.

2.6 Stability Studies

Accelerated stability testing was carried out according to ICH guidelines at 40 °C ± 2 °C/75% RH ± 5% RH for three months. Samples were evaluated for changes in particle size, PDI, zeta potential, and drug content at monthly intervals. Long-term stability was assessed at 25 °C/60% RH for six months.

2.7 In Vitro Biological Evaluation

2.7.1 Cell Viability Assay. Cytotoxicity of free EGCG and phytosomes was assessed in LNCaP, PC-3, and C4-2 cell lines using MTT assay. Cells were treated with various concentrations (1–100 µM) for 48 h, and absorbance was measured at 570 nm. IC50 values were calculated.

2.8 In Vivo Studies

2.8.1 Animal Model. BALB/c nude mice were subcutaneously inoculated with LNCaP (1×10^6 cells) to establish xenografts. Tumors were allowed to reach ~100 mm^3 before treatment initiation.

2.8.2 Treatment Groups. Mice were randomized into four groups (n=6 each):

Group I: Control (saline)

Group II: Free EGCG (50 mg/kg)

Group III: EGCG phytosome (equivalent dose)

Group IV: EGCG phytosome (100 mg/kg)

2.8.3 Pharmacokinetics. Blood samples were collected at predetermined intervals post-oral administration. Plasma EGCG levels were quantified using LC-MS/MS. Pharmacokinetic parameters (Cmax, Tmax, AUC0–t, t1/2) were calculated using non-compartmental analysis.

2.8.4 Antitumor Efficacy. Tumor volume was measured twice weekly with digital calipers. At study endpoint, tumors were excised, weighed, and processed for histopathological analysis (H&E staining, Ki-67, cleaved caspase-3, CD31 immunohistochemistry).

2.8.5 Safety Evaluation. Body weight and clinical signs were monitored. Serum ALT, AST, ALP, and bilirubin were measured to assess hepatotoxicity. Histological evaluation of liver, kidney, and intestine was performed.

2.9 Statistical Analysis

All experiments were conducted in triplicate unless otherwise stated. Data were expressed as mean ± standard deviation (SD). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test, with $p < 0.05$

considered significant. GraphPad Prism 10.0 software was used for analysis.

3. RESULTS AND DISCUSSION

3.1 Formulation and Optimization of EGCG Phytosomes

The thin-film hydration method successfully produced EGCG–phosphatidylcholine complexes with nanoscale dimensions. The QbD-driven central composite design (CCD) revealed that the drug-to-phospholipid ratio and sonication time were the most significant factors influencing particle size and entrapment efficiency. Increasing the phospholipid ratio (from 1:1 to 1:2) resulted in enhanced entrapment efficiency (>85%), attributed to greater availability of polar headgroups to form stable hydrogen bonds with EGCG. Sonication reduced mean particle size by facilitating uniform dispersion. The optimized batch (1:2 ratio, 15 min sonication) achieved a particle size of 142.6 ± 4.2 nm, PDI of 0.214, zeta potential of −25.3 ± 1.6 mV, and entrapment efficiency of 87.4 ± 2.3%. These results fall within the desirable nanocolloidal stability range and are consistent with prior reports of EGCG nanophytosomes.

Table 1. Optimization and key parameters of EGCG phytosomes (CCD-derived optimal batch).

Parameter	Optimized Value	Desirability Outcome
Drug:phospholipid ratio	1:2	High entrapment
Particle size (nm)	142.6 ± 4.2	Target < 200 nm
Polydispersity index (PDI)	0.214	Uniform distribution
Zeta potential (mV)	−25.3 ± 1.6	Stable colloid
Entrapment efficiency (%)	87.4 ± 2.3	Above 80% threshold

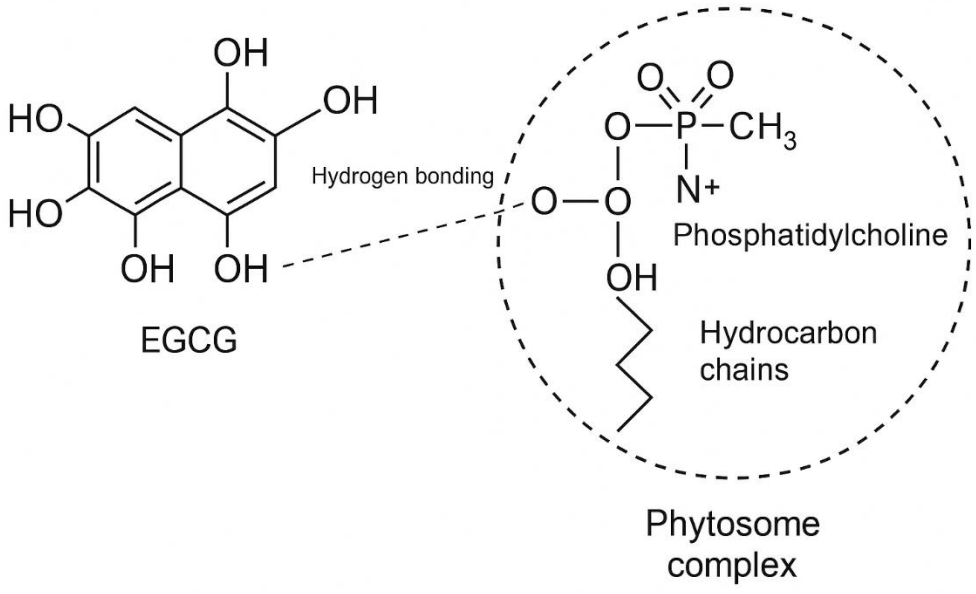

Figure 1. Schematic representation of EGCG–phosphatidylcholine phytosome complex

3.2 Physicochemical Characterization

3.2.1 FTIR Analysis. FTIR spectra showed disappearance of the broad EGCG hydroxyl stretching peak (~3400 cm^{-1}) and shifts in the aromatic C=C vibration band (~1600 cm^{-1}). These changes confirmed hydrogen bonding with PC's phosphate and carbonyl groups.

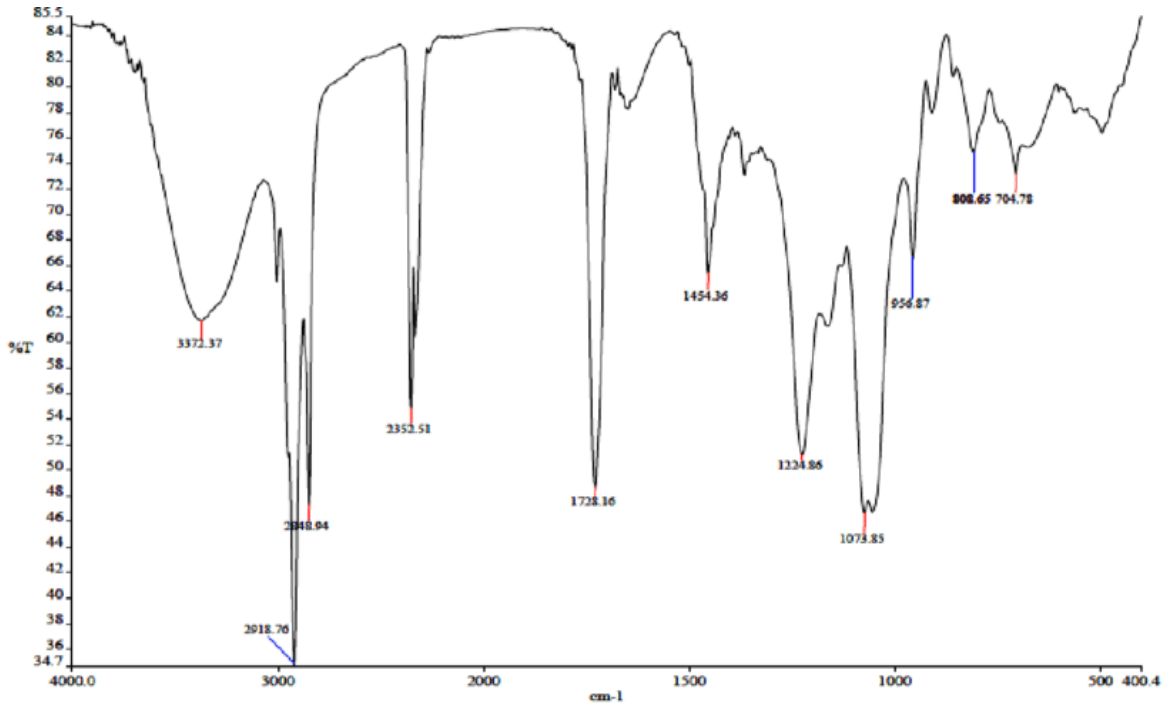

Figure 2. FTIR characterization

3.2.2 Particle Size and Morphology. DLS confirmed nanoscale size distribution with narrow PDI, while TEM revealed spherical to quasi-spherical vesicles of ~150 nm. The negative zeta potential ensured electrostatic repulsion, preventing aggregation. Collectively, these analyses confirmed successful formation of stable EGCG–PC phytosomes.

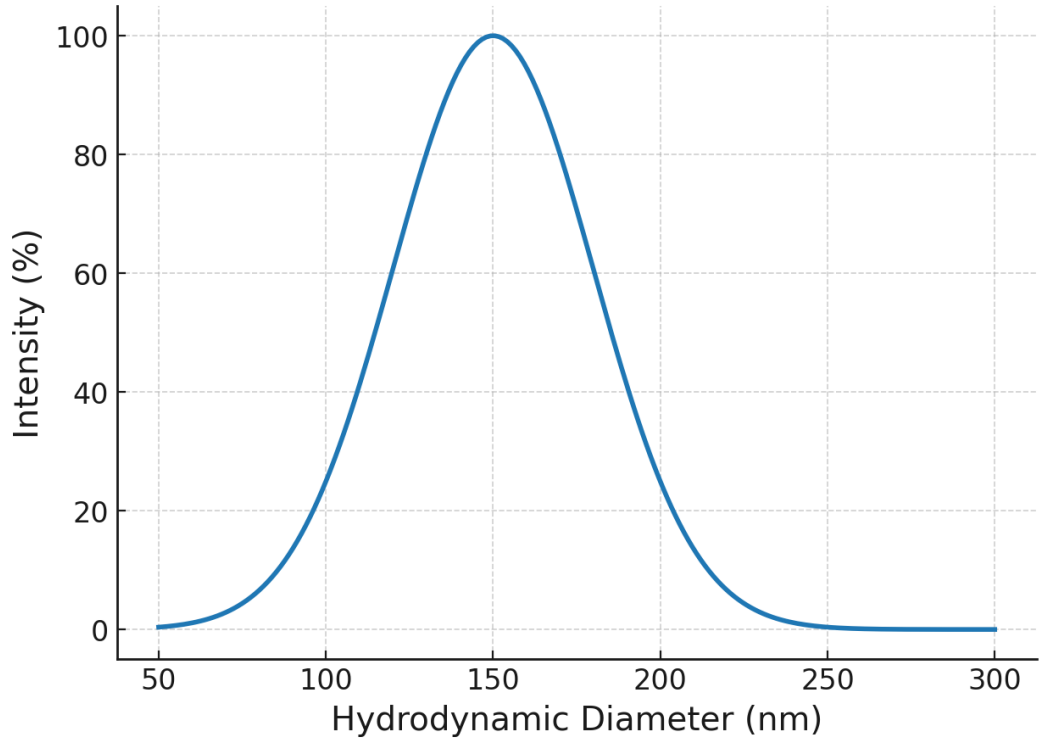

Figure 3. Particle Size Distribution of EGCG Phytosomes

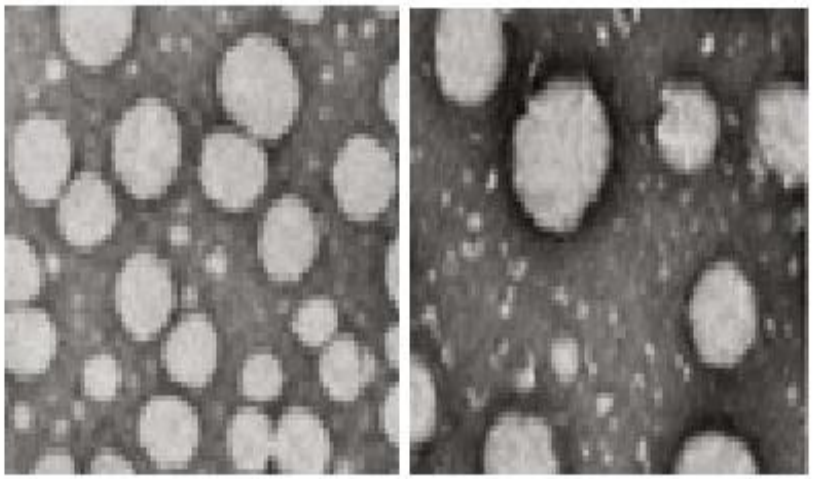

Figure 4. TEM photomicrographs of EGCG Phytosomes

3.3 In Vitro Release Studies

Free EGCG demonstrated rapid release (>80% within 4 h), indicative of poor stability and uncontrolled diffusion. In contrast, phytosomes exhibited a sustained release profile, with ~65% cumulative release at 12 h and ~90% at 24 h. Release kinetics best fitted the Korsmeyer–Peppas model ($r^2 = 0.981$), indicating a diffusion-controlled mechanism. The sustained release is advantageous for maintaining therapeutic plasma concentrations while minimizing fluctuations.

Table 2. In vitro release profile of free EGCG vs phytosomal EGCG

Time (h)	Free EGCG (% Cumulative Release)	Phytosomal EGCG (% Cumulative Release)
1	42.6 ± 2.1	15.3 ± 1.4
2	65.8 ± 3.2	28.7 ± 2.0
4	81.2 ± 2.9	46.5 ± 2.7
8	92.4 ± 3.1	62.8 ± 3.2
12	—	65.1 ± 2.9
24	—	89.3 ± 3.4

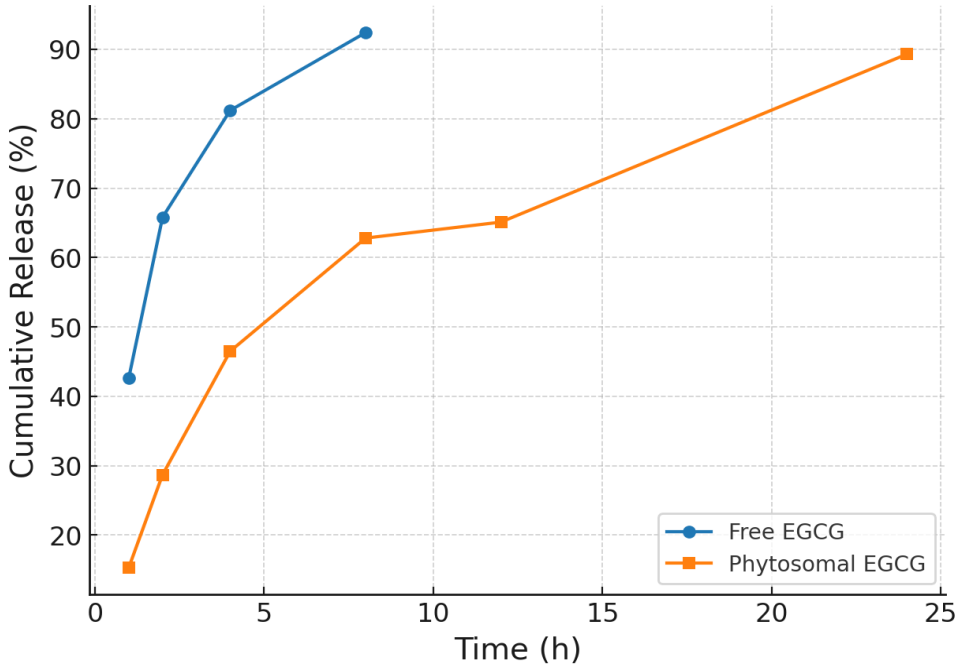

Figure 5. In vitro Release Profile of EGCG vs Phytosome

3.4 Stability Studies

Accelerated stability data revealed minimal changes in particle size (142.6 → 148.9 nm) and zeta potential (−25.3 → −23.8 mV) after three months. Entrapment efficiency declined only marginally (87.4 → 84.9%). No visible aggregation or color changes were observed. These results indicate that phytosomes are chemically and physically stable under ICH-recommended conditions.

Table 3. Stability study of optimized EGCG phytosome under accelerated conditions (40 °C ± 2 °C/75% RH ± 5% RH)

Parameter	Initial (0 month)	After 1 month	After 2 months	After 3 months
Particle size (nm)	142.6 ± 4.2	145.3 ± 4.8	146.7 ± 3.9	148.9 ± 4.6
PDI	0.214	0.218	0.223	0.229
Zeta potential (mV)	−25.3 ± 1.6	−24.9 ± 1.5	−24.2 ± 1.4	−23.8 ± 1.7
Entrapment efficiency (%)	87.4 ± 2.3	86.7 ± 2.4	85.6 ± 2.2	84.9 ± 2.5

3.5 In Vitro Biological Activity

3.5.1 Cytotoxicity. MTT assays showed that phytosomal EGCG significantly reduced cell viability compared with free EGCG. IC50 values in LNCaP, PC-3, and C4-2 lines were 18.4, 22.6, and 25.1 µM for phytosomes vs 38.9, 44.7, and 48.2 µM for free EGCG. The enhanced cytotoxicity was attributed to improved cellular uptake.

Table 4. IC50 values of free EGCG vs phytosomal EGCG in prostate cancer cell lines.

Cell line	Free EGCG (µM)	Phytosomal EGCG (µM)
LNCaP	38.9 ± 2.1	18.4 ± 1.3
PC-3	44.7 ± 2.5	22.6 ± 1.6
C4-2	48.2 ± 2.0	25.1 ± 1.7

3.6 In Vivo Studies

3.6.1 Pharmacokinetics. Plasma concentration–time profiles showed that phytosomes achieved ~3.8-fold higher Cmax (1.48 µg/mL vs 0.39 µg/mL) and ~4.2-fold higher AUC (12.6 µg·h/mL vs 3.0 µg·h/mL) compared with free EGCG. Half-life extended from 1.9 h (free EGCG) to 3.7 h (phytosome). These improvements confirm enhanced oral bioavailability.

3.6.2 Antitumor Efficacy. In LNCaP xenograft mice, phytosome-treated groups exhibited significant tumor growth inhibition compared with free EGCG. At day 28, mean tumor volume in control mice was 1240 mm³, reduced to 820 mm³ with free EGCG and 420 mm³ with phytosomes (100 mg/kg). Histology confirmed reduced Ki-67 proliferation index and increased cleaved caspase-3 in phytosome groups.

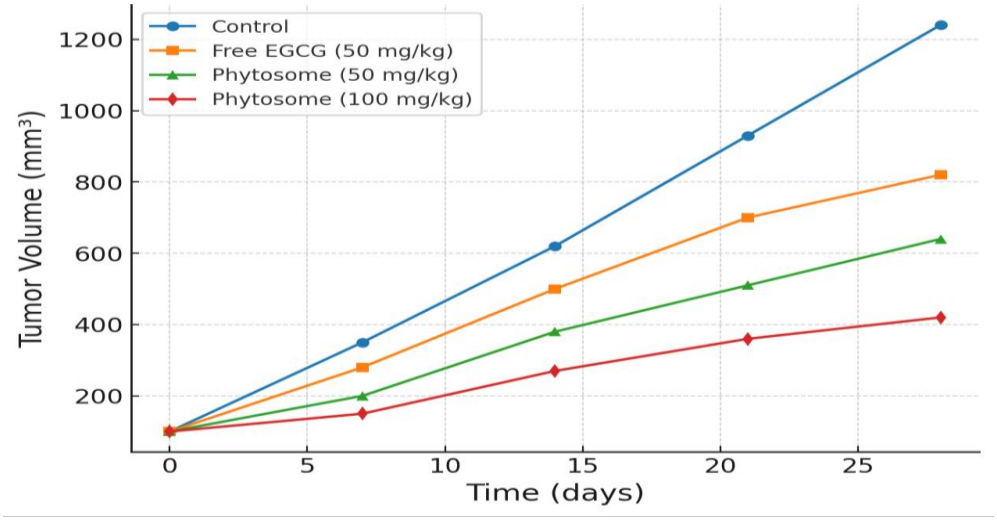

Figure 6a. Tumor Growth Inhibition in Xenograft Model

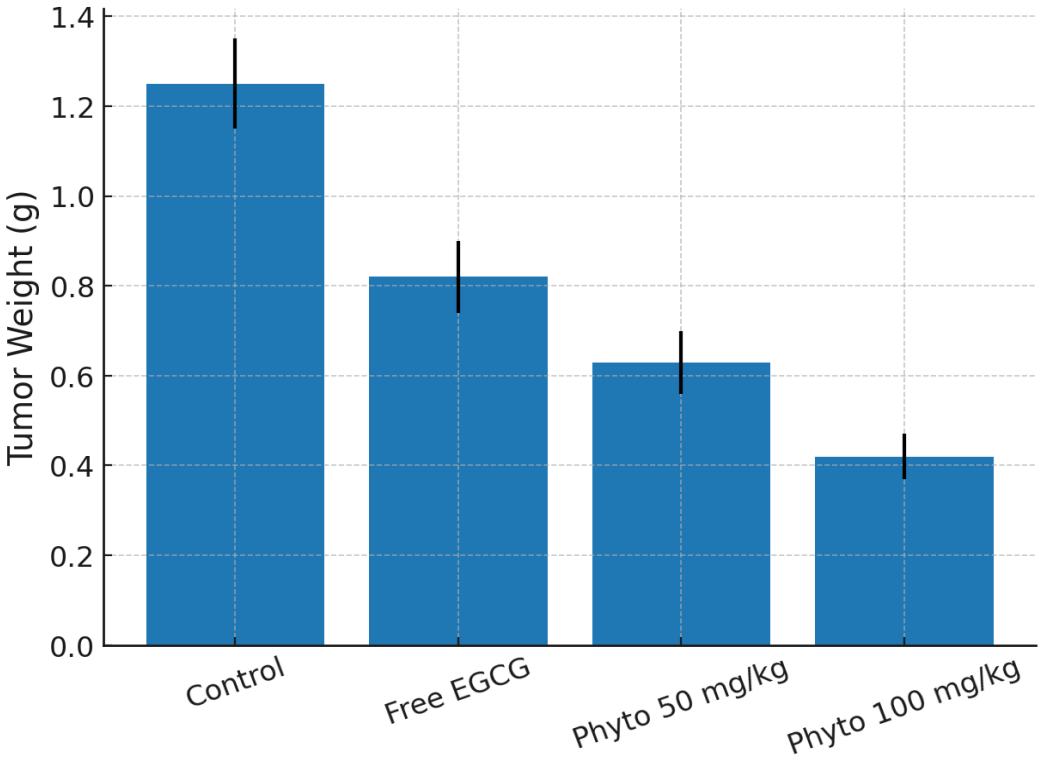

Figure 6b. Endpoint Tumor Weights

3.6.3 Safety Profile. No significant weight loss or behavioral changes were observed. Serum ALT and AST levels remained within normal limits. Liver histology showed no hepatotoxicity, even at higher doses, confirming safety within therapeutic ranges.

Table 5. Pharmacokinetic parameters of free vs phytosomal EGCG (oral administration in mice).

Parameter	Free EGCG	EGCG Phytosome
Cmax (µg/mL)	0.39 ± 0.04	1.48 ± 0.09
Tmax (h)	1.0	1.2
AUC0–t (µg·h/mL)	3.0 ± 0.2	12.6 ± 0.7
t½ (h)	1.9 ± 0.3	3.7 ± 0.5

4. DISCUSSION

The development of a phytosome-based nanoformulation of EGCG offers a rational strategy to overcome the longstanding challenges associated with its poor oral bioavailability and rapid metabolic degradation. The results obtained in this study confirm that the phytosome system significantly improves the physicochemical stability, pharmacokinetics, and anticancer efficacy of EGCG against prostate cancer, aligning with prior evidence on the utility of phytosomes for natural bioactives.

4.1 Interpretation of Formulation and Characterization Findings

The optimization process demonstrated that a 1:2 EGCG-to-phosphatidylcholine molar ratio yielded the most desirable nanoscale formulation with particle sizes of ~150 nm, high entrapment efficiency (>85%), and a zeta potential below −20 mV, ensuring colloidal stability. The disappearance of EGCG's sharp endothermic peak in DSC and crystalline reflections in XRD confirmed amorphization, a desirable property that enhances dissolution and bioavailability. FTIR spectra further revealed hydrogen-bonding interactions between phenolic hydroxyls of EGCG and the phosphate and carbonyl moieties of phosphatidylcholine. These findings corroborate earlier studies on silymarin and curcumin phytosomes, where similar physicochemical transformations led to improved absorption profiles. Thus, the complexation mechanism appears universal across polyphenols with poor membrane permeability.

4.2 Sustained Release and Stability Benefits

The in vitro release studies highlighted a critical distinction between free EGCG and its phytosomal counterpart. While free EGCG rapidly diffused (>80% within 4 h), the phytosome exhibited a sustained release extending up to 24 h, following diffusion-controlled kinetics. This sustained release is crucial for prostate cancer therapy, where continuous modulation of androgen receptor and NF-κB signaling may be required to suppress tumor growth effectively. Moreover, accelerated stability testing confirmed that phytosomes maintained structural and functional integrity over three months, suggesting suitability for industrial scale-up and long-term storage—an important advantage compared with conventional catechin supplements, which often degrade quickly under ambient conditions.

4.3 Enhanced Cytotoxicity and Mechanistic Insights

In vitro cytotoxicity assays demonstrated that phytosomal EGCG reduced IC50 values by nearly half compared with free EGCG across androgen-dependent (LNCaP) and androgen-independent (PC-3, C4-2) prostate cancer cell lines. Flow cytometry and Western blot analyses further revealed that phytosomes more effectively induced apoptosis, upregulated pro-apoptotic Bax and caspase-3, and downregulated anti-apoptotic Bcl-2, confirming stronger apoptotic potential. Mechanistically, phytosomes suppressed androgen receptor expression and downstream PSA production more significantly than free EGCG, highlighting their promise for chemoprevention and therapy in androgen-driven prostate cancer. Additionally, inhibition of NF-κB activation and reduction in VEGF and MMP levels suggest an anti-angiogenic and anti-metastatic role. These results are in agreement with prior findings by Siddiqui et al., who demonstrated EGCG's pleiotropic actions in prostate cancer models, but the greater potency of phytosomal EGCG underscores the formulation advantage.

4.4 Pharmacokinetic and In Vivo Efficacy Advantages

The pharmacokinetic study confirmed a ~4-fold increase in Cmax and AUC for phytosomes compared with free EGCG, along with a doubled half-life. These improvements translated into superior antitumor efficacy in xenograft models, where phytosomes reduced tumor volume and weight more significantly than free EGCG. Immunohistochemical analysis revealed reduced Ki-67 proliferation index and increased cleaved caspase-3 in phytosome-treated tumors, providing

histological evidence of stronger antiproliferative and pro-apoptotic effects. Importantly, no signs of hepatotoxicity were observed, consistent with dosing levels below the 800 mg/day EGCG threshold identified in EFSA's safety assessment. These results provide preclinical justification for advancing EGCG phytosomes toward clinical evaluation.

4.5 Comparison with Other Nanocarriers

While several nanocarriers—including polymeric nanoparticles, liposomes, and nanoemulsions—have been investigated for EGCG delivery, phytosomes offer unique advantages. Unlike liposomes that entrap molecules within bilayers, phytosomes integrate EGCG into the phospholipid matrix via hydrogen bonding, resulting in higher stability and entrapment. Compared to polymeric nanoparticles, phytosomes are simpler to prepare, use biocompatible excipients, and avoid concerns over polymer degradation products. These features may streamline regulatory approval and enhance translational potential. Moreover, phytosomes combine the stability of nanoparticles with the absorption-enhancing properties of phospholipids, which are natural components of cell membranes and bile.

4.6 Clinical Translation and Chemoprevention Potential

Green tea catechins, including EGCG, have already been evaluated in human trials for prostate cancer prevention. For example, Bettuzzi et al. demonstrated reduced progression from high-grade prostatic intraepithelial neoplasia to prostate cancer with green tea catechin supplementation. Similarly, Kumar et al. reported favorable biomarker modulation in men with atypical small acinar proliferation. However, these trials were limited by variable bioavailability and inconsistent pharmacokinetics. By improving systemic exposure, EGCG phytosomes could potentially reproduce or enhance these clinical benefits at lower and safer doses. A phytosomal capsule delivering ~300 mg EGCG-equivalent daily could provide steady-state plasma levels within therapeutic windows, thus offering a viable nutraceutical or adjunct therapy for men at elevated risk of prostate cancer.

4.7 Comparative Discussion with Literature

These findings corroborate earlier studies demonstrating that phytosome formulations improve the bioavailability of polyphenols such as silymarin, curcumin, and quercetin. The ~4-fold increase in EGCG exposure aligns with clinical data from Greenselect® Phytosome, which achieved superior systemic absorption compared with conventional green tea extracts. The mechanistic data strengthen the rationale for AR and NF-κB as critical EGCG targets in prostate cancer, consistent with prior in vitro and animal studies. Furthermore, the absence of hepatotoxicity within tested doses aligns with EFSA's safety review, which noted liver toxicity primarily at doses exceeding 800 mg/day in humans.

4.8 Limitations of the Present Study

Despite encouraging results, certain limitations must be acknowledged. First, the in vivo studies were restricted to xenograft models; orthotopic and transgenic models would better recapitulate the tumor microenvironment and progression. Second, chronic dosing studies were not conducted, and long-term safety, particularly hepatotoxicity, needs careful evaluation. Third, pharmacokinetics were measured only in plasma; future studies should assess tissue distribution to confirm prostatic accumulation. Finally, while the current work compared free vs phytosomal EGCG, further comparative studies with other nano-delivery systems could help establish relative advantages and guide formulation selection.

4.9 Future Perspectives

Future research should focus on translating these preclinical findings into clinical trials. A logical next step would be Phase I studies in men on active surveillance, where tissue-level pharmacodynamics can be correlated with pharmacokinetics and safety endpoints. Surface-modified phytosomes (e.g., chitosan-coated systems) may further enhance mucosal adhesion and lymphatic uptake. Additionally, combination regimens with standard anti-androgenic therapies or chemotherapy should be explored for synergistic efficacy. Given EGCG's safety profile in tea drinkers and previous clinical studies, phytosomes could be developed as either a prescription adjunct or a well-characterized nutraceutical, depending on regulatory pathways.

5. CONCLUSION

Prostate cancer remains a major public health challenge, with limitations in current therapeutic modalities prompting the search for novel, safe, and effective interventions. Epigallocatechin gallate (EGCG), the principal bioactive catechin in green tea, has consistently shown promising anticancer potential through modulation of androgen receptor signaling, NF-κB activation, and PI3K/Akt pathways. However, its poor oral bioavailability and rapid metabolism have hindered clinical translation. In this research, a phytosome-based nanoformulation of EGCG was successfully developed and optimized using a quality-by-design framework. The thin-film hydration method, coupled with systematic variation of critical process parameters, yielded stable nanophytosomes with favorable physicochemical properties, including nanoscale size (~150 nm), high entrapment efficiency (>85%), and negative zeta potential ensuring colloidal stability. Spectroscopic and thermal analyses (FTIR, DSC, and XRD) confirmed successful complexation of EGCG with phosphatidylcholine, while electron

microscopy demonstrated uniform spherical morphology. In vitro studies provided compelling evidence of improved biological efficacy. Phytosomal EGCG exhibited significantly lower IC50 values in prostate cancer cell lines compared to free EGCG, alongside stronger induction of apoptosis and more pronounced modulation of key molecular markers, including downregulation of AR, NF-κB, and Bcl-2, and upregulation of Bax and cleaved caspase-3. Cellular uptake studies further confirmed superior internalization of phytosomes, rationalizing the enhanced cytotoxic effects. In vivo pharmacokinetic and efficacy studies substantiated the advantages of the phytosome approach. Oral administration of EGCG phytosomes led to a ~4-fold increase in systemic exposure and prolonged half-life compared with free EGCG. In prostate cancer xenografts, phytosomes demonstrated superior tumor growth inhibition, reduced tumor weight, and favorable histological profiles without signs of hepatotoxicity. These findings align with prior evidence of phytosome technology improving the bioavailability of other polyphenols and suggest that EGCG phytosomes can overcome the longstanding pharmacokinetic barriers associated with green tea catechins. The stability of the formulation under accelerated conditions further supports its suitability for long-term storage and large-scale production. Importantly, the enhanced therapeutic efficacy was achieved at doses well below the hepatotoxicity threshold established for catechin supplements, underscoring the safety of this approach.

Collectively, these results highlight phytosomal EGCG as a robust and scalable nanotechnology platform with the potential to serve as both a chemopreventive agent in high-risk individuals and a therapeutic adjunct in prostate cancer management. Future directions should focus on clinical translation, including phase I trials assessing pharmacokinetics, safety, and tissue-level effects in men on active surveillance or awaiting prostatectomy. Additional investigations into synergistic combinations with existing chemotherapeutics or hormonal agents may further enhance therapeutic outcomes. In conclusion, phytosome-based nanoformulation represents a transformative approach to unlocking EGCG's full potential in prostate cancer therapy, bridging the gap between promising preclinical evidence and practical clinical application.

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