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Development of Phlorizin-Loaded Lipid–Polymeric Hybrid Nanoparticles: Pharmacokinetic and Drug-Likeness Prediction with In Vitro Anti-Senescence Assessment

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Development of Phlorizin-Loaded Lipid–Polymeric Hybrid Nanoparticles: Pharmacokinetic and Drug-Likeness Prediction with *In Vitro* Anti-Senescence Assessment

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Abstract

Drug delivery systems powered by nanotechnology have greatly progressed pharmaceutical science by enhancing the solubility, stability, and therapeutic effectiveness of bioactive compounds. In this study, lipid–polymeric hybrid nanoparticles (LPHNPs) containing Phlorizin were created through a one-step nanoprecipitation method to address the compound's natural limitations in solubility and bioavailability. Thorough characterization was conducted, encompassing evaluations of particle size, zeta potential, morphology, entrapment efficiency, and in vitro drug release profile. Dynamic light scattering assessments revealed a Z-average particle size of 425.7 nm and a polydispersity index (PDI) of 0.272, showing consistency in size distribution. The nanoparticles showed a zeta potential of −28.8 mV, suggesting strong colloidal stability. Scanning electron microscopy showed clearly defined, solid nanoparticles at the nanoscale. The formulation reached an entrapment efficiency of 99.62% and exhibited prolonged drug release for as long as 20 hours, adhering to near-zero-order kinetics. The anti-senescence effect was assessed in high glucose-exposed 3T3-L1 murine adipocytes through the use of SA-β-Gal staining. The Phlorizin nanosuspension offered dose-dependent defense against cellular senescence, demonstrated by a decrease in SA-β-Gal-positive cells at higher concentrations. Morphological assessment verified the return to a typical fibroblast-like cell structure. Together, these findings suggest that Phlorizin-loaded LPHNPs have significant potential as a nanocarrier system to improve therapeutic delivery and reduce glucose-triggered adipocyte senescence.

Keywords: Phlorizin, Lipid–Polymeric Hybrid Nanoparticles, Nanoprecipitation, Anti-senescence, 3T3-L1 adipocytes, SA-β-Gal.

1 Introduction

Cellular senescence is a complex biological process defined by permanent cell cycle arrest, metabolic dysregulation, and distinct morphological changes (1). This process is critically implicated in organismal aging and the development of various metabolic diseases, such as obesity, diabetes mellitus, and cardiovascular disorders. Sustained hyperglycemia has been shown to intensify oxidative stress and inflammatory mechanisms, which in turn accelerate the onset of premature senescence within adipose tissue. Consequently, there is growing scientific interest in devising therapeutic approaches aimed at counteracting glucose-induced cellular aging and promoting the restoration of normal cellular activity(2).

Nanotechnology-based drug delivery systems are highly effective strategies to enhance the therapeutic potential of poorly soluble bioactive compounds (3). Lipid–polymeric hybrid nanoparticles (LPHNPs) have been amongst the most studied nanosystems, owing to the structural advantages of polymeric nanoparticles combined with the biocompatibility of lipid-based carriers(4). The hybrid systems consist of a polymeric core that ensures mechanical stability and sustained drug release, surrounded by a lipid shell that increases drug loading, cellular uptake and biological compatibility. These unique properties make LPHNPs promising carriers for phytochemicals and hydrophobic therapeutic agents(5).

Phlorizin is a naturally occurring dihydrochalcone flavonoid predominantly derived from the bark of apple trees and other members of the Malus species(6). It is known for its potent inhibition of sodium-glucose cotransporters SGLT1 and SGLT2 and thus represents an important compound in the research on glucose metabolism. Apart from its antihyperglycemic effects, phlorizin is also an antioxidant, anti-inflammatory and potential anti-senescence agent (7,8). Recent studies have shown that phlorizin can reduce oxidative stress-induced senescence and decrease senescence-associated β-galactosidase activity in diverse cellular models(9).

Regardless of the promising pharmacological activities of phlorizin, poor aqueous solubility, low permeability, rapid metabolism and extremely low oral bioavailability severely restrict its therapeutic application. Phlorizin is classified as a Biopharmaceutics Classification System (BCS) Class IV drug, meaning it has low solubility and low permeability. Such limitations call for the development of advanced delivery systems that can improve their stability, bioavailability, and sustained therapeutic action.

Lipid-polymeric hybrid nanoparticles (LPHNPs) are a promising platform to tackle these problems(10). The encapsulation of phlorizin in the lipid-polymeric matrix may enhance the drug protection, reduce the premature degradation, enhance the cellular uptake and provide controlled release characteristics. Moreover, the nano scale size of LPHNPs could enable enhanced intracellular delivery and prolonged therapeutic activity (11).

In the current study, Phlorizin-loaded lipid-polymeric hybrid nanoparticles were prepared by using a single-step nanoprecipitation method and characterized for physicochemical and in vitro anti-senescence potential. The prepared nanoparticles were characterized for particle size, zeta potential, morphology, entrapment efficiency and drug release profile. Further biological

activities were evaluated against high glucose induced senescence in 3T3-L1 murine adipocytes by SA-β-Gal staining. The present study investigated the potential of Phlorizin-loaded LPHNPs as a next-generation nanocarrier system for treating metabolic stress-induced cellular senescence(12,13).

Therefore, the aim of this study was to develop phlorizin loaded LPHNs by a one-step nanoprecipitation method and to evaluate their physicochemical characteristics and in vitro anti-senescence activity. The prepared nanoparticles were characterized for particle size, zeta potential, morphology, entrapment efficiency and drug release profile. Further biological activities were evaluated against high glucose induced senescence in 3T3-L1 murine adipocytes by SA-β-Gal staining. The present study investigated the potential of Phlorizin-loaded LPHNPs as a next-generation nanocarrier system for treating metabolic stress-induced cellular senescence.

2 Materials and Methods

2.1 Materials

The active therapeutic ingredient was phlorizin. The polymer component was polyvinylpyrrolidone (PVP), the lipid component was soy lecithin, the emulsifier was Tween 80, the organic solvents were ethanol and acetone, and the aqueous phase was distilled water (14). High glucose DMEM medium supplemented with antibiotics and fetal bovine serum was used to cultivate murine 3T3-L1 preadipocytes. 25 mM glucose was used to induce a high-glucose stress. Phlorizin nanosuspension was assessed at 31.25, 62.5, 125, 250, and 500 µg/ml. The positive control was resveratrol. The β-Galactosidase Staining Kit for Senescence was used to assess senescence. Image J software was used to count cells that tested positive for SA-β-Gal.

2.2 Determination of λmax

To prepare a solution of the appropriate concentration for spectrophotometric analysis, phlorizin was carefully weighed and dissolved in ethanol. A UV-visible spectrophotometer was used to examine the solution between 200 and 400 nm. The wavelength associated with maximal absorbance (λmax) was noted.

2.3 Preparation of Calibration Curve

2.3.1 Calibration Curve in Ethanol and Phosphate Buffer (pH 7.4)

An accurately weighed 10 mg of phlorizin was transferred into a 10 mL volumetric flask, and the volume was made up to 10 mL with ethanol to obtain a concentration of 1000 µg/mL. Working standard solutions of concentrations in the range of 10-20 µg/mL were prepared and analyzed at a wavelength of 285 nm. The stock solution of phlorizin was prepared in phosphate buffer (pH 7.4), and serial dilutions were prepared. Absorbance was measured at 323 nm, and a calibration curve was plotted.

2.4 Formulation of Phlorizin-Loaded LPHNPs

A single-step nanoprecipitation method was used for the preparation of phlorizin-loaded lipid polymeric hybrid nanoparticles (LPHNPs)(15,16). The organic phase was prepared by dissolving phlorizin (20 mg), polymer (100 mg), and lipid (40 mg) in ethanol/acetone (1:1, v/v) and sonicated to ensure complete solubilization and homogeneity. To prepare the aqueous phase, Tween-80 (10 mg) was dissolved in distilled water with continuous stirring. The organic phase was then added to the aqueous phase with constant stirring at 700–800 rpm. The rapid diffusion of the ethanol–acetone mixture into water led to spontaneous precipitation of polymers and lipids, forming the nanoparticles. To guarantee that the solvent had completely evaporated, the resulting nanoparticle suspension was agitated for an hour at room temperature. (17)(18).

2.5 Characterization of Formulation

Comprehensive characterization of the developed Phlorizin-loaded lipid–polymeric hybrid nanoparticles was carried out to evaluate their physicochemical properties, structural integrity, surface characteristics, colloidal stability, and suitability as an efficient nanocarrier system.

2.5.1 Particle Size and Polydispersity Index

Particle size and polydispersity index (PDI) are critical parameters influencing nanoparticle stability, drug release, cellular uptake, and biodistribution. The particle size distribution and PDI of the prepared formulation were determined using Dynamic Light Scattering (DLS). The nanoparticle dispersion was appropriately diluted with distilled water to avoid multiple scattering effects and analyzed at 25 °C(19).

The optimized formulation exhibited a Z-average particle size of 425.7 nm with a PDI value of 0.272, indicating relatively uniform particle distribution and acceptable homogeneity. The narrow distribution profile suggested minimal aggregation and effective stabilization of the nanoparticles within the colloidal system. The obtained nanoscale size range is considered suitable for enhanced cellular interaction and sustained drug delivery applications.

2.5.2 Zeta Potential Analysis

The zeta potential and particle size were measured using a laser scattering method (Malvern Nano S90, Malvern Instruments, United Kingdom) at 25 °C and a detection angle of 90°. The samples were diluted with distilled water to get the right scattering strength. Zeta potential analysis was performed to evaluate the surface charge and physical stability of the nanosuspension. Surface charge plays an important role in maintaining colloidal stability by preventing particle aggregation through electrostatic repulsion.

The prepared Phlorizin-loaded LPHNPs showed a zeta potential value of −28.8 mV, indicating good colloidal stability and sufficient repulsive force between nanoparticles. The negative surface charge may be attributed to the presence of lipid and polymeric components in the formulation. The obtained zeta potential value confirms the formation of a stable nanosuspension system with reduced aggregation tendency during storage.

2.5.3 Preliminary Microscopic Examination

The prepared formulation was initially examined under a light microscope to evaluate the general morphology and particle distribution pattern. Microscopic analysis revealed a dense and uniformly distributed nanosuspension with minimal visible aggregation. The particles appeared discrete and well dispersed throughout the medium, indicating successful nanoparticle formation during the nanoprecipitation process.

2.5.4 Scanning Electron Microscopy (SEM)

The surface morphology of the phlorizin-LPHNPs was investigated. Surface morphology and shape were analyzed by scanning electron microscopy (JSM 6490 LV, JEOL, Japan). The samples were gold-sputter-coated, and the morphology was characterized at a high magnification of 1.02 KX with an acceleration voltage of 15.0 kV. The nanoparticle dispersion was mounted on an aluminum stub, air-dried, and observed under appropriate magnification.

SEM micrographs revealed the presence of solid, discrete, and well-defined nanoparticles with smooth surface morphology. The particles exhibited nanoscale dimensions ranging from 119.7 nm to 334.1 nm. The observed morphology confirmed successful encapsulation and formation of lipid–polymeric hybrid nanoparticles. The absence of significant particle aggregation further supported the stability and homogeneity of the formulation.

2.5.5 Stability Assessment of Phlorizin-Loaded LPHNPs

Stability assessment is an essential parameter in nanoparticle formulation development as it determines the ability of the nanosystem to maintain its physicochemical properties during storage. The stability of the prepared Phlorizin-loaded LPHNPs was primarily evaluated based on particle size distribution, polydispersity index, zeta potential value, and visual appearance of the nanosuspension. The optimized formulation showed good physical stability with no visible signs of phase separation, precipitation, or sedimentation. The nanosuspension maintained its characteristic homogeneous opalescent appearance throughout the observation period. The zeta potential value of −28.8 mV provided sufficient electrostatic stabilization, thereby minimizing particle aggregation and enhancing colloidal stability.

The relatively low PDI value further indicated uniform particle distribution and reduced instability associated with particle clustering. The lipid–polymeric hybrid architecture also contributed significantly to formulation stability by combining the mechanical strength of the polymeric core with the biocompatibility and protective properties of the lipid shell. +The sustained release profile obtained during in vitro drug release studies additionally confirmed the structural integrity and stability of the nanoparticles. The formulation successfully retained the drug within the lipid–polymeric matrix and demonstrated controlled release over 20 hours without evidence of rapid burst release or premature drug leakage.

Overall, the characterization and stability studies confirmed that the developed Phlorizin-loaded LPHNPs possessed suitable physicochemical properties, excellent colloidal stability, and structural integrity, making them a promising nanocarrier system for therapeutic delivery.

2.6 Evaluation

2.6.1 Entrapment Efficiency

The ultrasonication method was used to assess the encapsulation efficiency of phlorizin-LPHNs. In short, a cooling centrifuge was used to spin 5 mL of the prepared LPHN suspension at 10,000 RPM for 1 hour at 10 °C. After centrifugation, particulate matter was removed by carefully decanting the supernatant and passing the remaining liquid through Whatman filter paper. A double-beam UV-Vis spectrophotometer (Model: GI 5700, Genius) was used to measure the concentration of free (unentrapped) phlorizin at 285 nm after the cleared supernatant had been suitably diluted with distilled water. The following formula was then used to determine the percentage encapsulation efficiency (% EE):

$$\% EE = \frac{Total\ drug\ amount - Free\ drug\ amount}{Total\ amount} \times 100$$

2.6.2 *In Vitro* Drug Release Study

The dialysis bag method was used to evaluate Phlorizin LPHNPs' in vitro drug release. To put it briefly, 5 mL of the nanoparticle formulation was added to a dialysis bag after one end was secured with a thread. The other end was knotted and placed in a beaker filled with 50 milliliters of pH 7.4 phosphate buffer. A magnetic stirrer set to 37 ± 0.5 °C was used to hold the beaker containing the dialysis bag containing the formulation. To maintain sink conditions, a sample (2 mL) was removed at predetermined intervals and replaced with an equivalent volume of buffer (pH 6.8) in the beaker. A UV-Vis spectrophotometer was used to quantify the phlorizin content at 285 nm (20,21).

2.6.3 *In Vitro* Anti-Senescence Evaluation

The anti-senescence potential of phlorizin-loaded lipid–polymeric hybrid nanoparticles (F1) was evaluated in an in vitro model of high glucose-induced cellular senescence in 3T3-L1 murine pre-adipocytes(22). The study was designed to assess the potential of the test nanoparticles to reduce glucose-induced senescence, which was assessed by Senescence-associated β-galactosidase (SA-β-Gal) staining. 3T3-L1 cells were cultured in high-glucose DMEM supplemented with 10% FBS and 1% antibiotic-antimycotic under standard conditions (37°C, 5% CO2). Cells were treated with 25 mM glucose to induce cellular senescence. The phlorizin-loaded LPHNPs (F1) were used at five different concentrations (31.25, 62.5, 125, 250, and 500 µg/ml) along with the senescence inducer for 24 h. Controls consisted of untreated cells (negative control) and cells treated with 25 µM resveratrol (positive control) plus 25 mM glucose. Cells were fixed and stained with a Senescence β-Galactosidase Staining Kit after treatment. The percentage of SA-β-Gal positive cells was measured, and the morphological changes were observed by an inverted microscope(23).

2.7 Tools and Software

Numerous virtual laboratory environments, including Swiss ADME and ADMETlab 3, have been assigned to offer the

pharmacokinetic profiles of therapeutically active drug choices. ADMETlab 3 was selected because it can predict a number of drug-likeness metrics and most ADME features(24). Based on the physicochemical factors, Swiss ADMET displayed an oral bioavailability graph. The graph employs six characteristics with predetermined ranges that promote oral bioavailability: size, unsaturation, lipophilicity, insolubility, polarity, and flexibility.

3. Results and Discussion

3.1 Determination of λmax

The UV-Visible spectrophotometric scan of Phlorizin exhibited a distinct absorption peak at 285 nm in ethanol (Fig.1). The identification of λmax at 285 nm is characteristic of the dihydrochalcone structure of Phlorizin and was used for further analytical measurements.

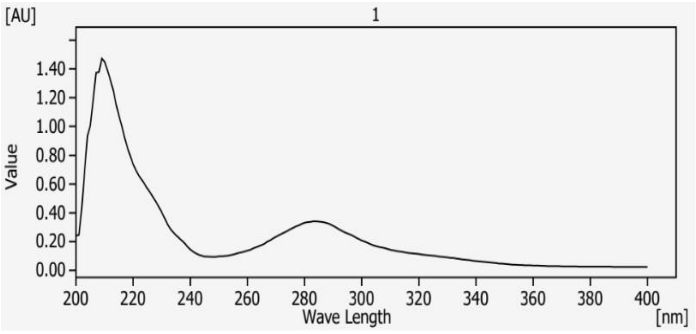


Fig 1. UV spectra of the phlorizin drug in ethanol

3.2 Calibration Curve Studies

The calibration curve in ethanol demonstrated a linear relationship between concentration and absorbance (Fig. 2). Similarly, the calibration curve in phosphate buffer (pH 7.4) (Fig. 3) exhibited satisfactory linearity suitable for in vitro release studies.

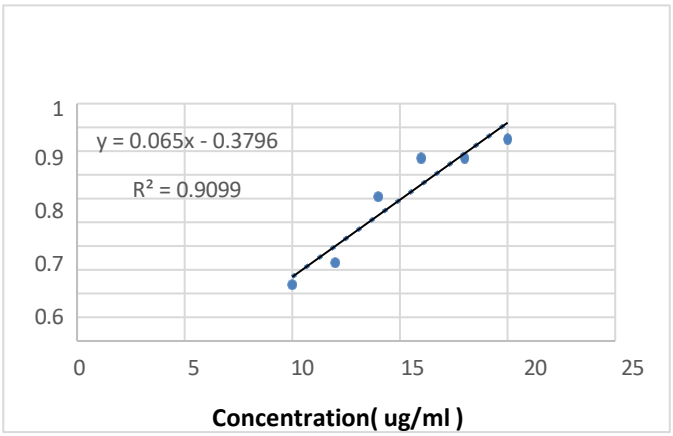


Fig 2. Calibration curve of Phlorizin in Ethanol

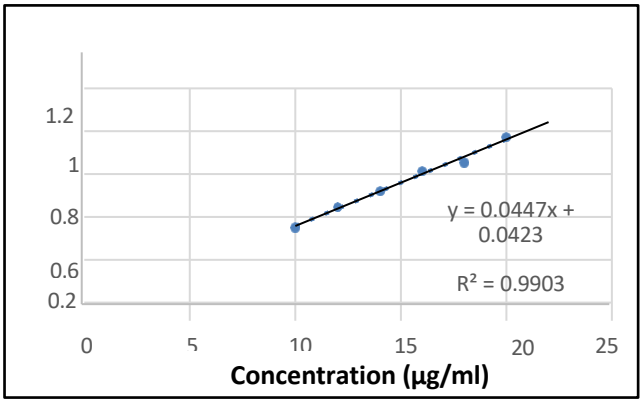


Fig 3. Calibration curve of Phlorizin in Phosphate buffer (pH 7.4)

3.3 Formulation of Phlorizin LPHNPs

Phlorizin-loaded LPHNPs were synthesized using a single-step nanoprecipitation technique. The formulation formed a homogeneous opalescent dispersion with a bluish-white tint, indicating stable colloidal nanoparticles. A drug, polymer and lipid ratio of 1:5:2 was essential for structural integrity and drug encapsulation.

3.4 Particle Size and Polydispersity Index

With a Z-average diameter of 425.7 nm and a PDI of 0.272, dynamic light scattering examination demonstrated adequate homogeneity and little aggregation. The median particle size was indicated by the D50 value of 241.30 nm, which demonstrated appropriateness for drug delivery activities (Table 1) (Fig. 4). Percentile distribution of the prepared nanoparticles were represented in Table 2.

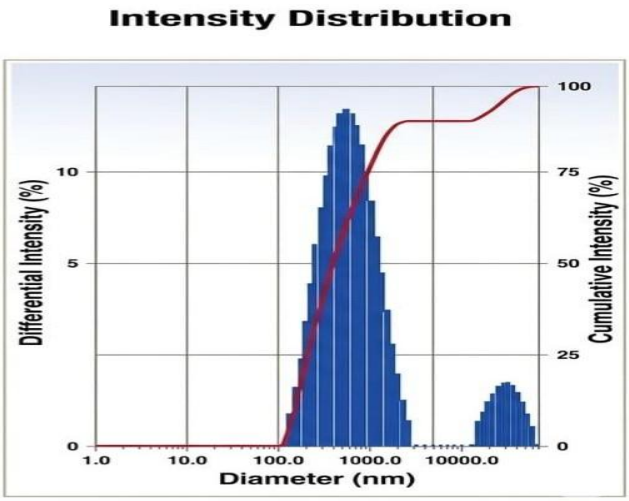


Fig 4. Particle Size Distribution

Table 1. Particle Size Parameters by DLS

Parameter	Observed Value
Z-Average Diameter	425.7 nm
Polydispersity Index (PDI)	0.272
Diffusion Constant (D)	1.155×10^{-8}
| Scattering Intensity	28690 cps
Temperature	25.0°C

Table 2. Percentile Distribution of Particle Size

Percentile	Diameter (nm)
D10	126.70
D50	241.30
D90	2467.20

3.5 Zeta Potential Analysis

Strong electrostatic repulsion and good physical stability of the nanosuspension were shown by the zeta potential of −28.8 mV (Fig.5). The negative surface charge may be attributed to ionizable functional groups on the nanoparticle surface from the lipid and polymeric components. The observed zeta potential value reflects adequate electrostatic repulsion among the nanoparticles, which effectively prevents particle aggregation and ensures homogeneous dispersion throughout the formulation. Because of increased repulsive interactions between particles, nanoparticle systems with zeta potential values larger than ±25 mV are generally regarded as physically stable. The stability of the produced LPHNPs is thus confirmed by the measured value of −28.8 mV, which also shows that they can stay distributed for a long time without experiencing major agglomeration. The superior stability offered by the optimized zeta potential plays a critical role in preserving particle integrity, extending shelf life, and ensuring reproducible drug release performance. Collectively, the zeta potential data confirm the potential of the developed nanoparticulate system as a reliable and stable platform for sustained therapeutic delivery.

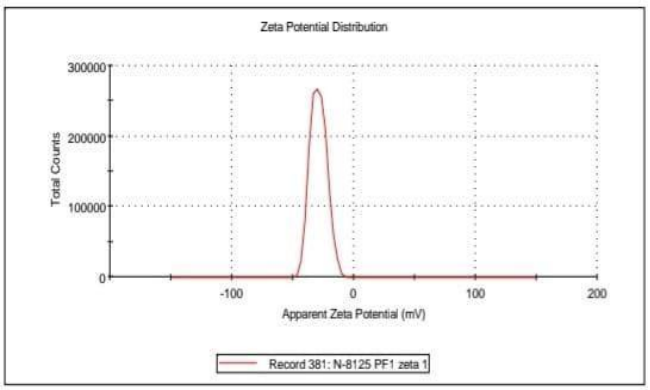


Fig 5. Zeta potential distribution

3.6 Microscopic and SEM Analysis

Light microscopic examination revealed a dense and highly uniform distribution with minimal aggregation (Fig. 6). SEM analysis of Phlorizin-loaded lipid polymer hybrid nanoparticles revealed nanosized particles with predominantly spherical to irregular morphologies. The nanoparticles were slightly agglomerated.

This can be explained by interparticle interactions during the drying of the sample. The particle size ranged from 119–334 nm, with an average diameter of 233 nm (Fig. 7). The particles exhibited relatively smooth surfaces with no distinct crystalline structures, indicating successful entrapment of Phlorizin within the lipid–polymer matrix. The nano-scale size and morphology indicate that the formulation can be used for improved drug delivery applications.

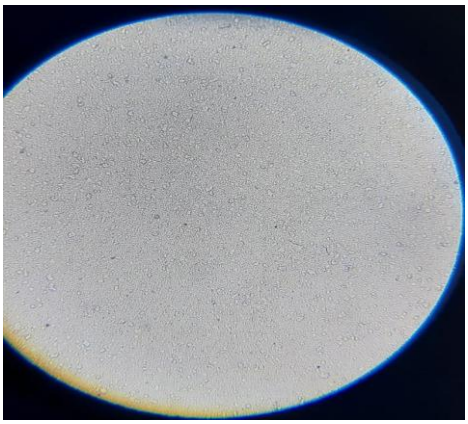


Fig 6. Light microscopic image of Phlorizin LPHNPs

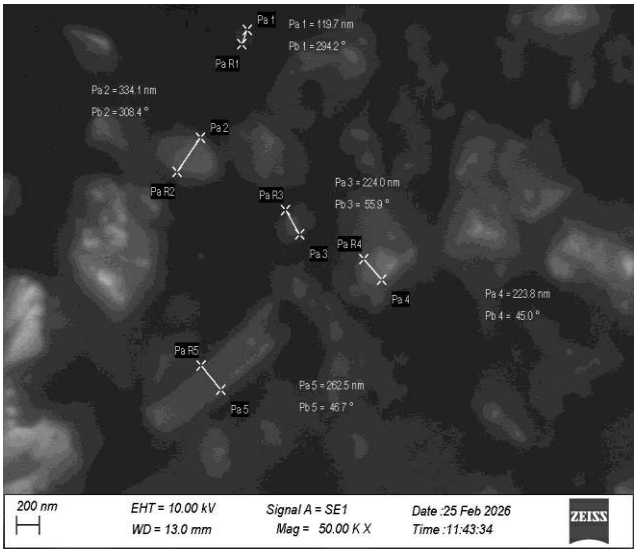


Fig 7. SEM image of the Phlorizin LPHNPs

3.7 Entrapment Efficiency

The formulation showed an extremely high entrapment efficiency (EE) of 99.62%, indicating the excellent potential of the lipid-polymeric hybrid nanoparticle (LPHNP) system to entrap the drug in its matrix. Such high EE suggests that the drug is highly compatible with the lipid–polymeric components, which could be due to the presence of favorable hydrophobic interactions and the drug's ability to efficiently partition into the carrier system during formulation. The optimized lipid–polymer composition likely limited drug leakage and prevented drug diffusion into the external aqueous phase during preparation.

The entrapment of drugs is almost complete which is good in terms of improving therapeutic performance as it offers maximum drug loading and minimum wastage of active pharmaceutical ingredient. Moreover, high entrapment efficiency is beneficial for sustained drug release behavior, improved stability and bioavailability of poorly soluble compounds like phlorizin. The selected preparation method was also efficient in generating a stable nanosystem with homogeneous drug distribution. The overall obtained value of EE confirms the suitability of the developed LPHNP formulation as a successful carrier for drug delivery applications.

3.8 In Vitro Drug Release Study

Phlorizin-loaded LPHNPs showed a biphasic release pattern in the in vitro drug release investigation, with a regulated initial release and a sustained release over a 20-hour period. Surface-associated drug molecules may be responsible for the release of about 15.97% of the drug over the first two hours. Effective prolonged release behavior was demonstrated by the formulation's 53.8% drug release at 10 hours and full release at 20 hours. Phlorizin's penetration through the lipid-polymer matrix and the slow deterioration of the carrier system could be the cause of the sustained release profile. A consistent and regulated drug release pattern independent of drug concentration was suggested by the release kinetics' excellent connection with zero-order kinetics. These results show that the created LPHNPs are appropriate for long-term therapeutic effects in sustained drug delivery applications (Fig. 8).

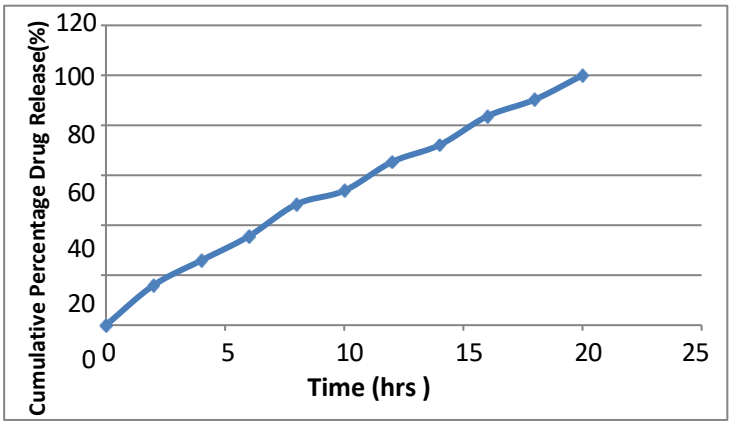


Fig 8. Plot of Cumulative Percentage Drug Release (%) vs. Time (hrs)

3.9 In Vitro Anti-Senescence Evaluation

High glucose (25 mM) treatment significantly increased the number of SA-β-Gal-positive senescent cells compared to the untreated control, confirming successful induction of premature senescence. Co-treatment with phlorizin-loaded LPHNPs (F1) resulted in a clear, dose-dependent reduction in the percentage of β-galactosidase-positive cells. The inhibitory effect became more pronounced with increasing concentrations, with the highest tested concentration (500 µg/ml) showing the strongest reduction in senescence markers. The nanoparticles demonstrated comparable or superior activity relative to the resveratrol positive control at several concentrations. Phlorizin-loaded lipid–polymeric hybrid nanoparticles (F1) effectively attenuated high glucose-induced cellular senescence in 3T3-L1 pre-adipocytes in a dose-dependent manner. These findings indicate that the nanoformulation of phlorizin retains and potentially enhances its anti-senescence properties, making it a promising candidate for further development in managing senescence-associated conditions linked to metabolic stress. Further studies on molecular mechanisms, long-term efficacy, and in vivo translation are warranted. Microscopic examination revealed that glucose-treated cells exhibited typical senescent morphology, including enlarged and flattened cell shapes. Treatment with F1 noticeably improved cellular morphology, with cells appearing healthier and less senescent in a concentration-dependent manner (Fig. 11).

Table 7. Percentage of SA-β-Gal-positive 3T3-L1 cells

Group	Mean ± S.E.M. (%)
Untreated (UTD)	3.73 ± 0.85
Glucose 25mM	78.40 ± 1.90
RSV 25µM	5.28 ± 0.17
LPNS 31.25 µg/ml	52.36 ± 0.25
LPNS 62.5 µg/ml	51.08 ± 0.21
LPNS 125 µg/ml	43.49 ± 0.22
LPNS 250 µg/ml	6.92 ± 0.17
LPNS 500 µg/ml	3.14 ± 0.26

Treatment with high glucose (25 mM) dramatically increased the number of SA-β-Gal-positive senescent cells compared with the untreated control, indicating that premature senescence was successfully induced. The percentage of β-galactosidase-positive cells was markedly reduced in a dose-dependent manner after co-treatment with phlorizin-loaded LPHNPs (F1). As concentrations increased, the inhibitory impact became more noticeable; the greatest decline in senescence markers was observed at the highest tested concentration of 500 µg/ml. At various doses, the nanoparticles showed similar or better efficacy than the resveratrol positive control. In 3T3-L1 pre-adipocytes, phlorizin-loaded lipid–polymeric hybrid nanoparticles (F1) successfully and dose-dependently reduced high glucose-induced cellular senescence (Table 7).

These results suggest that phlorizin's anti-senescence capabilities are preserved and may be enhanced in its nanoformulation, making it an appropriate choice for future research and development in the treatment of senescence-related disorders associated with metabolic stress. It is necessary to conduct additional research on in vivo translation, long-term effectiveness, and molecular pathways. Under a microscope, glucose-treated cells exhibited typical senescent morphology, including flattened, expanded cell shapes. Cells treated with F1 showed a concentration-dependent improvement in cellular shape, looking healthier and less senescent (Fig. 10).

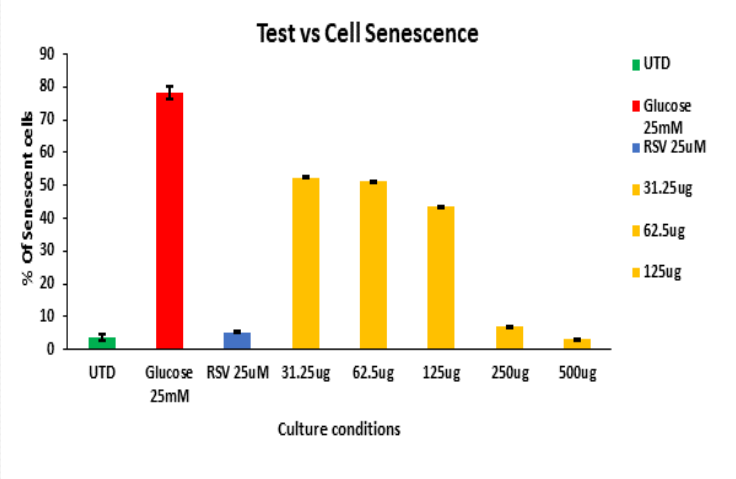


Fig 10. Mean % 3T3L1 cells positive for β-Galactosidase Stain after exposure to the test compound and controls for 24 hours.

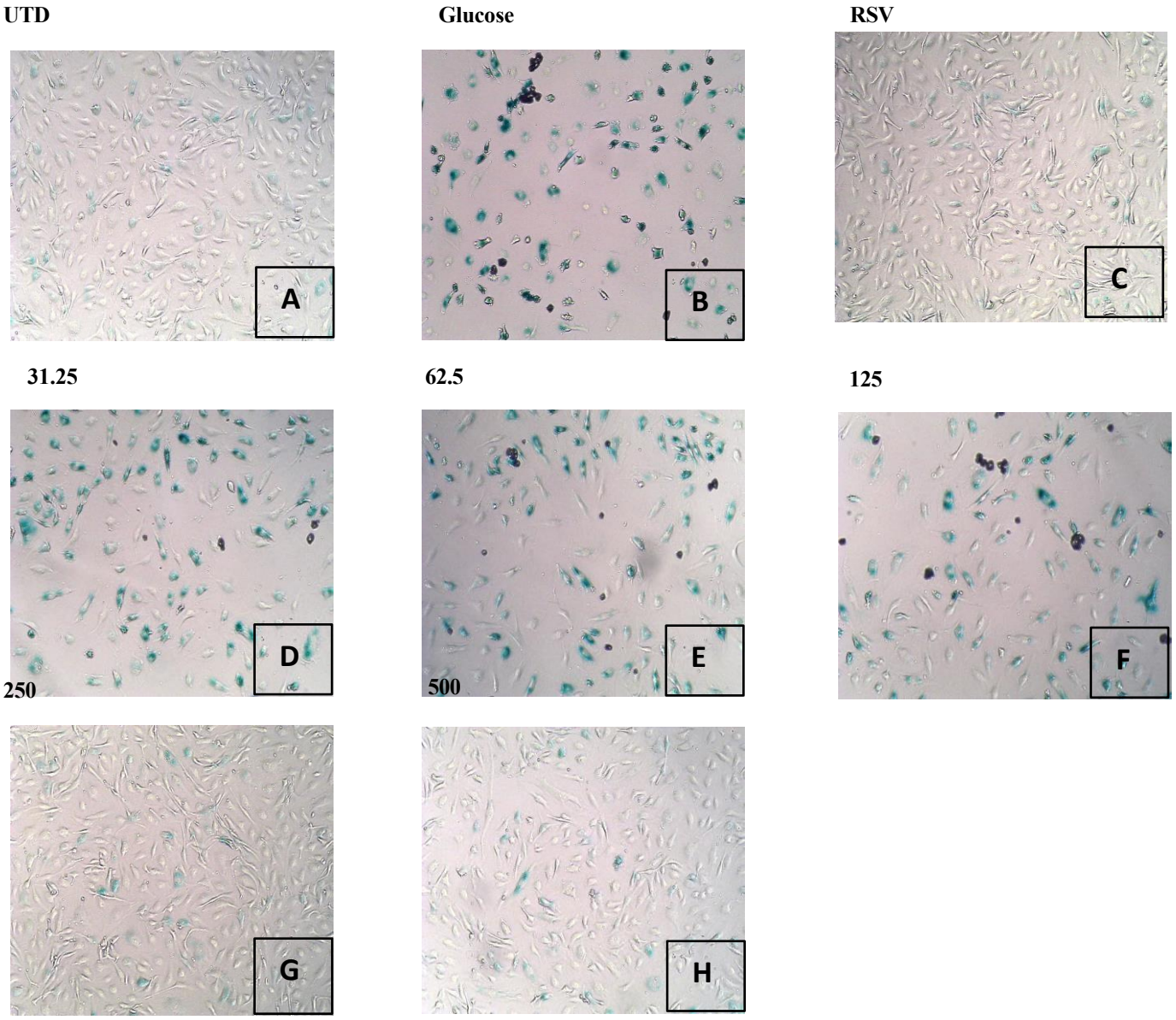


Fig 11. Phase-contrast micrographs (20×) of 3T3-L1 cells showing dose-dependent morphological protection by phlorizin nanosuspension (PNS) against high glucose (25 mM)-induced senescence. (A) Untreated: normal fibroblast-like morphology. (B) Glucose: enlarged, flattened senescent cells (arrows). (C) RSV 25 µM + Glucose: morphological rescue. (D-H) PNS 31.25-500 µg/ml + Glucose: progressive recovery, complete at 250-500 µg/ml. Scale bar = 100 µm.

4.Analysis of Pharmacokinetic Properties of Phlorizin LPHNs

The SwissADME and ADMETLab 3.0 online prediction systems were used to assess Phlorizin's pharmacokinetic and drug-likeness characteristics of Phlorizin. The analysis provided a comprehensive assessment of its physicochemical properties, lipophilicity, solubility, pharmacokinetic behavior, and medicinal chemistry profile. Phlorizin demonstrated low lipophilicity, with a consensus Log P value of 0.06, high polarity indicated by a TPSA value of 177.14 Å², and a molecular weight of 436.41 g/mol. Although Phlorizin is predicted to be water-soluble, it exhibits poor gastrointestinal absorption and lacks permeability across the blood-brain barrier (BBB). According to the BOILED-Egg model, the compound's high polarity and elevated TPSA value position it outside the optimal range for both gastrointestinal absorption and BBB penetration (Fig. 12).

Phlorizin was also found to be affected by P-glycoprotein (P-gp), which could cause it to remain less inside cells and reduce the amount absorbed when taken by mouth. Also, it did not block important CYP450 enzymes, which means it is less likely to interfere with other drugs. The absence of PAINS and Brenk alerts showed it has a good chemistry and safety profile.

Overall, Phlorizin showed poor ability to pass through membranes and limited absorption even though it dissolves well in water and has acceptable safety features. These findings support developing lipid–polymer hybrid nanoparticles to improve how well it is absorbed and delivered as medicine. The radar plot results agree with earlier studies showing Phlorizin's low permeability and poor stomach absorption (Fig. 13). Therefore, enhancing drug encapsulation, permeability, and sustained drug release through the incorporation of Phlorizin into lipid–polymeric hybrid nanoparticles (LPHNPs) may address these limitations. The physicochemical analysis supports the development of a nanoparticle-based delivery system to improve the therapeutic efficacy of Phlorizin. Summary of pharmacokinetic properties of phlorizin analyzed by ADMET Lab 3.0 were depicted in Table 8.

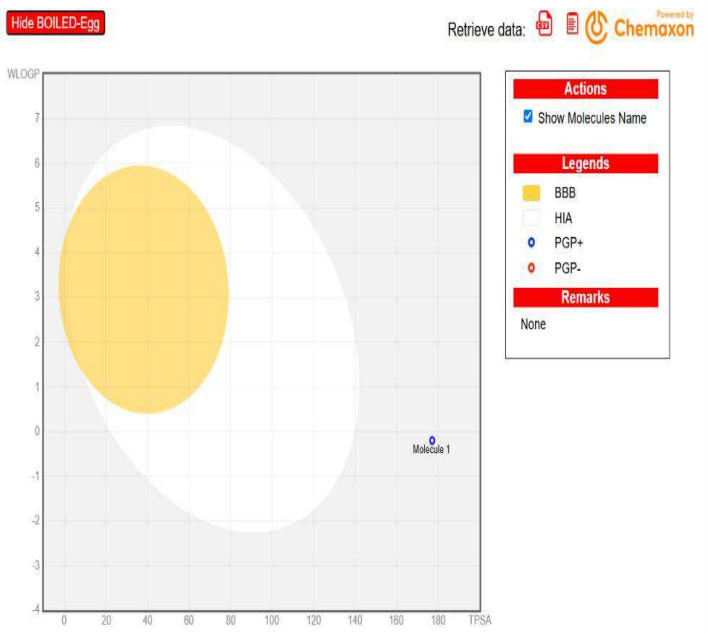


Fig.12 Boiled Egg model of Phlorizin



Fig.13 SWISSADME Radar of Phlorizin

Table 8: Summary of pharmacokinetic properties of phlorizin analyzed by ADMETLab 3.0

Category	Parameter	Observed Data
General Information	Molecule Name	Phlorizin
	Molecular Formula	$C_{21}H_{24}O_{10}$
	Molecular Weight	436.41 g/mol
Physicochemical Properties	Heavy Atoms	31
	Aromatic Heavy Atoms	12
	Fraction Csp3	0.38
	Rotatable Bonds	7
	H-Bond Acceptors (HBA)	10
	H-Bond Donors (HBD)	7
	Molar Refractivity	106.14
	TPSA	177.14 Å²
Lipophilicity	iLOGP	1.25
	XLOGP3	0.54
	WLOGP	-0.20
	MLOGP	-1.42
	SILICOS-IT LogP	0.13
	Consensus LogP	0.06
Water Solubility	Log S (ESOL)	-2.71
	ESOL Solubility	8.50e-01 mg/ml ; 1.95e-03 mol/l
	ESOL Class	Soluble
	Log S (Ali)	-3.83
	Ali Solubility	6.43e-02 mg/ml ; 1.47e-04 mol/l
	Ali Class	Soluble
	Log S (SILICOS-IT)	-1.66
	SILICOS-IT Solubility	9.59e+00 mg/ml ; 2.20e-02 mol/l
	SILICOS-IT Class	Soluble
Pharmacokinetics	GI Absorption	Low
	BBB Permeation	No
	P-gp Substrate	Yes
	CYP1A2 Inhibition	No

	CYP2C19 Inhibition	No
	CYP2C9 Inhibition	No
	CYP2D6 Inhibition	No
	CYP3A4 Inhibition	No
	Skin Permeation (Log Kp)	-8.58 cm/s
Drug-likeness	Lipinski Rule	Yes; 1 violation (NHorOH >5)
	Ghose Filter	Yes
	Veber Rule	No; 1 violation (TPSA >140)
	Egan Rule	No; 1 violation (TPSA >131.6)
	Muegge Rule	No; 2 violations (TPSA >150, H-don >5)
	Bioavailability Score	0.55
Medicinal Chemistry	PAINS Alert	0 alert
	Brenk Alert	0 alert
	Lead-likeness	No; 1 violation (MW >350)
	Synthetic Accessibility	4.93

5. Conclusion

Phlorizin-loaded LPHNPs, an innovative nanocarrier technology for a long time therapeutic applications, were effectively created and thoroughly characterized in the current study. Positive physicochemical properties of the improved formulation included excellent entrapment efficiency, strong colloidal stability, nanoscale particle size, and a sustained drug release profile lasting up to 20 hours. The formulation's capability to sustain extended and reliable drug availability is demonstrated by the regulated release behavior, which adhered to zero-order kinetics.

Additionally, the biological evaluation in 3T3-L1 murine adipocytes demonstrated significant dose-dependent attenuation of high glucose-induced cellular senescence, highlighting the potential protective effect of the developed formulation against glucose-mediated cellular damage. The enhanced therapeutic performance may be attributed to improved drug encapsulation and sustained release provided by the lipid–polymer hybrid matrix.

In conclusion, these results indicate that the practical approach for preparing phlorizin-loaded LPHNPs may enhance drug delivery and help prevent glucose-induced adipocyte senescence. Nevertheless, additional in vivo studies and comprehensive evaluations are required to validate the anti-senescence effects, pharmacokinetics, and therapeutic relevance of this nanoparticulate system.

Author Contributions

Salamath Nisha B- SNB, Gandhimathi R- GR, Indumathi P - IP, Karthikeyan Elumalai- KE, Marina Juliet A- MJA, Vijayaraj Surendran- VS, Gnanamithra Thinakaran -GT, and Joel Mart E- JME.

VS and SNB: Conceptualization, Methodology, Investigation, Data Curation

MJA, IP and KE: Formal Analysis, Writing – Original Draft.

GR, GT, and JME: Methodology, Formal Analysis, Validation, Drug-Likeness Prediction, Writing – Review & Editing, Investigation, Validation, Data Curation

SNB and KE: Computational Studies, Visualization, Software, Investigation, Writing – Review & Editing.

GT and IP: Supervision, Resources, Project Administration, Writing – Review & Editing

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data will be provided on reasonable request.

Ethics declaration:

Not applicable.

Reference:

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