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Luteolin loaded liposomes shows anti-psoriatic activity in mice tail model for psoriasis

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Management for psoriasis, a chronic inflammatory skin disease, is complicated. The goal is to investigate how luteolin-loaded liposomes might be used to create a novel psoriasis treatment strategy. In this current research, luteolin loaded liposomes (LLL) were prepared by using ether injection method. The formulation was characterized with UV-Visible spectroscopy, ATR-FTIR, DSC, measurement of particle size, zeta potential, and poly dispersity index. Moreover, wavelength at absorbance maxima (λ-max) of luteolin in a phosphate buffer solution with pH value 7.4 was determined. The *in vivo* anti-psoriatic activity was determined in a mouse tail model. The development of a calibration curve made direct measurement in UV-Vis possible. The presence of groups such as carboxyl, amine, esters, hydroxyl, nitrile, and nitro were determined in the ATR-FTIR. DSC is indeed used to evaluate the interactions and thermal stability of the compounds. In this current study, in the characterization of liposomes the particle size has a great impact. The optimal zeta potential of -20.8 mV is because of dissociation of carboxylic acid groups on the surface of cholesterol nanoparticles and it varies depending on the formulation. If nanoparticles are homogeneous, the polydispersity index (PDI) will be typically less than 0.351. In *in vivo* model for psoriasis, topical application of LLL resulted in significant reduction of psoriatic symptoms such as scaling, redness, and overall disease severity. The results demonstrate the potential of LLL for the management of psoriasis.

Keywords: Liposomes, Luteolin, Mice tail model, Psoriasis

Psoriasis, majorly affecting the skin, is a chronic autoimmune disease. It is majorly caused by T lymphocytes majorly associating with elevated levels of phosphorylated NF-κB, that has an important role in keratinocyte dysfunction and responses from the immune system[1]. Despite its widespread incidence, accurate data about its occurrences are difficult to procure as there is lack of mandatory case reporting. As per the studies done previously approximately 3% of the population worldwide are affected with psoriasis[2]. Studies claim that different genes play important roles in regulation of the immune system and growth of the skin cells, as both are significant in the development of the disease[3]. However, this disease is multi-factorial, with factors like environment, weather, stress, infections, and skin injuries often acing as triggers and exacerbating the symptoms. There are certain medications, like, lithium, beta-blockers, antimalarials, tetracyclines, and non-steroidal anti-inflammatory drugs, are also associated with the onset and psoriasis worsening. Generally, it is in five forms, with plaque psoriasis, or psoriasis vulgaris is the most common.

Luteolin is a flavonoid that is found in various tea, fruits, and vegetables. Its effective anti-inflammatory, antioxidant, and antimutagenic properties have created interest as a promising therapeutic candidate for psoriasis[4]. Study shows that luteolin can inhibit the activation of human keratinocytes and regulate NF-κB signalling. It particularly suppresses TNF-induced IL-6, IL-8, and VEGF production in keratinocytes and HaCaT cells. Additionally, it regulates gene expression of RELA and disrupts the NF-κB pathway by limiting phosphorylation, DNA binding, and nuclear translocation. And, it blocks IL-6-driven Th17 cell maturation and selectively reduces proliferation of HaCaT cell[5]. Its lipid solubility enhances its potential for topical formulations targeting psoriasis.

The disadvantages of traditional topical therapies have been solved with modern drug delivery systems

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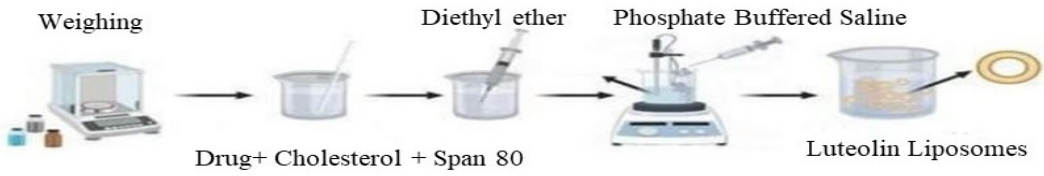

Graphical abstract

including restriction of drug penetration and dose-dependent toxicity. Along with the advanced drug delivery system nanoparticles, polymeric nanoparticles, lipid-based nanoparticles, and vesicular nanoformulations, including liposomes, niosomes, ethosomes, transfersomes, and invasomes, have shown significant potential[6]. A recent research developed a gel with curcumin-loaded polymeric nanoparticles for transdermal application. The formulation has achieved an entrapment efficiency of 78.45% and showed long-term skin retention, resulting in substantial therapeutic effects in a psoriatic mouse model.

Liposomes are vesicles of nanoscale with one or more lipid bilayers surrounding a hydrophilic core. It is composed primarily of phospholipids, cholesterol, and sometimes surfactants, ranging from a few to hundreds of nanometers, making them ideal for biomedical applications, specifically drug delivery. Their composition exhibits drug-loading capacity, stability, and biological interactions. Surface charges like neutral, positive, or negative, affects cellular uptake and bio distribution. Because of their biocompatibility and ability to encapsulate both hydrophobic and hydrophilic molecules, liposomes can enhance bioavailability, drug protection, and therapeutic efficacy. Ligand and PEGylation attachment further optimize their targeting capabilities and circulation time[7]. They serve multiple functions, primarily as drug carriers that protect therapeutics from enzymatic degradation and enhance absorption. They enable targeted drug delivery, decreasing the side effects and increasing efficacy. Beyond drug transportation, liposomes are used in imaging by encapsulating contrast agents, in vaccines to enhance immune responses, and also in gene therapy as vectors for DNA or RNA. In cosmeceuticals, they boost the absorption of bioactive ingredients. Their versatility makes liposomes valuable in pharmaceuticals, diagnostics, regenerative medicine, and skincare[8].

Liposomes have exhibited promising future in enhancing transdermal drug delivery for psoriasis. The celastrol-loaded liposomes increase in-vitro permeability and effectively decrease redness and scaling in psoriatic mouse models by improving celastrol's water solubility and skin penetration[9]. Likewise, there is a study that developed a liposomes gel for cyclosporine delivery, that achieved significantly higher permeability (50.57%) compared to a cyclosporine suspension (10.13%) in ex-vivo rat skin studies. These results conclude the promise of liposomes and other innovative formulations in enhancing drug delivery and therapeutic effectiveness for psoriasis treatment[10]. Topical therapies are the most important choice for psoriasis treatment due to their low toxicity and increased patient compliance.

Materials and Methods

Chemicals and reagents

Luteolin powder, freshly prepared, is ready for use at BLD Pharmatech (India) Pvt. Ltd., located at Hyderabad, Telangana, India. Cholesterol is available at Central Drug House (P) Ltd. Sorbitan monooleate (SPAN 80®), extra pure grade and Ethyl alcohol (Diethyl Ethyl hydroxide, 99.5%, extra pure AR) are manufactured by Sisco Research Laboratories Pvt. Ltd., Mumbai, Maharashtra, India. Methanol is manufactured by SD Fine Chemicals Ltd., located at Mumbai, Maharashtra, India. Additional use of manufacturing methods helps to improve the quality of the analysis.

Preparation of luteolin liposomes

Luteolin-loaded liposomes were formulated using a modified injection technique[11], where a nonionic surfactant (Span 80) and cholesterol were added in different ratios. The process started with dissolving cholesterol and surfactant in 6 mL of diethyl ether, followed by the addition of 2 mL of methanol with a premeasured amount of luteolin. The organic phase was subsequently introduced into 15 mL of phosphate buffer (pH 7.4) with regulated flow rate of 1 mL/min under ambient hydration conditions.

After injection, the dispersion was continuously stirred with a magnetic stirrer, while the temperature

was maintained between 60°C and 65°C for 10 min to promote proper liposome formation.

Characterization of liposomes

The pharmaceutical properties of liposomes are prepared for evaluation, considering size distribution, surface charge, dispersion index, infrared spectroscopy, and ultraviolet analysis.

Particle size, zeta potential, polydispersity index

Dynamic light scattering (DLS) with the Anton Paar Litesizer 500 was used to analyze the size of liposomal particles (nm), surface charge (mV), and polydispersity index (PDI). At 633 nm wavelength, 173° detection angle, 1.33 refractive index and 0.8872 cP viscosity under controlled temperature of 25°C the measurements were taken. The samples were diluted at 1:1,000 ratio with Milli-Q water before analysis. The measurement of particle size was performed in triplicate and the data was presented in the form mean ± range (nm). Zeta potential evaluations were repeated three times, with values reported as mean ± standard deviation (mV)[12].

Fourier- transform infrared spectroscopy (FT-IR)

The spectra of FT-IR of cholesterol, luteolin, and luteolin liposomes were analyzed at a temperature (25 ± 1)°C using a standard detection method with a resolution of 2 cm^{-1} (Bruker FTIR Alpha-II, DST PURSE).A diamond crystal is placed at an angle of 45° using the internal reflection method. The spectrum is overexposed and the average of the 32 scans has a resolution of 21. Corrections of the spectral elements are applied to the surface of wavenumbers 4000 to 400 cm^{-1} and a selection process for the analysis of the band. Image adjustment is the result of Opus logic.

Differential scanning calorimetry (DSC)

The differential scanning method was performed using DSC-Q 200 (AICTE-NAFETIC) model. The system includes a Thermal Analyzer (TA-60), a heat detector (DSC-60), and a Flow Controller (FCL-60), with regulation managed by NETZSCH Proteus thermal software.

Determination of λ_{max} of Luteolin

By dissolving 10 mg of luteolin in 10 mL of Phosphate Buffered Saline (PBS) at apH of 7.4, luteolin stock solution was prepared resulting in an initial concentration of 1000 µg/mL. To obtain a working solution (100 µg/mL), 1 mL of this stock was diluted with PBS to a total volume of 10 mL. Subsequently, 1 mL of 100 µg/mL solution was further diluted in 10 mL of PBS, achieving a final luteolin concentration of 10 µg/mL. The maximum absorption wavelength (λ_{max}) of this final solution was analyzed using a UV-Visible spectrophotometer (Lab India UV/Vis Spectrometer UV 3000, DST-BEURS) in the wavelength range of 200–400 nanometer.

Preparation of Luteolin standard curve

Dilute this solution to prepare concentrations of 10, 20, 30, 40 and 50 µg/mL. Ensure accurate dilution by using the correct volumes and calculations. Measure the pH of each prepared solution using a pH meter or pH indicator strips. Adjust as necessary to maintain the pH at 7.4 for all concentrations. Set the UV-visible spectrophotometer to λ_{max} of 261 nm.

Rinse the spectrophotometer with PBS solution to zero the instrument. Measure the absorbance of each prepared PBS solution at 261 nm. Record the absorbance values for each concentration. Be sure to record any adjustments made during the process to maintain the pH at 7.4.

Experimental animals

The study was conducted at JSS College of Pharmacy, Ooty. The Institutional Animal Ethical Committee (IAEC) approval number for the conduct of this study is JSSCP/OT/IAEC/42/23-24. The animals used for this experiment are mature and healthy male albino mice, with a weight of 25-30 grams and no visible problems. The animals were fed a standard laboratory diet and had unlimited access to drinking water. This is the first step in the ventilation and temperature control process and the first adjustment period for acclimatization to laboratory conditions before the start of the experiment.

Mice tail model for psoriasis

The tail test, developed by Jarrett and Spearman, is, depending on the nature of the techniques, a sensitive and very reliable morphometric method. It allows to evaluate the quantitative effects of antipsoriatic treatments on the difference between epidermal processes and changes in the psoriatic process[13].

Rationale

This is the basis for the induction of orthokeratosis in areas of the adult row with normal differences in parameters.

Method

Eighteen healthy adult male Wistar albino mice, each weighing about 25 to 30 grams and free from significant skin lesions, were selected for the study.

The characteristics, according to reference standards, herbal extracts, and formulations, are established by the local authority during the next 14 days. All the tested samples were dissolved in water before application. After 14 days of application, the process of sacrificing was done with anesthesia. Each set of queue samples were stored in individual containers filled with a 10% premol solution prepared in saline[17]. Prior to analysis, longitudinal histological sections of the queue are prepared and stained for hematoxylin and eosine[14]. The histopathological procedures are prepared by the Department of Veterinary Pathology, Chennai Veterinary College.

The animals are divided into three groups and for the administration the catering functions are used. Group II is a service that is part of the solvent, and functions within the administration and solvency of the application. Group II is a receipt for the standard of care, dithranol, which is useful as a reference in the results of antipsoriatic effects. Group III, with liposomal formulation experiments, determines the evaluation of the effects on the conditions, caused by the relationship with the solvent and the standard property.

Horizontal length of hair follicles – The horizontal length of individual hair follicles adjacent to the sebaceous glands was measured. The study included 6 animals per group, divided into 3 groups.

Horizontal arrangement of developed granules within each scale was evaluated. For each treatment group, 10 scales in each animal were analyzed, with 6 animals one group, resulting in a total number of 60 measurements per each treatment.Vertical thickness of epidermis, extending from dermo-epidermal junction to deepest layer of the stratum corneum, was measured. This assessment included 5 measurements per body, 10 scales per each animal, and 5 measurements per scale, with 6 animals in each treatment group, totaling 60 measurements per treatment[15-17].

Histopathology

The methods used to depict the features of psoriasis are based on the features of histopathological examination using established protocols and methods used to determine the history of psoriasis. On the seventh day, the first euthanasia takes place after a short period before returning for analysis. The tissue samples show 5 micrometer (um) thick slices and sections. These sections were stained using hematoxylin and eosin (H&E), a commonly utilized technique in histopathology to distinguish cellular structures. The staining is examined in a Leica DM 2000 microscope and the microscopic images are used for analysis and further investigation. The clinical pathology studies are prepared by the Department of Veterinary Pathology, Chennai Veterinary College, and the slides are evaluated.

Results and Discussion

Preparation of luteolin liposomes

Liposomes were prepared by using ether injection technique, which is an easy method that can be utilized in the laboratory production of liposomes. Before mixing the lipid mixture, the aqueous phase is started; the temperature gradient facilitates the rapid evaporation of the ether, which favors the spontaneous formation of vesicles and liposomes. The homogenization speed and sonication time were optimized at 65°C for 10 min with 18,000 rpm.

Characterization of liposomes

Fourier transform infrared spectroscopy (ATR-FTIR)

The drug-excipient compatibility studies for the solid mixtures of drug and excipients were evaluated through FT-IR analysis, which demonstrated excellent compatibility. This was confirmed by the absence of physical or chemical interactions among the components.

Differential scanning calorimetry (DSC)

It is used to investigate interactions and thermal stability of compounds. It is performed to study the thermal characteristics and phase transitions of substances. It was used to evaluateinteractions and thermal stability of luteolin. DSC experiments were carried out to explore the thermal characteristics of pure drugs. The thermograms of luteolin displayed a distinct endothermic peakat 337.85°C (Fig. 1).

Determination of λ_{max} of Luteolin

UV-spectral assessment was conducted to identify peak for luteolin absorption wavelength (λ_{max}) at pH 7.4 in phosphate-buffered saline (PBS). The luteolin λ_{max} was recorded at 261 nm.

Preparation of Luteolin standard curve

At 7.4 Ph, the concentration and absorbance of luteolin in PBS were measured, and a calibration curve was generated with absorbance on Y-axis and concentration on X-axis (Fig. 2). The slope and intercept of the resulting graph were calculated as 0.01 and 0.001, respectively.

Mice tail model for psoriasis

Screening for Psoriasis control activity

The evaluation of the statistical parameters of the principle, the degree of orthokeratosis, indicators of

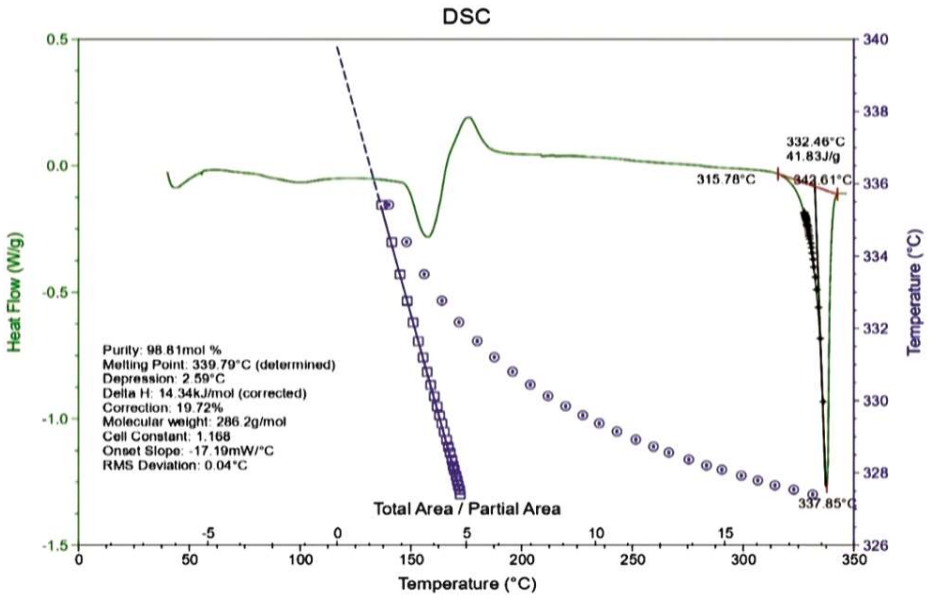

Fig. 1 — Thermogram of Luteolin

Table 1 — Effect of luteolin liposomes on degree of orthokeratosis and relative epidermal thickness and drug activity in the mouse tail test

S. No.	Groups	% Orthokeratosis	Drug Activity	% Relative Epidermal Thickness
1.	Control	17.30 ±4.09	0	100.00 ± 10.7
2.	Standard Dithranol (0.1%)	85.36±3.56***	75.87	98.98±4.6
3.	Luteolin Liposomes	85.07±3.36***	76.49	120.7±12.8

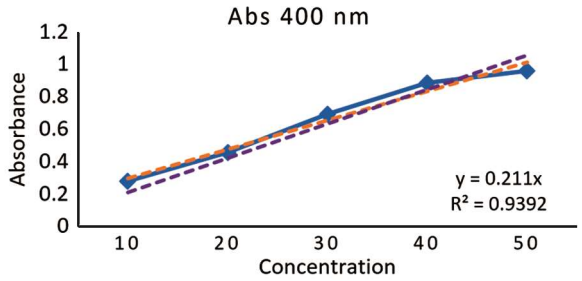

Fig. 2 — Calibration curve of luteolin

the extras and the formulations on which the effectiveness is significant (P< 0.0004) for the difference between the group reports. The effectiveness of introspective analysis of 'medical activity' and general parameters for group reporting (Table 1 and Fig. 3).

Statistics analysis

The data was presented as mean ± standard deviation and analyzed with one-way ANOVA (Analysis of Variance) with Tukey's post hoc test. Computations were performed using GraphPad Prism 5, with significance threshold set at P< 0.05.

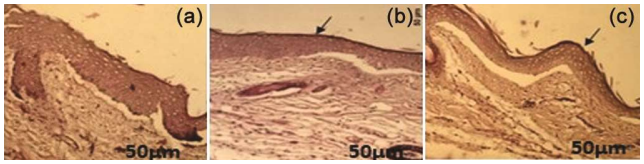

Fig. 3 — Histopathological sections of mouse tail skin treated topically for 14 days, (set the original microscope magnification 40x). (a) Control, (b) Dithranol 0.1% and (c) Luteolin Liposomes (semi solid form). Note that: (a) the granular layer is less developed in most parts of the control specimen; (b) Dithranol 0.1% induces orthokeratosis is clearly seen over the whole horizontal length of the scale as a black layer; (c) a well-developed granular layer is also seen, in mouse tail skin treated with Luteolin Liposomes

Discussion

Luteolin-encapsulated liposomes have exhibited notable anti-psoriatic effects in a mice tail model for psoriasis. As a dermal inflammatory disease with a chronic character, psoriasis is improved with the incorporation of nanocarriers such as liposomes, with targeted drug delivery and increased bioavailability of pharmaceutical agents such as luteolin. The naturally found flavonoid is considered valuable owing to high anti-inflammatory, antioxidant,

and immunomodulatory properties and is a potential drug in treating psoriasis.

To assess the performance of luteolin-loaded liposomes, a wide array of analytical methods was adopted. The presence of luteolin in the liposomal system and chemical interactions with the drug and the lipid components was ensured with the implementation of Attenuated Total Reflection - Fourier Transform Infrared Spectroscopy (ATR-FTIR). Differential Scanning Calorimetry (DSC) provided information on the thermal properties and stability of the formulation, confirming successful drug encapsulation without compromising liposome integrity.

The wavelength of luteolin in phosphate buffer solution (pH 7.4), where luteolin is maximally absorbed, was identified in order to correctly calculate its concentration in the process of analytical evaluation. The calibration curve of luteolin in PBS, having pH 7.4 was prepared in order to get accurate drug contents and release profile analyses. The method of calibration is suitable and efficient in monitoring luteolin delivery in bio-systems.

The development and optimizing process was multi-factorial in basis with consideration of a wide array of factors in a formulation and their mutual interactions. The process made optimizing process of the liposomal system possible based on balancing properties such as particle size, zeta potential, and polydispersity index (PDI).

In the mouse tail model of psoriasis, scaling symptoms, erythema, and overall appearance of the disease increased with topically applied luteolin-loaded liposomes. The reason was increased dermal permeation and steady and slow luteolin release provided by the drug-delivery system in the liposomes. The future potential in the treatment of psoriasis with treatments based on nanotechnology is demonstrated in this research and makes way for future treatment and clinical trials.

Conclusion

The luteolin-loaded liposomal system was extremely effective in treating psoriasis in the mouse tail model and is thought to be because of enhanced bioavailability and a longer drug release profile. The incorporation and stability characterization of luteolin was validated with the usage of ATR-FTIR and DSC. Furthermore, λ_{max} measurement and construction of a standard curve ensured luteolin concentration measurement. These results emphasize the potential of luteolin-loaded liposomes in treating psoriasis and warrant investigation in a clinical setup.

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Conflict of interest

All authors declare no conflict of interest.

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