"FORMULATION, CHARACTERIZATION AND IN VITRO EVALUATION OF BETULINIC ACID-LOADED LIPID NANOCARRIERS FOR ENHANCED BIOAVAILABILITY AND SUSTAINED RELEASE IN LUNG CANCER THERAPY"

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ABSTRACT:
Betulinic Acid, a bioactive compound with significant anticancer potential, suffers from poor solubility and low bioavailability, limiting its therapeutic efficacy. This study aimed to develop Betulinic Acid-loaded lipid nanocarriers (BALNCs) to enhance drug solubility, bioaccessibility, and controlled release. With particle diameters between 42 and 44 nm, a polydispersity index (PDI) of approximately 0.26, and a zeta potential of approximately 20 mV, the nanocarriers were effectively synthesised and characterised, guaranteeing stability. High encapsulation efficiency (82–86%) and controlled loading capacity were achieved, demonstrating effective drug entrapment. Bioaccessibility studies showed a significant improvement in Betulinic Acid availability, with BALNC formulations achieving >46% bioaccessibility within 100 minutes, compared to the poor solubilization of the pure drug solution. Stability assessments in simulated gastrointestinal fluids confirmed the structural integrity of BALNCs under gastric conditions, while intestinal conditions led to size increases due to digestion. The in vitro drug release profile supported improved solubility and absorption by showing an early burst release followed by continuous drug release, reaching approximately 90% after 6 hours. These findings demonstrate how well lipid nanocarriers may transport betulinic acid while resolving issues with its solubility and bioavailability. This work advances lipid-based nanocarrier techniques for the oral administration of poorly soluble bioactive chemicals and lays a solid basis for future in vivo research and clinical applications.

Keywords: Betulinic Acid, Lipid nanocarriers, Bioaccessibility improvement, Chemotherapeutic agents, Nanostructured Carriers, Oral drug delivery.

INTRODUCTION:

Despite improvements in detection and treatment, cancer continues to be one of the world's leading causes of death, and its burden is growing globally. Conventional chemotherapy, though widely used, is often associated with severe systemic toxicity, poor tumor selectivity, multidrug resistance, and limited therapeutic efficacy. These challenges necessitate the development of alternative therapeutic strategies that can selectively target cancer cells while minimizing side effects. One such promising candidate is Betulinic Acid, a naturally occurring pentacyclic triterpenoid with potent anticancer activity [1-4].

In contrast to conventional chemotherapeutic agents, betulinic acid specifically induces apoptosis via the mitochondrial pathway, resulting in cytochrome c release, caspase activation, and programmed cell death. It has also been extensively studied for its selective cytotoxicity against a variety of cancers, including small cell lung cancer, melanoma, neuroblastoma, and testicular cancer. Additionally, betulinic acid exhibits anti-angiogenic and anti-metastatic properties, preventing tumour growth by modifying important signalling pathways like PI3K/Akt, NF-κB, and MAPK. These properties make it a highly attractive candidate for cancer therapy [5, 6]. Beyond its anticancer potential, Betulinic Acid has also demonstrated anti-inflammatory, antiviral, and antioxidant properties, further expanding its therapeutic scope. It helps manage chronic inflammatory diseases and neurological disorders by suppressing NF-κB activation and inhibiting pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β). Its antiviral activity has been reported against HIV, hepatitis B virus (HBV), and influenza, where it disrupts viral replication and maturation. Additionally, its antioxidant effects contribute to cellular protection against oxidative stress, suggesting potential applications in cardiovascular and neuroprotective therapies [1, 3, 7-10]. Despite its promising pharmacological benefits, the clinical application of Betulinic Acid is significantly limited by

its poor aqueous solubility, low bioavailability, and inefficient intestinal absorption. As a highly lipophilic compound, it exhibits low dissolution rates in physiological fluids, leading to inadequate systemic absorption and therapeutic efficacy. Moreover, rapid metabolism and clearance further reduce its in vivo bioavailability, preventing sustained therapeutic effects. These limitations underscore the need for advanced drug delivery systems that can enhance Betulinic Acid's solubility, absorption, and bioavailability [8, 9, 11-13].

The solubility and bioavailability issues with hydrophobic medications, such as betulinic acid, may be resolved with the use of lipid-based nanocarriers. These nanocarriers encapsulate lipophilic drugs within a lipid matrix, enhancing drug solubilization, protecting against enzymatic degradation, and improving gastrointestinal permeability. Furthermore, lipid nanocarriers can improve tumour accumulation, extend circulation duration, and enable controlled drug release via passive targeting mechanisms including the increased permeability and retention (EPR) effect [14-16]. Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) are two of the lipid-based delivery technologies that work well for oral medication delivery. The solid lipids that make up SLNs are stable at body temperature and form nanoparticles that provide enhanced stability and extended drug release. However, their highly crystalline structure sometimes limits drug loading capacity. NLCs, on the other hand, incorporate a mixture of solid and liquid lipids, creating a more flexible lipid matrix that enhances drug loading efficiency, prevents premature crystallization, and improves bioavailability [6, 17]. Recent studies have demonstrated that Betulinic Acid-loaded lipid nanocarriers (BALNCs) significantly enhance drug solubility, bioaccessibility, and dissolution rates, thereby improving intestinal absorption and systemic availability. By encapsulating Betulinic Acid within lipid nanocarriers, it is possible to achieve sustained release, minimize premature drug degradation, and improve overall therapeutic efficacy. Additionally, improved gastrointestinal processing is made possible by lipid nanocarriers, which combine with digestive enzymes and bile salts to create mixed micelles that improve drug absorption [7].

In light of these benefits, the goal of this research is to create and describe lipid nanocarriers (BALNCs) loaded with betulinic acid in order to enhance drug solubility, bioaccessibility, and sustained release. To guarantee stability and ideal drug loading, the study assesses important factors such as particle size, polydispersity index (PDI), zeta potential, encapsulation efficiency (EE), and loading capacity (LC). Furthermore, the bioaccessibility of BALNCs is assessed using simulated gastric and intestinal fluids to predict their absorption potential. Furthermore, to ascertain the dissolving profile of betulinic acid under intestinal simulations, in vitro drug release studies are carried out, offering vital information about its sustained release behaviour. By systematically analyzing these parameters, this research aims to demonstrate the feasibility of lipid-based nanocarriers as an effective strategy to enhance Betulinic Acid bioavailability, facilitating its clinical translation for cancer therapy and other therapeutic applications. This study contributes to the advancement of nanotechnology-driven drug delivery systems, addressing the limitations of conventional formulations and offering a novel approach to improving the therapeutic potential of Betulinic Acid.

In order to improve solubility, bioavailability, and controlled release, this study creates Betulinic Acid-loaded Lipid Nanocarriers (BALNC), which will increase the effectiveness of treatment for small cell lung and testicular malignancies. To fill in preclinical and clinical gaps, stability in simulated gastrointestinal fluids, bioaccessibility, and drug release kinetics are evaluated. By overcoming the drawbacks of traditional formulations, this study increases medication delivery based on nanotechnology for more efficient cancer treatment.

MATERIAL AND METHODS:

Drugs, chemicals and other reagents:

Medium-chain triglyceride (MCT) and Imwitor 900 K, commonly used as lipid excipients to enhance drug solubility and stability, were generously provided as complimentary samples by BASF SE, Ludwigshafen, Germany. As a phospholipid stabiliser in the formulation, Lipoid SPC-3, a highly pure type of phosphatidylcholine derived from soybeans, was acquired from Avanti Polar Lipids, Inc., Alabaster, Alabama, USA. The surfactants Tween 80 (Polysorbate 80) and Span 20, known for their emulsification and dispersion-enhancing properties, were procured from Sigma-Aldrich, India. Betulinic Acid, a pentacyclic triterpenoid exhibiting significant pharmacological potential, served as the active pharmaceutical ingredient and was sourced from Sigma-Aldrich. Additional excipients, such as ethanol and chloroform, were used as solvents during the preparation of lipid nanocarriers. Furthermore, phosphate-buffered saline (PBS) was utilized for stability and in vitro release studies, ensuring a physiologically relevant environment. To ensure accuracy, repeatability, and dependability in the experimental processes, all additional chemicals and reagents, such as acetone and methanol, were of analytical grade.

Fabrication of Betulinic Acid loaded lipid nanocarriers:

Hot homogenisation and ultrasonication were used to create the Nanostructured Lipid Nanocarriers (LNC), which were tailored to the distinct lipid compositions of each formulation [18]. The heat homogenisation method was used to create lipid nanocarriers (LNCs), which were then ultrasonically sonicated to achieve nanoscale dispersion. The lipid phase was first made by precisely weighing and heating the liquid lipid (medium-chain triglyceride, or MCT) and solid lipid (Imwitor 900 K) to about 75°C, making sure the solid lipid melted completely. To guarantee even distribution, the medication was dissolved in the molten lipid phase while being constantly stirred for formulations containing betulinic acid (BALNC-1, BALNC-2, and BALNC-3). Simultaneously, the aqueous phase was prepared by dissolving the surfactants (Tween 80 and Span 20) and lecithin in distilled water, which was also maintained at 75°C to prevent premature lipid solidification. A first pre-emulsion was then formed by introducing the lipid phase gradually into the aqueous phase while homogenising at a high speed of 10,000 rpm for 10 minutes using an Ultra-Turrax homogeniser. The pre-emulsion was ultrasonically sonicated for five minutes at 60% amplitude using a probe sonicator with an on/off pulse cycle (30 seconds pulse-on and 10 seconds pulse-off) to further reduce particle size and guarantee uniform dispersion. The resulting nanoemulsion was then cooled to room temperature under gentle stirring, allowing the lipid phase to recrystallize and form stable lipid nanocarriers. For additional characterisation, the finished dispersions were kept in glass vials at 4°C. This method ensured the development of lipid nanocarriers with optimized particle size, uniform drug encapsulation, and improved stability, enhancing their suitability for drug delivery applications [18, 19].

Table 1. Formulation of lipid nanocarriers containing blank and Betulinic Acid:

Formulation	Solid Lipid (%)	Liquid Lipid (%)	Tween 80 (%)	Lecithin (%)	Span 20 (%)	Water (%)	Mono/Di/Tri Glycerides (%) *	Betulinic Acid (mg)
Blank-lipid nanocarriers (BLNC)	4.0	2.0	1.5	1.0	0.5	89.0	2.0	0
Betulinic Acid - loaded lipid nanocarriers 1 (BALNC-1)	4.0	2.0	1.5	1.0	0.5	88.5	2.0	0.5

Betulinic Acid - loaded lipid nanocarriers 2 BALNC-2)	5.0	3.0	1.5	1.5	0.5	86.0	2.5	1.0
Betulinic Acid - loaded lipid nanocarriers 3 BALNC-3)	6.0	4.0	2.0	2.0	1.0	83.0	3.0	2.0

***Note:** The percentage of various lipids employed in the production process was used to determine the mono-, di-, and triglyceride content of LNCs (Lipid Nanocarriers). BLNCs are lipid nanocarriers with a blank nanostructure. BALNCs are lipid carriers with a nanostructure that contain betulinic acid.

Measurement of Particle size and zeta potential:

Dynamic light scattering (DLS) was used to assess the lipid nanocarriers' particle size and zeta potential using a Malvern Zetasizer Nano ZS90 (Malvern Instruments, UK). Prior to analysis, the samples were suitably diluted with deionised water to avoid multiple scattering effects. The intensity-weighted mean diameter was utilised to calculate particle size, and the polydispersity index (PDI) was employed to evaluate the homogeneity of the size distribution. Electrophoretic light scattering (ELS) was used to detect the zeta potential in order to assess the stability and surface charge of the nanocarriers. To guarantee precision and repeatability, every measurement was carried out at 25°C, and every sample was examined three times [20].

Evaluation of Encapsulation efficiency (EE) and loading capacity (LC):

An indirect approach based on centrifugation was used to measure the loading capacity (LC) and encapsulation efficiency (EE) of betulinic acid in lipid nanocarriers. To separate the unencapsulated medication from the nanocarriers, the nanocarrier dispersion was spun using a chilled centrifuge set to 15,000 rpm for 30 minutes at 4°C. After collecting the supernatant, UV-Vis spectrophotometry was used to measure the amount of free betulinic acid at the predefined λmax. The following formulas were used to determine EE and LC:

$$EE\ (\%) = \text{Total Drug} - \text{Free Drug in Supernatant} / \text{Total Drug} \times 100$$

$$LC\ (\%) = \text{Total Drug} - \text{Free Drug in Supernatant} / \text{Total Lipid} + \text{Drug} \times 100$$

Every measurement was carried out in triplicate, and the mean ± standard deviation (SD) was used to express the results. The effectiveness of drug entrapment and the ability of lipid nanocarriers to efficiently encapsulate betulinic acid for enhanced therapeutic performance were both revealed by this study [21].

Lipid digestion in vitro under circumstances modelled by the stomach and intestines:

In order to evaluate the digestion behaviours and release profile of lipid nanocarriers, in vitro lipid digestion tests were conducted to mimic the physiological conditions of the gastrointestinal (GI) tract, specifically the stomach and intestines. Simulated intestinal fluid (SIF) and simulated gastric fluid (SGF) made in accordance with USP recommendations were used in the investigation [22]. To simulate gastric digestion, the lipid nanocarrier dispersion was first incubated in SGF (pH 1.2) containing pepsin at 37°C with constant stirring. After a predefined period, the medium was neutralized to pH 6.8, and SIF containing pancreatin and bile salts was introduced to simulate intestinal digestion. By quantifying the release of free fatty acids using titration with sodium hydroxide (NaOH), the degree of lipid hydrolysis was measured in order to track the digestive process. By gathering aliquots and using an assay to analyse the drug content, the drug release profile of betulinic acid was evaluated at various time intervals. The stability, digestion kinetics, and bioaccessibility of lipid nanocarriers loaded

with betulinic acid under GI circumstances that were simulated were all investigated in triplicate to assure reproducibility.

In vitro bioaccessibility:

The degree to which betulinic acid is available for absorption after digestion was assessed by in vitro bioaccessibility tests. These studies simulated human gastrointestinal conditions to predict the release of Betulinic Acid from lipid nanocarriers and its subsequent readiness for intestinal absorption. Intestinal and stomach digestion were the two successive stages of the digestion process. Lipid nanocarriers were incubated in pepsin-containing simulated gastric fluid (SGF, pH 1.2) at 37°C with constant stirring during the gastric phase. To start intestinal digestion, simulated intestinal fluid (SIF) containing pancreatin and bile salts was added after the medium had been neutralised to pH 6.8 for a predetermined amount of time. The bioaccessible fraction of Betulinic Acid was determined by centrifuging the digestion mixture at 15,000 rpm for 30 minutes to separate the micellar phase, which represents the absorbable form of the drug. UV-Vis spectrophotometry was used to measure the amount of betulinic acid in the micellar fraction, and the following formula was used to determine bioaccessibility:

Bioaccessibility (%) = Drug in Micellar Fraction / Total Drug in Formulation × 100

All experiments were conducted in triplicate to ensure reproducibility. This assessment provided crucial insights into the efficiency of lipid nanocarriers in enhancing Betulinic Acid bioavailability by improving its solubilization and gastrointestinal absorption [23, 24].

Drug release under simulated intestinal conditions:

The drug release behavior of Betulinic Acid-loaded lipid nanocarriers (BALNC) was evaluated under simulated intestinal conditions to mimic physiological drug dissolution and absorption. To simulate enzymatic digestion in the small intestine, the investigation was carried out using simulated intestinal fluid (SIF, pH 6.8) that was made in accordance with USP requirements and contained pancreatin and bile salts. A dialysis membrane (MWCO 12-14 kDa) containing a specified volume of lipid nanocarrier dispersion was submerged in 100 mL of SIF that was kept at 37°C while being continuously stirred at 100 rpm. To maintain sink conditions, aliquots of the release medium were taken out and replaced with an equivalent volume of new SIF at predetermined intervals. The concentration of Betulinic Acid released was determined using UV-Vis spectrophotometry at its predetermined λmax (222 nm). In order to ascertain the drug release mechanism, the cumulative drug release percentage was computed, and the release kinetics were examined by fitting the data to mathematical models such as zero-order, first-order, Higuchi, and Korsmeyer-Peppas models. To guarantee precision and repeatability, every experiment was carried out in triplicate. This study provided essential insights into the release profile of Betulinic Acid from lipid nanocarriers, contributing to the optimization of formulation strategies for enhanced intestinal absorption and therapeutic efficacy [25].

Statistical analysis:

To warrant the quality and dependability of the experimental results, statistical analysis was carried out. Every measurement was carried out in triplicate, and the mean ± standard deviation (SD) was used to express the results. To find significant differences between formulations, data were analysed using Tukey's post hoc test after one-way analysis of variance (ANOVA). P-values below 0.05 were regarded as statistically significant. The best-fit model explaining the release mechanism was found by using regression analysis on drug release kinetics. GraphPad Prism software, Version 8, was used for all statistical calculations, guaranteeing accurate data interpretation and validation of experimental results.

RESULTS AND DISCUSSION:

Physicochemical characterization:

The physicochemical stability and drug entrapment properties of Betulinic Acid-loaded lipid nanocarriers (BALNCs) were assessed by analysing their particle size, polydispersity index (PDI), zeta potential, encapsulation efficiency (EE), and loading capacity (LC) both prior to and following lyophilization.

Particle Size and Polydispersity Index (PDI):

The blank lipid nanocarriers (BLNC) exhibited a mean particle size of 56 ± 4.59 nm, whereas Betulinic Acid-loaded formulations (BALNC-1, BALNC-2, and BALNC-3) showed slightly smaller sizes ranging from 42 to 44 nm, indicating that drug incorporation did not significantly impact particle size. The PDI values for all formulations remained around 0.26–0.27, suggesting a narrow size distribution and uniform formulation. However, upon lyophilization, a substantial increase in particle size was observed, with lyophilized BALNCs (L-BALNC-1, L-BALNC-2, and L-BALNC-3) showing particle sizes between 184 and 186 nm. This increase in size is expected due to aggregation during the freeze-drying process, which is common for lipid-based nanocarriers.

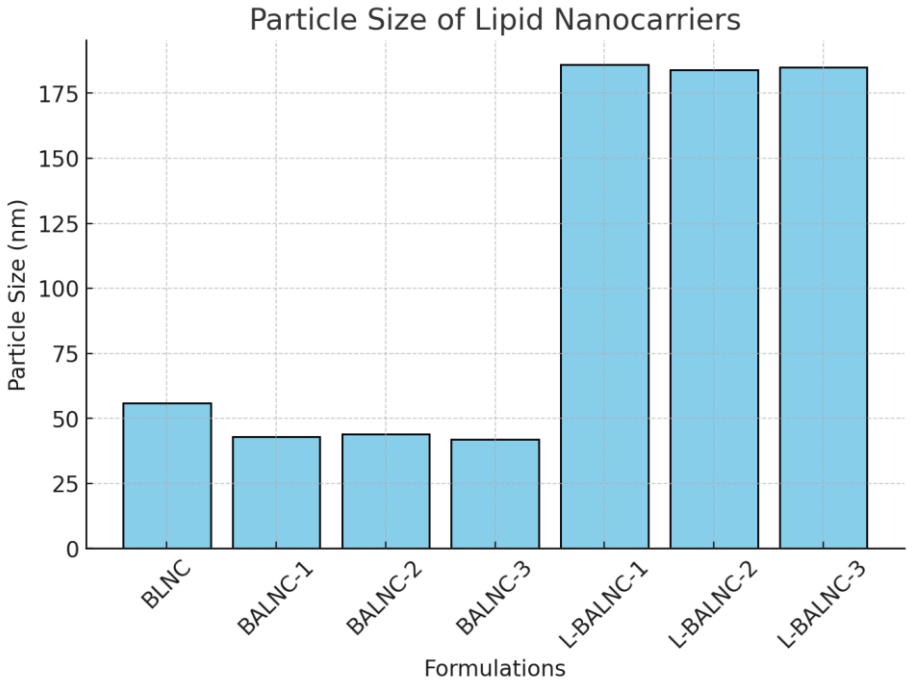

Figure 1. Particle Size (nm) distribution

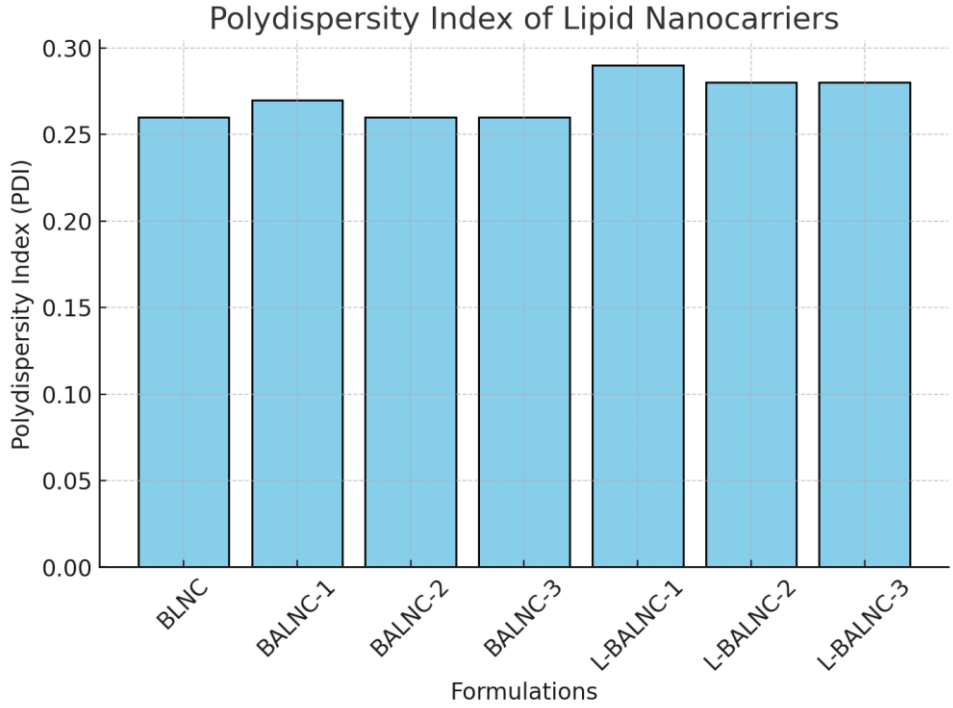

Figure 2. Polydispersity Index (PDI)

Zeta Potential and Stability:
The surface charge (zeta potential) of the lipid nanocarriers ranged between 19 and 21 mV, with no significant changes between blank and Betulinic Acid-loaded formulations. These values indicate moderate colloidal stability, as zeta potentials above ±20 mV generally prevent excessive aggregation. However, zeta potential values for lyophilized formulations were not determined (n.d.), possibly due to structural changes affecting charge distribution post-lyophilization.

Encapsulation Efficiency (EE) and Loading Capacity (LC):
The encapsulation efficiency (EE) for Betulinic Acid-loaded formulations was consistently high, ranging from 82% to 86%, suggesting effective entrapment of the drug within the lipid matrix. The loading capacity (LC) remained relatively stable across formulations, with values between 0.77% and 0.81%, indicating that the lipid nanocarrier system could efficiently accommodate Betulinic Acid. Post-lyophilization, a slight increase in EE was noted (85%–86%), possibly due to changes in the lipid matrix during freeze-drying that enhanced drug entrapment.

Table 2. The loading capacity, surface charge, encapsulation effectiveness, particle size, and polydispersity index of lipid nanocarriers loaded with betulinic acids and lyophilised:

Details of Formulation and Codes	Size (nm)	PDI	Zeta Potential (mV)	EE (%)	LC (%)
Blank-lipid nanocarriers (BLNC)	56 ± 4.59	0.26	21	-	-
BALNC-1	43 ± 3.86	0.27	20	83 ± 2.76	0.81 ± 0.14
BALNC-2	44 ± 3.59	0.26	21	82 ± 2.69	0.79 ± 0.15
BALNC-3	42 ± 3.68	0.26	19	83 ± 2.44	0.77 ± 0.14
Lyophilized					

L-BALNC-1	186 ± 7.45	0.29	n.d.	85± 2.58	0.78 ± 0.12
L-BALNC-2	184 ± 6.92	0.28	n.d.	85± 2.78	0.81 ± 0.14
L-BALNC-3	185 ± 7.85	0.28	n.d.	86± 2.90	0.78 ± 0.16

Note: * Encapsulation efficiency is represented by EE, loading capacity by LC, and polydispersity index by PDI.Not determined, but statistically significant, $P < 0.05$ (n.d.). To identify LNCs (Lipid Nanocarriers), the proportion of different lipids employed in the synthesis process was used. Lipid nanocarriers having a blank nanostructure are known as BLNCs. BALNCs are nanostructured lipid carriers loaded with betulinic acid.

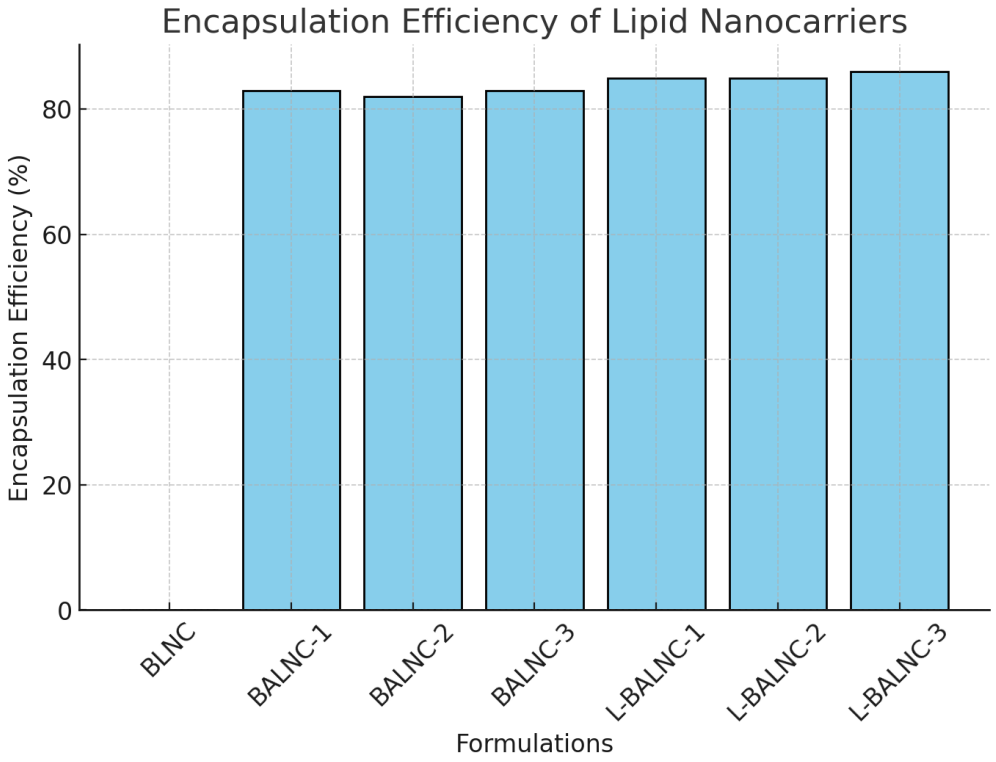

Figure 3. Encapsulation Efficiency (%)

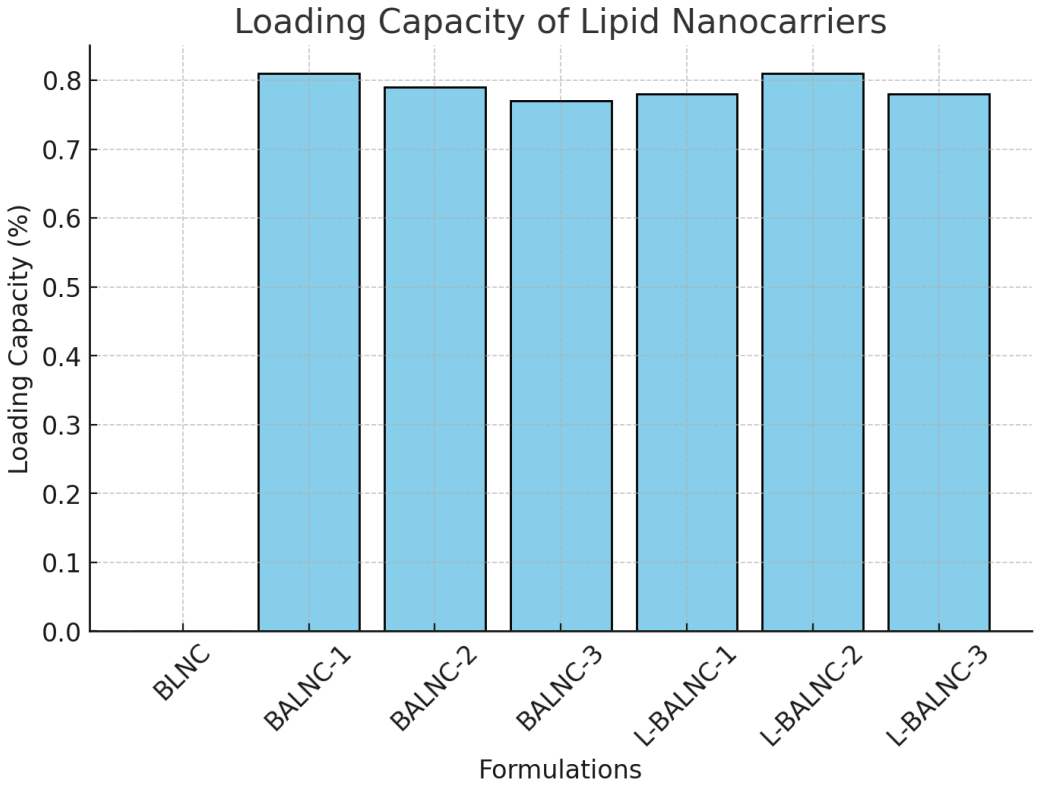

Figure 4. Loading Capacity (%)

Lipid nanocarrier stability in gastrointestinal fluid simulation:

In particular, simulated gastric fluid (SGF, pH 2.0) and simulated intestinal fluid (SIF, pH 7.0) were used to assess the stability of betulinic acid-loaded lipid nanocarriers (BALNC). The structural integrity and behaviour of the nanocarriers in the gastrointestinal system can be inferred from the variations in particle size under various situations. At the initial stage, all formulations exhibited relatively similar particle sizes, ranging from 286 ± 3.25 nm (BALNC-1) to 291 ± 2.97 nm (BALNC-3), indicating uniformity in formulation. The slight variation in particle size among the different BALNC formulations suggests that minor differences in composition did not significantly impact the initial size of the nanocarriers.

Stability in Simulated Gastric Fluid (SGF, pH 2.0):

Upon exposure to SGF, the particle size of all formulations decreased significantly, with values reducing to 170 ± 3.22 nm (BALNC-1), 158 ± 3.51 nm (BALNC-2), and 169 ± 3.47 nm (BALNC-3). This reduction in size suggests partial lipid solubilization and possible structural rearrangement of the nanocarriers under acidic conditions. The stability in SGF indicates that the nanocarriers are not prone to excessive aggregation or degradation in the stomach, making them suitable for oral drug delivery.

Stability in Simulated Intestinal Fluid (SIF, pH 7.0):

In contrast, when transferred to SIF (pH 7.0), a substantial increase in particle size was observed, with sizes exceeding 1050 nm for all formulations. The marked expansion in size (1052 ± 16.36 nm for BALNC-1, 1059 ± 15.86 nm for BALNC-2, and 1065 ± 14.45 nm for BALNC-3) suggests nanoparticle aggregation or potential lipid digestion due to the presence of bile salts and pancreatic enzymes in the intestinal environment. This behaviour indicates that the nanocarriers undergo structural modifications upon intestinal exposure, likely due to digestion-mediated destabilization or drug release. The results demonstrate that BALNC formulations maintain stability in the gastric environment, with a reduction in particle size

potentially enhancing their bioavailability. However, the significant increase in size in SIF suggests structural instability, possibly due to enzymatic digestion or particle aggregation. This finding emphasizes the need for further optimization, such as incorporating stabilizers or modifying lipid composition, to enhance intestinal stability and improve controlled drug release. Overall, these lipid nanocarriers show promising stability in the acidic gastric environment but may require formulation adjustments to prevent excessive size enlargement and aggregation in the intestinal phase.

Table 3: Stability of lipid nanocarriers in simulated gastrointestinal fluid:

Parameter	Particle size (nm ± SD)		
	BALNC-1	BALNC-2	BALNC-3
Initial size	286 ± 3.25	288 ± 2.79	291 ± 2.97
Size in SGF (pH 2.0)	170 ± 3.22	158 ± 3.51	169 ± 3.47
Size in SIF (pH 7.0)	1052 ± 16.36	1059 ± 15.86	1065 ± 14.45

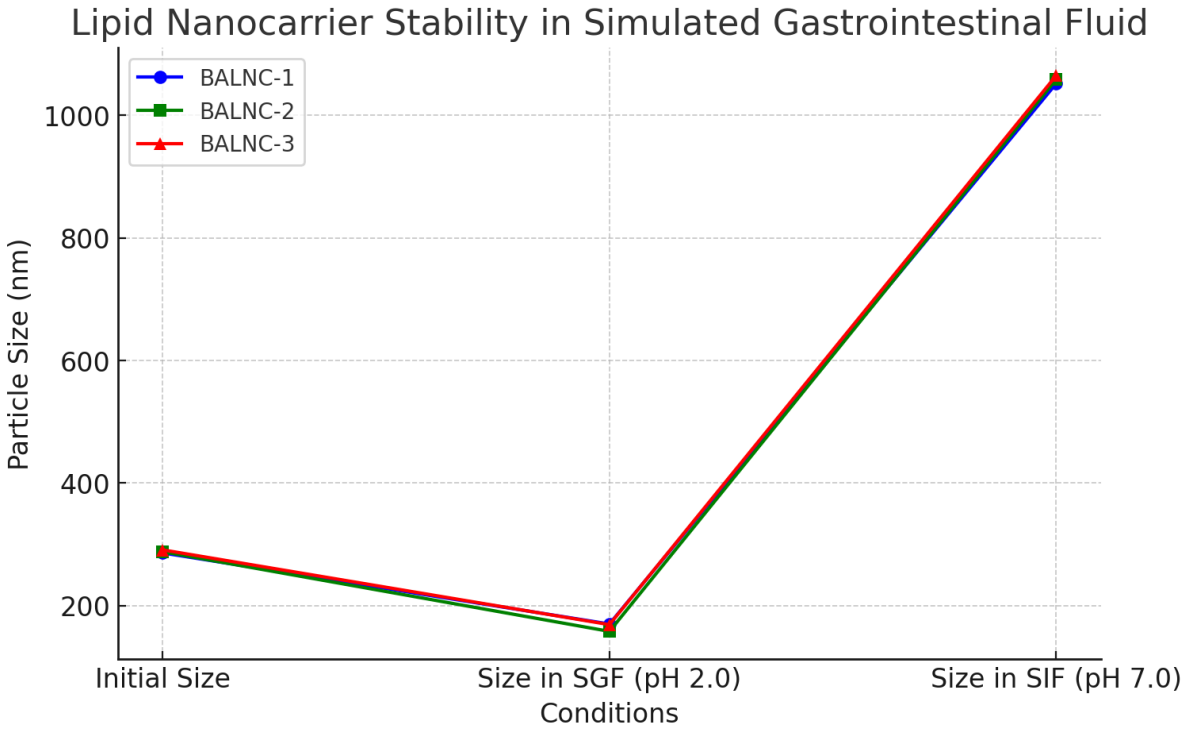

Figure 5. Lipid nanocarrier stability in SGF and SIF. Simulated Gastric Fluid (SGF) has a pH of 2.0. * denotes statistically significant changes (P < 0.05), and the pH of Simulated Intestinal Fluid (SIF) is 7.0.

Bioaccessibility study:

The bioaccessibility study evaluated the extent to which Betulinic Acid becomes available for absorption after digestion in simulated gastrointestinal conditions. The results compared the bioaccessibility of a pure Betulinic Acid solution (BAS) with Betulinic Acid-loaded lipid nanocarriers (BALNC-1, BALNC-2, and BALNC-3) at different time intervals (25, 50, and 100 minutes). At the initial 25-minute mark, the pure Betulinic Acid solution (BAS) exhibited very low bioaccessibility (5.5 ± 0.15%), indicating poor solubility and limited availability for absorption. In contrast, all lipid nanocarrier formulations showed significantly higher bioaccessibility values, with BALNC-1 (35.5 ± 1.19%), BALNC-2 (34.6 ± 1.13%), and BALNC-3 (35.3 ± 1.15%). This suggests that lipid nanocarriers enhance the solubilization of Betulinic Acid in the gastrointestinal environment, improving its potential for absorption. By

50 minutes, there was a gradual increase in bioaccessibility across all formulations. The BAS showed a slight improvement (6.9 ± 0.13%), but it remained significantly lower than the lipid nanocarriers. The BALNC formulations exhibited bioaccessibility ranging from 42.8% to 45.6%, indicating sustained release and improved drug dispersion. Among them, BALNC-1 had the highest bioaccessibility (45.6 ± 1.17%), suggesting that formulation composition might influence the drug release profile. At 100 minutes, the bioaccessibility of the pure Betulinic Acid solution remained very low (8.0 ± 0.17%), confirming its poor solubility in physiological conditions. In contrast, the lipid nanocarriers reached their highest bioaccessibility levels, with BALNC-1 (46.8 ± 1.20%), BALNC-2 (44.8 ± 1.11%), and BALNC-3 (44.7 ± 1.19%). Although there was only a marginal increase from the 50-minute mark, the results indicate that the lipid nanocarriers successfully enhanced the solubilization and bioaccessibility of Betulinic Acid over time. The results clearly demonstrate that lipid nanocarriers significantly enhance the bioaccessibility of Betulinic Acid compared to its pure solution, which exhibited minimal solubilization. The encapsulation within lipid nanocarriers facilitated better dispersion, protection from degradation, and improved dissolution in the simulated gastrointestinal environment. All of the BALNC formulations demonstrated greater bioaccessibility, despite minor differences, demonstrating the potential of lipid-based delivery methods to increase the oral bioavailability of poorly soluble medications such as betulinic acid. Further optimization, such as exploring different lipid compositions or stabilizers, may further enhance bioaccessibility and absorption efficiency.

Table 4: The ability of lipid nanocarriers to be bioavailable expressed as percentage bioaccessibility:

	Bioaccessibility (% ± SD)			
Time (minutes)	**Betulinic Acid Solution (BAS)**	**BALNC-1**	**BALNC-2**	**BALNC-3**
25	5.5 ± 0.15	35.5 ± 1.19	34.6 ± 1.13	35.3 ± 1.15
50	6.9± 0.13	45.6 ± 1.17	43.7 ± 1.16	42.8 ± 1.16
100	8.0 ± 0.17	46.8± 1.20	44.8 ± 1.11	44.7 ± 1.19

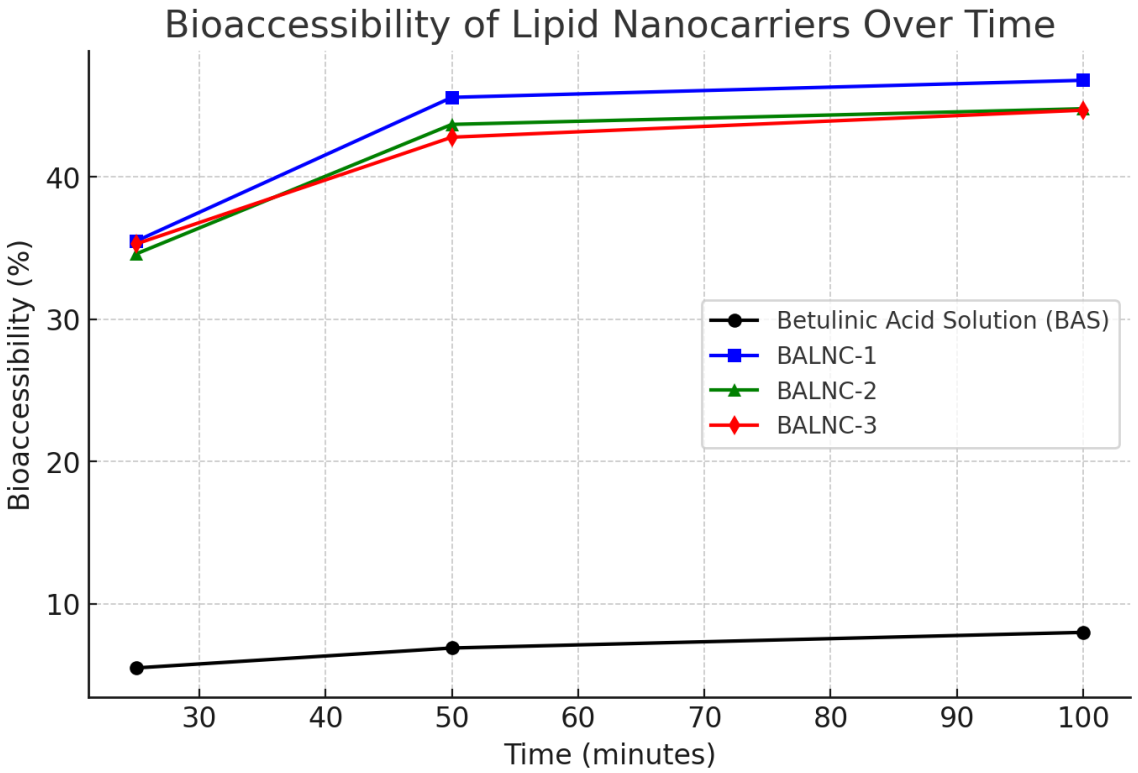

Figure 6. The time-dependent bioaccessibility of betulinic acid in SIF.

Drug release under simulated intestinal conditions:

The drug release study evaluated the release profile of Betulinic Acid from lipid nanocarriers (LNCs) in simulated intestinal fluid (SIF, pH 7.0) over a period of six hours. The results provide insight into the sustained release properties of the formulations (BALNC-1, BALNC-2, and BALNC-3), which are crucial for improving drug bioavailability and therapeutic efficacy. At 0 hours, no drug release was detected, confirming that Betulinic Acid remained encapsulated within the lipid nanocarriers at the start of the experiment. By the first hour, approximately 17.3–17.7% of the drug was released across all formulations, indicating an initial burst release phase. This rapid release suggests that a portion of Betulinic Acid may be present near the surface of the nanocarriers, allowing for immediate diffusion. By the second hour, drug release increased significantly to ~38.6%, reflecting continuous diffusion and dissolution of the lipid matrix in the intestinal environment. Between 3 to 5 hours, the drug release exhibited a sustained pattern, with values rising from ~60% at 3 hours to ~82% at 5 hours. This phase indicates a controlled release mechanism, likely driven by lipid degradation and gradual diffusion of the encapsulated drug from the nanocarrier core. The similarity in release profiles among BALNC-1, BALNC-2, and BALNC-3 suggests that formulation composition did not significantly affect release kinetics, indicating a well-optimized system. At 6 hours, the drug release reached ~89.4–89.8%, indicating near-complete release of Betulinic Acid. The absence of a plateau before the 6-hour mark suggests that the lipid matrix allowed for efficient drug diffusion without excessive retardation. This prolonged release behavior is advantageous for improving Betulinic Acid's therapeutic efficacy, ensuring its gradual availability for intestinal absorption. The drug release study confirms that lipid nanocarriers provide a sustained and controlled release profile for Betulinic Acid, significantly enhancing its dissolution in simulated intestinal conditions. The initial burst release ensures rapid drug availability, while the sustained release phase prolongs therapeutic effects. The effectiveness of lipid-based delivery methods in increasing the bioavailability of betulinic acid is demonstrated by the significant cumulative release (~90%) in just 6 hours. To fine-tune the release kinetics for

particular therapeutic uses, additional optimisation could be investigated, such as altering the lipid composition or surfactant content.

Table 5: Release of the drug in intestinal simulation circumstances:

Time (hour)	Betulinic Acid Release from LNC (% ± SD)		
	BALNC-1	BALNC-2	BALNC-3
0	0	0	0
1	17.76 ± 1.44	17.35 ± 1.32	17.33 ± 1.27
2	38.56 ± 1.71	38.62 ± 1.30	38.61 ± 1.29
3	60.35 ± 1.33	60.35 ± 1.45	60.28 ± 1.32
4	72.87 ± 1.61	73.28± 1.47	73.19 ± 1.41
5	82.25 ± 1.37	82.26 ± 1.52	82.26 ± 1.56
6	89.44 ± 1.46	89.77 ± 1.63	89.46 ± 1.77

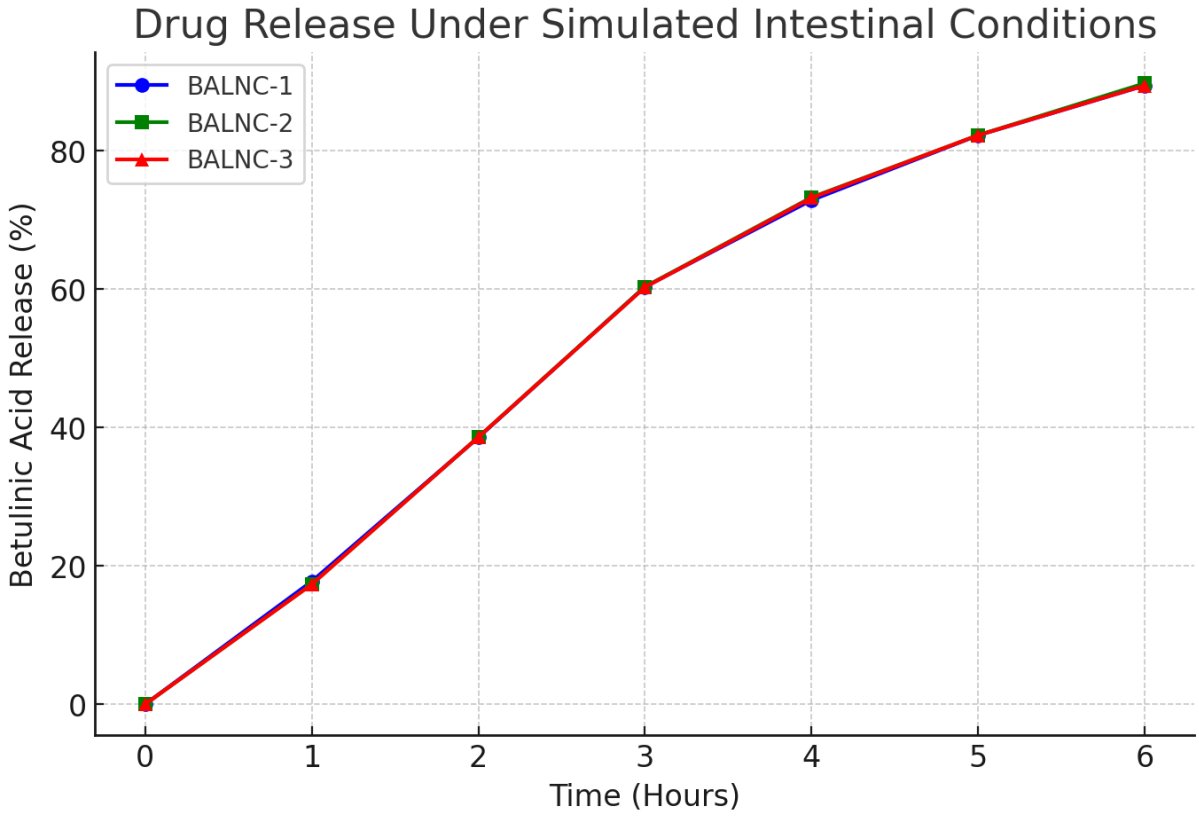

Figure 7. In vitro release profile of betulinic acid from nanocarriers in an intestinal environment devoid of enzymes (mean ± SD; n = 3). BALNC is an acronym for lipid nanocarriers loaded with betulinic acid.

CONCLUSION:

The present study successfully developed and characterized Betulinic Acid-loaded lipid nanocarriers (BALNCs) to enhance the solubility, bioaccessibility, and controlled release of Betulinic Acid. The formulations exhibited small particle sizes (42–44 nm), low polydispersity index (PDI ~0.26), and moderate zeta potential (~20 mV), ensuring stability and uniformity. Encapsulation efficiency was consistently high (82–86%), demonstrating the nanocarriers' ability to effectively entrap the drug. The bioaccessibility study revealed that lipid nanocarriers significantly improved drug availability compared to the pure Betulinic Acid solution, with

bioaccessibility exceeding 46% after 100 minutes. Stability studies in simulated gastrointestinal fluids indicated that the nanocarriers maintained their structural integrity in gastric conditions but exhibited increased particle size in intestinal fluids, suggesting interaction with bile salts and enzymatic digestion. With almost 90% of the drug released within 6 hours, the in vitro drug release investigation validated a sustained release profile, underscoring the possibility of long-lasting therapeutic effects. All of these results highlight how well lipid-based drug delivery methods work to increase betulinic acid's stability, bioavailability, and solubility. The study backs up the possible use of lipid nanocarriers in clinical settings to improve betulinic acid's therapeutic effectiveness, especially when it comes to oral drug administration. Further investigations, including in vivo pharmacokinetic studies and optimization of formulation parameters, are necessary to translate these promising in vitro findings into clinical applications.

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