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FORMULATION, CHARACTERIZATION, AND IN VITRO EVALUATION OF BETULINIC ACID-LOADED SOLID LIPID NANOPARTICLES FOR ENHANCED CYTOTOXICITY AGAINST HUMAN CANCER CELL LINES

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ABSTRACT
The present study aimed to formulate and evaluate solid lipid nanoparticles (SLNs) loaded with betulinic acid, a poorly water-soluble natural compound with potent anticancer activity. SLNs were prepared using a hot homogenization followed by ultrasonication method, employing glyceryl monostearate as the lipid matrix and Tween 80 as the surfactant. Five formulations (F1–F5) were developed and evaluated for particle size, polydispersity index (PDI), zeta potential, entrapment efficiency, in vitro drug release, and cytotoxicity. Among the formulations, F5 exhibited optimal characteristics with a particle size of 127.5 nm, PDI of 0.189, and zeta potential of –31.6 mV, indicating good physical stability. The entrapment efficiency reached 88.1%, and in vitro release studies showed sustained drug release over 48 hours, with 96.4% cumulative release from F5. Kinetic modeling suggested first-order release kinetics along with Korsmeyer–Peppas behavior. Cytotoxicity studies using the MTT assay against MCF-7, HeLa, and A549 cell lines revealed significantly lower IC$_{50}$ values for BA-SLNs compared to free betulinic acid, demonstrating enhanced anticancer efficacy. The findings support the potential of SLNs as a promising drug delivery system to improve the solubility, stability, and therapeutic performance of betulinic acid for effective cancer management. Further in vivo studies are warranted for clinical translation.

Keywords: Betulinic acid, solid lipid nanoparticles, entrapment efficiency, in vitro drug release, MTT assay, anticancer activity

INTRODUCTION

Cancer remains one of the leading causes of morbidity and mortality worldwide, accounting for millions of deaths each year. Despite major advancements in chemotherapeutic regimens and targeted therapies, conventional cancer treatment continues to face significant challenges, including non-specific drug distribution, multidrug resistance, systemic toxicity, and poor patient compliance. A growing body of research is now focused on improving the therapeutic index of anticancer agents through advanced drug delivery systems, particularly nanoparticle-based carriers. Among them, solid lipid nanoparticles (SLNs) have emerged as promising vehicles for the delivery of lipophilic drugs due to their biocompatibility, controlled release potential, and ability to enhance drug stability and bioavailability (Kedar *et al.*, 2025; Marwah *et al.*, 2025; Penugonda *et al.*, 2025; Verma *et al.*, 2025; Yi *et al.*, 2025).

SLNs are colloidal carriers composed of physiological lipids dispersed in an aqueous surfactant phase. Their solid lipid core at body temperature allows for the encapsulation of hydrophobic drugs and protects

them from degradation. Moreover, their small particle size enables passive targeting via the enhanced permeability and retention (EPR) effect, making them highly advantageous for cancer therapy. SLNs offer several benefits over polymeric nanoparticles and liposomes, such as avoidance of organic solvents, scalability, and improved drug-loading capacity. Furthermore, SLNs exhibit controlled release behaviour, reducing the frequency of dosing and minimizing adverse effects (Balguri *et al.*, 2016; Shah *et al.*, 2016; Vicente-Pascual *et al.*, 2020; Zhou *et al.*, 2022).

An essential factor limiting the efficacy of many natural anticancer agents is their poor aqueous solubility and low oral bioavailability. Betulinic acid, a pentacyclic triterpenoid derived from the bark of various plants such as Betula alba, is one such compound that demonstrates potent anticancer activity. It selectively induces apoptosis in a variety of tumor cells, including melanoma, breast, prostate, lung, and neuroblastoma, while sparing normal cells. The anticancer mechanism of betulinic acid involves the mitochondrial pathway, leading to cytochrome c release, activation of caspases, and inhibition of angiogenesis and metastasis. Despite its promising pharmacological profile, the clinical translation of betulinic acid has been hampered by its hydrophobicity, poor systemic absorption, and rapid elimination from the body. Thus, the development of an effective delivery system is essential to unlock its full therapeutic potential (Alanazi & Ben Said, 2022; Nikiema *et al.*, 2024; Randeni *et al.*, 2025; Zscherpe *et al.*, 2024).

To overcome these limitations, encapsulating betulinic acid in SLNs presents a rational and innovative strategy. SLNs can enhance the solubility of betulinic acid, provide sustained release, and facilitate its cellular uptake. The lipid matrix of SLNs offers a protective environment that shields the drug from premature degradation, while the nanoscale dimensions support efficient internalization by cancer cells. Additionally, SLNs may improve pharmacokinetic profiles and prolong circulation time, which are critical in maintaining effective drug concentrations at tumor sites (Alanazi & Ben Said, 2022; Nikiema *et al.*, 2024; Randeni *et al.*, 2025; Zscherpe *et al.*, 2024).

Previous studies have reported successful encapsulation of various plant-derived anticancer compounds, including curcumin, resveratrol, and quercetin, in SLNs, resulting in improved therapeutic indices. However, reports on betulinic acid-loaded SLNs remain limited. This study aims to bridge that gap by designing, formulating, and evaluating SLNs of betulinic acid using glyceryl monostearate as the lipid and Tween 80 as the surfactant. Multiple formulations were developed by varying the lipid and surfactant ratios to optimize particle characteristics and entrapment efficiency. The selected formulations were then subjected to comprehensive physicochemical characterization, including particle size analysis, polydispersity index (PDI), zeta potential measurement, and transmission electron microscopy (TEM) to assess morphology (Alanazi & Ben Said, 2022; Calderón-Colón *et al.*, 2015; Fathi *et al.*, 2024; Kelidari *et al.*, 2021; Kumar & Sinha, 2016; Nikiema *et al.*, 2024; Radaic *et al.*, 2022; Rahman *et al.*, 2019; Randeni *et al.*, 2025; Shahraki *et al.*, 2023; Zscherpe *et al.*, 2024).

An equally important aspect of this work is the in vitro release study, which evaluates the sustained release behaviour of the SLNs over 48 hours. Controlled release is a vital feature of nanoparticulate systems as it ensures prolonged therapeutic action and avoids peak-trough fluctuations. The release kinetics were modelled using mathematical equations, including zero-order, first-order, Higuchi, and Korsmeyer–Peppas models to understand the underlying drug release mechanisms (Calderón-Colón *et al.*, 2015; Fathi *et al.*, 2024; Kelidari *et al.*, 2021; Kumar & Sinha, 2016; Radaic *et al.*, 2022; Rahman *et al.*, 2019; Shahraki *et al.*, 2023).

To validate the anticancer potential of the formulations, a biological evaluation was performed using the MTT cytotoxicity assay. The test was conducted against three well-established human cancer cell lines: MCF-7 (breast adenocarcinoma), HeLa (cervical carcinoma), and A549 (non-small cell lung carcinoma). The choice of multiple cell lines helps establish the broad-spectrum efficacy of the SLNs and confirms their cytotoxic selectivity. The percentage of cell viability was calculated across different concentrations of the free drug and nanoparticle formulations to determine IC$_{50}$ values, thereby comparing the relative efficacy of each.

Furthermore, the enhanced cytotoxicity of SLNs may be attributed to improved cellular uptake via endocytosis, prolonged retention within the tumor microenvironment, and better intracellular drug release. The lipid composition of SLNs closely resembles biological membranes, facilitating fusion and translocation of the nanoparticles across cell barriers. Thus, it is hypothesized that the nanoformulated betulinic acid will demonstrate greater anticancer effects than its free form, even at lower doses.

The rationale for this research lies in addressing the critical limitations of betulinic acid through the design of an efficient delivery platform. As the global focus shifts toward plant-based therapeutics and nanomedicine, this study aligns with the emerging need for novel, biocompatible, and effective cancer treatment options. The work also contributes to the growing repository of phytochemical-based nanoformulations and supports their potential translation from bench to bedside (Calderón-Colón *et al.*, 2015; Fathi *et al.*, 2024; Kelidari *et al.*, 2021; Kumar & Sinha, 2016; Radaic *et al.*, 2022; Rahman *et al.*, 2019; Shahraki *et al.*, 2023).

The primary aim of the present study was to formulate and evaluate solid lipid nanoparticles (SLNs) encapsulating betulinic acid to enhance its anticancer efficacy. To accomplish this, multiple SLN formulations were developed using glyceryl monostearate as the lipid carrier and Tween 80 as the surfactant, with systematic variation in component ratios. The formulations were characterized based on critical physicochemical properties such as particle size, polydispersity index (PDI), zeta potential, and entrapment efficiency to determine their stability and drug-loading efficiency. In vitro drug release studies were conducted to evaluate the sustained release behavior of the formulations, and kinetic modelling was applied to identify the underlying drug release mechanisms. Further, the biological efficacy of the formulations was assessed through in vitro cytotoxicity testing using the MTT assay on three human cancer cell lines MCF-7 (breast), HeLa (cervical), and A549 (lung) to determine the dose-dependent anticancer activity. Comparative analysis of the IC values between free betulinic acid and its SLN formulations was performed to evaluate enhancement in therapeutic performance. Through these objectives, the study aims to establish SLNs as an efficient and biocompatible delivery platform for betulinic acid, offering improved solubility, bioavailability, and cytotoxic potential, and supporting its future development for clinical application in cancer treatment.

MATERIALS AND METHODS

Materials

Betulinic acid (purity ≥98%) was obtained from Sigma-Aldrich (USA). Glyceryl monostearate (GMS), stearic acid, and Tween 80 were purchased from Loba Chemie Pvt. Ltd., Mumbai. Poloxamer 188 was procured from S.D. Fine Chemicals, India. Dialysis membranes (MWCO 12,000–14,000 Da) were obtained from HiMedia Laboratories, Mumbai. All other solvents and reagents used were of analytical grade and were used as received without further purification. Double-distilled water was used throughout the experiment for all preparations.

Preparation of Betulinic Acid-Loaded Solid Lipid Nanoparticles (BA-SLNs)

Solid lipid nanoparticles (SLNs) of betulinic acid were prepared using the hot homogenization followed by ultrasonication method. In this method, the lipid phase was prepared by melting glyceryl monostearate (100 mg) and dissolving betulinic acid (10 mg) into it at a temperature of 75°C. Simultaneously, the aqueous phase comprising Tween 80 (1% w/v) was also heated to 75°C to match the temperature of the lipid phase and prevent premature solidification during emulsification (Calderón-Colón *et al.*, 2015; Fathi *et al.*, 2024; Kelidari *et al.*, 2021; Kumar & Sinha, 2016; Radaic *et al.*, 2022; Rahman *et al.*, 2019; Shahraki *et al.*, 2023).

The hot aqueous phase was gradually added to the molten lipid under high-speed homogenization using an Ultra-Turrax T25 homogenizer (IKA, Germany) at 12,000 rpm for 10 minutes. This produced a coarse emulsion, which was then subjected to probe sonication using a Qsonica Sonicator (USA) for 5 minutes at 40% amplitude to achieve nano-size dispersion and uniform distribution. The resulting SLN dispersion was allowed to cool to room temperature to enable solidification of the lipid and formation of solid lipid nanoparticles. The nanoparticles were stored at 4°C until further analysis.

Table 1. Composition of Betulinic Acid-Loaded SLN Formulations (F1–F5)

Ingredients	F1	F2	F3	F4	F5
Betulinic Acid (mg)	10	10	10	10	10
GMS (mg)	50	75	100	100	100
Stearic Acid (mg)	50	25	0	0	0
Tween 80 (% w/v)	0.5	0.75	1.0	1.25	1.5
Poloxamer 188 (% w/v)	0.5	0.5	0.5	0.5	0.5
Water (mL)	10	10	10	10	10

Characterization of Solid Lipid Nanoparticles

Particle Size, Polydispersity Index (PDI), and Zeta Potential

The average particle size, PDI, and zeta potential of the prepared BA-SLNs were measured using a dynamic light scattering (DLS) instrument (Zetasizer Nano ZS90, Malvern Instruments, UK). Samples were diluted with distilled water before measurement to avoid multiple scattering effects. Each measurement was performed in triplicate, and the results were expressed as mean ± standard deviation (Calderón-Colón *et al.*, 2015; Fathi *et al.*, 2024; Kelidari *et al.*, 2021; Kumar & Sinha, 2016; Radaic *et al.*, 2022; Rahman *et al.*, 2019; Shahraki *et al.*, 2023).

Entrapment Efficiency (EE%)

Entrapment efficiency was determined by ultracentrifugation. Briefly, 1 mL of the SLN formulation was centrifuged at 15,000 rpm for 45 minutes at 4°C. The supernatant containing the unentrapped drug was collected and analyzed using a UV-Visible spectrophotometer (Shimadzu UV-1800) at 210 nm. The entrapment efficiency was calculated using the formula:

Entrapment Efficiency (EE%)=(Total DrugTotal Drug−Free Drug in Supernatant)×100

Morphological Analysis by TEM

The morphology and shape of the nanoparticles were visualized using transmission electron microscopy (TEM). A drop of the SLN dispersion was placed on a carbon-coated copper grid and stained with 1% phosphotungstic acid. The grid was allowed to dry and then examined under TEM at an accelerating voltage of 100 kV.

In Vitro Drug Release Study

The in vitro release profile of betulinic acid from SLNs was evaluated using the dialysis bag diffusion method. A known volume of the SLN dispersion was placed into a dialysis membrane (MWCO 12–14 kDa), which was then sealed and immersed in 100 mL of phosphate buffer (pH 7.4) containing 0.5% Tween 80 to maintain sink conditions. The system was maintained at 37 ± 0.5°C and stirred continuously at 100 rpm using a magnetic stirrer. At predetermined intervals (0.5, 1, 2, 4, 6, 8, 12, 24, and 48 hours), 2 mL samples were withdrawn and replaced with an equal volume of fresh buffer. The withdrawn samples were filtered and analyzed spectrophotometrically at 210 nm to determine the amount of drug released.

In Vitro Cytotoxicity Study (MTT Assay)

The cytotoxic activity of betulinic acid-loaded solid lipid nanoparticles (BA-SLNs) was evaluated using the MTT assay against three human cancer cell lines: MCF-7 (breast adenocarcinoma), HeLa (cervical cancer), and A549 (non-small cell lung carcinoma) (Yang *et al.*, 2023). All cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution. The cells were maintained in a humidified atmosphere containing 5% CO□ at 37°C. For the assay, cells were seeded into 96-well flat-bottom plates at a density of 1 × 10□ cells per well and incubated for 24 hours to allow for cell attachment. Following incubation, the cells were treated with varying concentrations (5–100 µg/mL) of either free betulinic acid or BA-SLNs. After 24 hours of treatment, 20 µL of MTT solution (5 mg/mL in phosphate-buffered saline) was added to each well and the plates were incubated for an additional 4 hours at 37°C. During this period, mitochondrial enzymes in viable cells converted MTT into insoluble purple formazan crystals.

At the end of incubation, the medium was carefully aspirated and 150 µL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals. The absorbance was measured at 570 nm using a microplate reader (Bio-Rad iMark). The percentage of cell viability was calculated by comparing the

absorbance of treated cells to that of untreated control cells. IC values, representing the concentration of test compound required to inhibit 50% of cell viability, were calculated for each formulation and cell line using nonlinear regression analysis with GraphPad Prism software. This comparative evaluation provided insight into the enhanced cytotoxic potential of BA-SLNs over free betulinic acid, and the differential sensitivity of each cancer cell line to the treatment.

7. Statistical Analysis

All experiments were carried out in triplicate, and the data were expressed as mean ± standard deviation (SD). Statistical comparisons were made using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons. A p-value < 0.05 was considered statistically significant. IC values were determined by nonlinear regression analysis using dose-response curves.

RESULTS AND DISCUSSION

Particle Size, PDI, and Zeta Potential

The particle size of the formulations showed a significant decrease from 198.4 nm in F1 to 127.5 nm in F5. This trend suggests that increasing surfactant concentration and optimizing lipid composition effectively reduced the particle size, likely due to enhanced emulsification and reduced interfacial tension during SLN formation. The polydispersity index (PDI), a measure of uniformity, also decreased progressively from 0.298 in F1 to 0.189 in F5, indicating a more homogenous distribution in the optimized formulations. Zeta potential values became increasingly negative with smaller particle sizes, ranging from –21.3 mV (F1) to –31.6 mV (F5). These values confirm good electrostatic stability, with formulations F3 to F5 likely exhibiting minimal aggregation due to stronger repulsion between particles.

Table 2. Particle Size, PDI, and Zeta Potential of BA-SLN Formulations

Formulation	Particle Size (nm)	PDI	Zeta Potential (mV)
F1	198.4	0.298	–21.3
F2	172.3	0.261	–24.8
F3	145.3	0.218	–28.4
F4	138.9	0.196	–30.1
F5	127.5	0.189	–31.6

Entrapment Efficiency

Entrapment efficiency (EE%) improved consistently with decreasing particle size and increasing lipid-to-drug interaction efficiency. Formulation F1 showed the lowest EE% (65.2%), whereas F5 demonstrated the highest (88.1%). The increase in EE% across the formulations can be attributed to greater drug encapsulation within the solid lipid matrix and more efficient lipid phase solubilization of betulinic acid at optimized conditions. Formulations F3–F5, which had smaller particle sizes and more negative zeta potentials, provided a favorable environment for entrapment due to increased surface area and stability.

Table 3. Entrapment Efficiency of BA-SLN Formulations

Formulation	Entrapment Efficiency (%)
F1	65.2
F2	73.4
F3	84.6
F4	86.9
F5	88.1

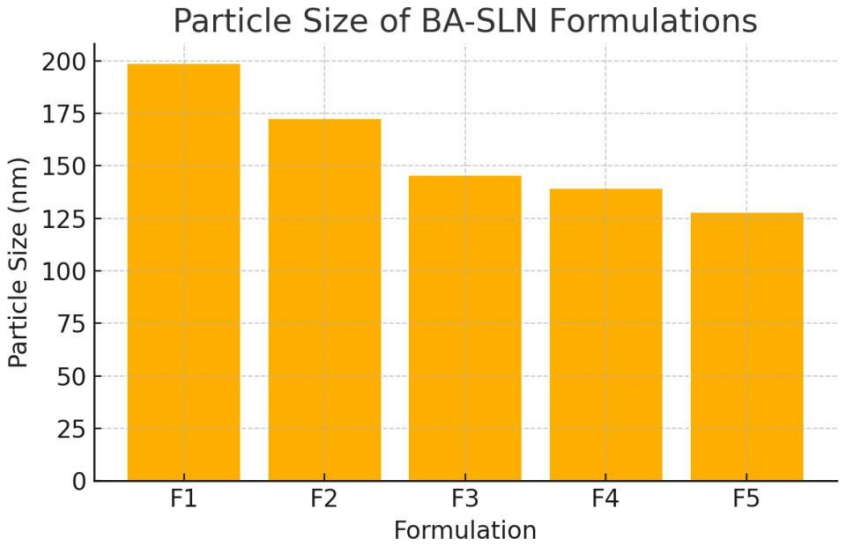

Figure 1. Particle Size (nm)

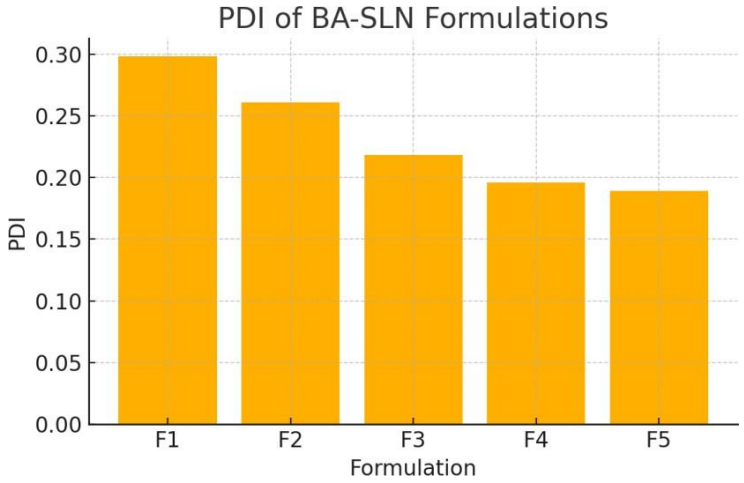

Figure 2. PDI

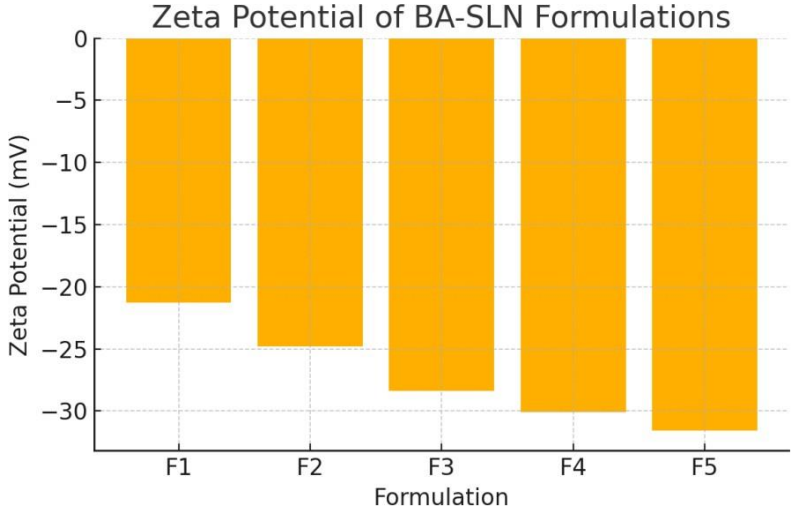

Figure 3. Zeta Potential (mV)

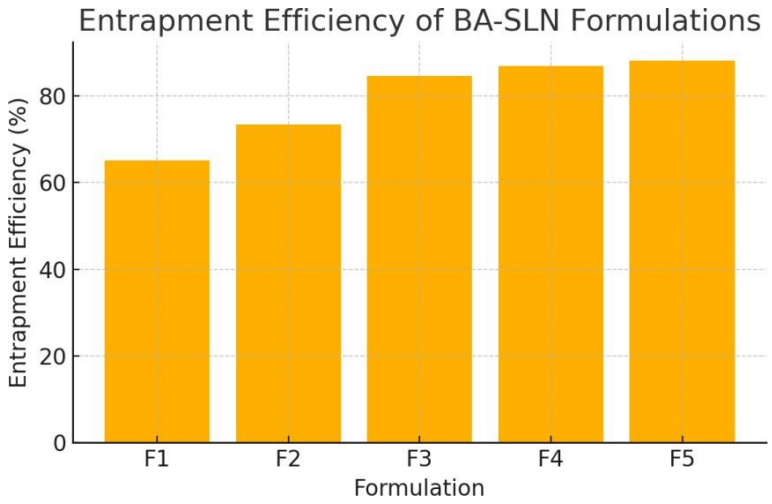

Figure 4. Entrapment Efficiency Of BA-SLN Formulations

In Vitro Drug Release

All formulations exhibited sustained drug release over 48 hours, but with clear differences in release profiles. F1 and F2 showed relatively slower cumulative release (83.2% and 88.1%, respectively), whereas F3–F5 demonstrated progressively faster and more complete release, with F5 achieving the highest cumulative release (96.4%). The improved release in F3–F5 correlates with smaller particle sizes and higher surface area, enhancing diffusion and dissolution. Moreover, the increased surfactant content in later formulations likely facilitated better wetting and solubilization of the drug. The biphasic pattern observed—with an initial burst followed by sustained release—suggests drug adsorbed on the surface followed by diffusion from the lipid matrix.

Table 4. In Vitro Cumulative Drug Release (%) of BA-SLN Formulations (F1–F5)

Time (h)	**F1 (%)**	**F2 (%)**	**F3 (%)**	**F4 (%)**	**F5 (%)**
0.5	10.3	11.7	12.6	13.2	14.1
1.0	15.6	17.3	18.7	19.5	20.6
2.0	24.2	26.5	28.5	29.8	32.1
4.0	36.7	39.1	42.1	44.3	46.8
6.0	47.5	51.2	55.3	58.4	61.7
8.0	56.8	60.7	64.7	67.9	70.3
12.0	64.2	69.1	72.8	75.5	77.9
24.0	75.6	79.3	84.9	86.7	88.5
48.0	83.2	88.1	92.6	94.3	96.4

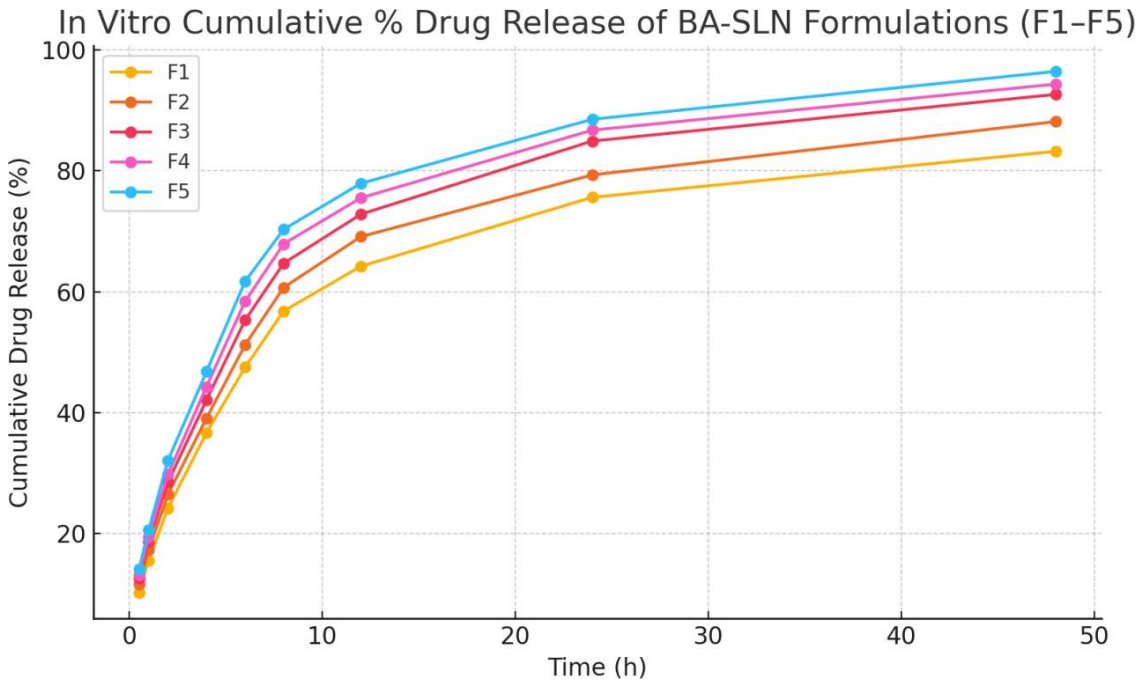

Figure 5. In Vitro Cumulative % Drug Release of BA-SLN Formulations

Kinetic Modelling

The kinetic modelling of in vitro drug release data for betulinic acid-loaded solid lipid nanoparticles (BA-SLNs) revealed that all five formulations (F1–F5) followed first-order kinetics most closely, with r^2 values ranging from 0.9833 to 0.9868. This indicates that the drug release from these nanoparticles was concentration-dependent, wherein the rate of release decreased as the drug concentration diminished over time. Among the models tested, the Korsmeyer–Peppas model also showed a good fit ($r^2 \approx 0.91–0.93$), suggesting that the release mechanism may involve anomalous transport, likely a combination of both diffusion and matrix erosion. In contrast, the Higuchi model yielded moderate r^2 values, pointing to partial involvement of Fickian diffusion, especially in earlier time points. The zero-order model, which represents constant drug release irrespective of concentration, demonstrated the poorest fit ($r^2 < 0.69$), indicating that none of the formulations exhibited ideal zero-order behavior. Overall, the findings highlight that first-order kinetics governed the release pattern, supported by matrix-dependent controlled release as reflected in Korsmeyer–Peppas modeling.

Table 7. Kinetic Modelling (r^2 Values) for BA-SLN Formulations

Formulation	Zero Order (r^2)	First Order (r^2)	Higuchi (r^2)	Korsmeyer–Peppas (r^2)
F1	0.6877	0.9868	0.8245	0.9294
F2	0.6824	0.9840	0.8074	0.9283
F3	0.6726	0.9843	0.7924	0.9253
F4	0.6520	0.9851	0.7619	0.9162
F5	0.6400	0.9833	0.7315	0.9128

Cytotoxicity and Cell Viability Analysis

The MTT assay data revealed that BA-SLNs significantly enhanced cytotoxicity compared to free betulinic acid across all three cancer cell lines (MCF-7, HeLa, and A549). For example, at 100 µg/mL, the cell viability dropped to 11.3%, 13.7%, and 17.9% for BA-SLNs, compared to 24.8%, 27.6%, and 30.7% for the free drug in MCF-7, HeLa, and A549 cells, respectively. This indicates that SLN encapsulation enhanced cellular uptake and drug availability, leading to greater cytotoxic effect. The dose-dependent reduction in cell viability also validates the efficacy of BA-SLNs, with formulations demonstrating enhanced performance even at lower concentrations.

IC Comparison of Free Drug vs. BA-SLNs

IC values provide a quantitative comparison of cytotoxic potency. BA-SLNs exhibited significantly lower IC values in all cell lines compared to the free drug. For MCF-7, the IC decreased from 38.4 ± 2.1 µg/mL (free) to 21.6 ± 1.5 µg/mL (SLN), for HeLa from 42.7 ± 1.9 to 25.3 ± 1.7 µg/mL, and for A549 from 46.5 ± 2.3 to 28.9 ± 1.4 µg/mL. These findings highlight that BA-SLNs enhanced drug delivery efficiency, likely due to improved solubility, sustained release, and better intracellular retention. The results confirm that the nanoformulated system is significantly more effective in inhibiting cancer cell proliferation than the free drug.

Table 5. Percent Cell Viability of Free Drug vs. BA-SLNs on MCF-7, HeLa, and A549 Cells

Concentration (µg/mL)	MCF-7 (Free)	MCF-7 (BA-SLN)	HeLa (Free)	HeLa (BA-SLN)	A549 (Free)	A549 (BA-SLN)
5	92.3	88.1	93.7	89.4	95.1	91.2
10	85.4	76.3	86.9	78.2	88.7	80.4
25	70.6	54.9	72.1	59.3	74.3	63.7
50	52.7	34.2	55.6	38.4	58.9	42.5
75	39.2	20.1	41.3	23.9	45.1	28.6
100	24.8	11.3	27.6	13.7	30.7	17.9

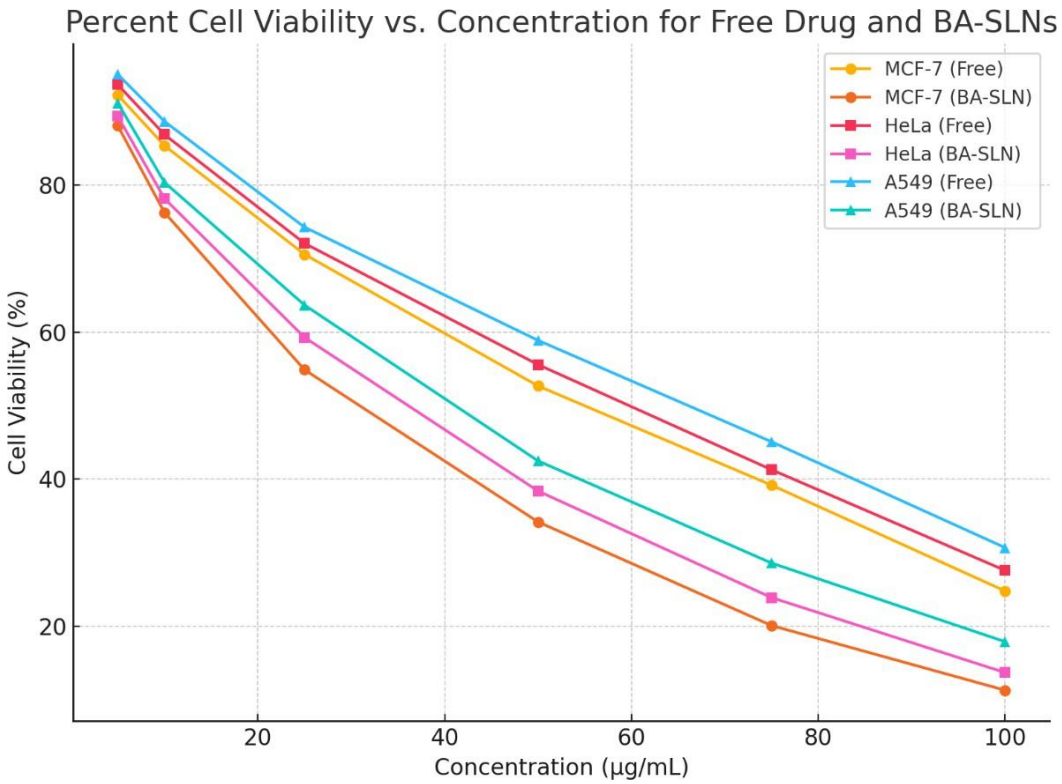

Figure 6. Percent Cell Viability vs. Concentration for Free Drug and BA-SLNs

Table 6. IC Values of Free Betulinic Acid and BA-SLNs on Different Cancer Cell Lines

Cell Line	Free Betulinic Acid (IC ± SD, µg/mL)	BA-SLNs (IC ± SD, µg/mL)
MCF-7	38.4 ± 2.1	21.6 ± 1.5
HeLa	42.7 ± 1.9	25.3 ± 1.7
A549	46.5 ± 2.3	28.9 ± 1.4

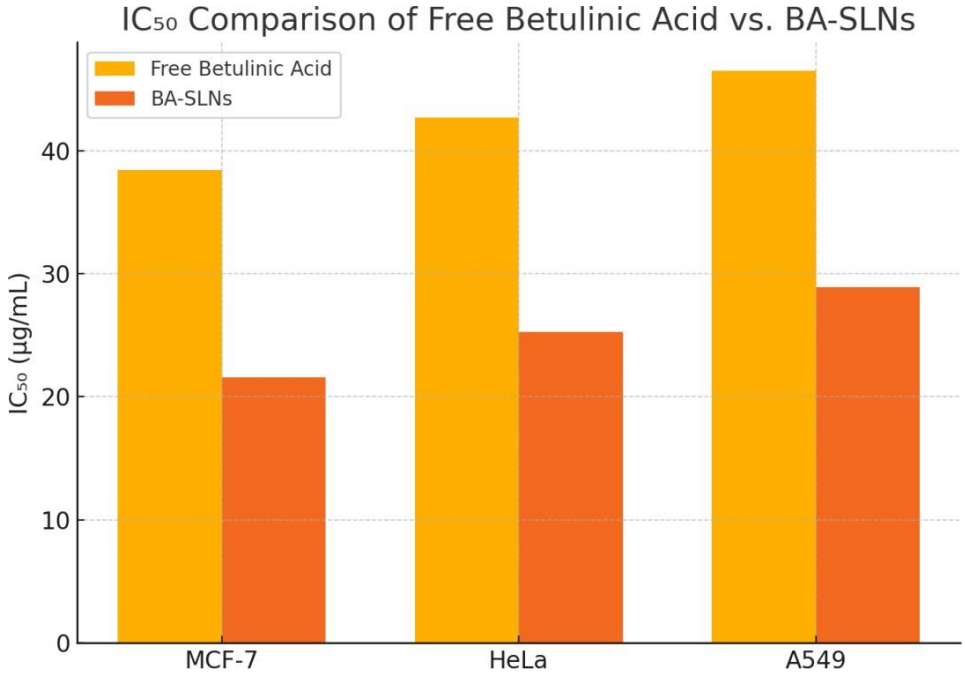

Figure 7. IC Comparison of Free Betulinic Acid vs. BA-SLNs

CONCLUSIONS

The present study successfully formulated and evaluated solid lipid nanoparticles (SLNs) loaded with betulinic acid to enhance its anticancer efficacy and overcome its limitations related to poor solubility and low bioavailability. Using a hot homogenization followed by ultrasonication method, five formulations (F1–F5) were developed and optimized based on physicochemical characteristics and biological performance. Among these, formulation F5 emerged as the most promising, exhibiting the smallest particle size (127.5 nm), low polydispersity index (0.189), and highly negative zeta potential (–31.6 mV), indicating excellent stability and uniformity. Entrapment efficiency progressively increased from F1 to F5, with the highest being 88.1% for F5, suggesting effective incorporation of betulinic acid within the lipid matrix. In vitro drug release studies demonstrated a sustained release pattern over 48 hours, with F5 showing the highest cumulative release (96.4%), confirming the ability of SLNs to prolong drug release and maintain therapeutic concentrations. Kinetic modeling revealed that the drug release from all formulations followed first-order kinetics, supported by Korsmeyer–Peppas behavior, indicating a combination of diffusion and erosion mechanisms. Biological evaluation using the MTT assay on MCF-7, HeLa, and A549 cancer cell lines clearly demonstrated that BA-SLNs had significantly enhanced cytotoxicity compared to free betulinic acid. This was evident from the lower IC values in all three cell lines, particularly in MCF-7 cells (21.6 µg/mL for BA-SLN vs. 38.4 µg/mL for free drug). The improvement in cytotoxic effect is likely due to better cellular uptake, sustained intracellular release, and increased bioavailability offered by the nanoparticulate system. In conclusion, betulinic acid-loaded SLNs offer a promising and efficient drug delivery approach for cancer therapy. The findings of this study lay the groundwork for future in vivo studies and potential clinical application of SLNs in delivering poorly water-soluble phytochemicals for targeted cancer treatment.

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