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Fabrication Lecithin Coated Liposomes Containing Guggul Extract for Psoriatic Treatment

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ABSTRACT

Psoriasis is a condition in which up to 78% of patients exhibit a significant association with underlying stress. Numerous herbal nano formulations, such as those incorporating curcumin, aloe vera, and neem, have demonstrated anti-inflammatory and immunomodulatory effects, which may be beneficial in the treatment of psoriasis. Further research is necessary to determine the efficacy and safety of these aspects, along with the ideal dose, treatment duration, and any adverse effects. This study work focuses on the development and assessment of guggul extract-loaded nanoliposomes formulated with sunflower lecithin to enhance the stability and efficacy of the formulation. The study examined different factors, including particle size, zeta potential, entrapment efficiency, in vitro drug loading percentage, and cytokine analysis, indicating that formulation GENL3 is both effective and stable

Keyword: *Liposomes, Psoriasis, Guggul, Lecithin, HPLC, Ortho phosphoric acid*

1. INTRODUCTION

Lecithin is a natural complex mixture of phosphatides that varies in color from light tan to dark reddish brown and in consistency from a fluid to a plastic solid [Jala et al., 2015]. Globally, lecithin is being used as emulsifiers, wetting and instantizing agents, viscosity modifiers, releasing agents, separating agents, and anti-dusting agents. They are also utilized in nutritional supplements and to extend the shelf life of food products. Lecithin is a lipid compound that is present in both the animal and plant origins. Lecithin is obtained from several sources such as sunflower seeds, soybeans, and egg yolks. Soy lecithin is the most prevalent among the three varieties. Nevertheless, it may not always be the optimal choice due to its genetic modification and high allergenicity. Lecithin is commonly derived from egg yolk. Additionally, it is unsuitable for anyone with egg allergies. Each lecithin necessitates the utilization of abrasive agents during the extraction procedure. Sunflower lecithin, whether used as an emulsifier or as a supplement, serves as an excellent substitute for both egg and soy lecithin due to its vegan nature, non-allergenic properties, non-GMO status, and the use of less aggressive extraction techniques that avoid the use of toxic chemicals [Jala et al., 2015]. Additionally, it possesses the ability to repair the skin.

Guggulu is a member of the Burseraceae family and can be categorized as either a shrub or a small tree. Guggulu is composed of volatile oil, gum resin, gugulipids, guggulsterones, guggulsterols, mukolol, and other steroids. Guggulu is widely employed in Ayurvedic medicine due to its astringent, antibacterial, expectorant, aphrodisiac, carminative, antispasmodic, and emmenagogue effects. Guggulu consists of essential oil, resin, gum, and bitter chemicals. The primary chemical components of guggul include Z-guggulsterone, E-guggulsterone, guggullignans I and II, gugglutetrols, mukulol, allylcembrol, c-27 guggulusterols I, II, III, Z-guggulusterol, E-guggulusterol, and others [Sarup et al., 2015]. These components are accountable for various pharmacological effects such as anti-inflammatory, analgesic, wound cleansing, and healing through their antibacterial properties [Ifra et al., 2022].

Psoriasis is a chronic skin disease caused by an overactive immune system, characterized by inflammation. It is linked to several health conditions like psoriatic arthritis, mental health issues, heart problems, and liver illnesses. Psoriasis is a condition characterized by inflammation that impacts approximately 2-3% of the global population [Goldminz et al., 2013]. Several factors have been linked to this, such as stress, previous streptococcal infection, interleukin upgrade, and a recently discovered candidate gene [Kamiya et al., 2019].

The present work aimed to develop the nanoliposomes loaded with guggul extract and sunflower lecithin which possesses non allergic, non-toxic and skin repaired properties used to treat psoriasis

2. MATERIAL AND METHODS

Material:

(8R,9S,10R,13S,14S)-17-Ethylidene-10,13-dimethyl-1,2,6,7,8,9,11,12,14,15decahydrocyclo penta [a]phenanthrene-3,16-dione (Guggulsterone Z) is purchased from Yucca enterprises, Mumbai.

3. METHODS

Chromatographic condition

The analysis was performed to determine the amount of drug sample using the Agilent Tech. (1100) equipment. Detection was carried out at a predetermined wavelength, controlled by CHEMSTATION 10.1 Software, with auto injector. The chromatographic separation was carried out on a Fortis C18 column (100 x 4.6 mm id with 2.5 mm particle size) using various mobile phase compositions at a different flow rate.

Preparation of mobile phase

Mobile phase was prepared by mixing HPLC grade acetonitrile, and distilled water with 0.10% Ortho phosphoric acid (OPA) in the ratio of 70:30 v/v. The content was sonicated for 15 min and filtered through a 0.45 µm membrane filter. Mixed solvents were degassed and used as a mobile phase.

4. METHODOLOGY

The optimized chromatographic separation was achieved on column Fortis C18 column (100 x 4.6 mm id with 2.5 mm particle size) by using HPLC grade acetonitrile, and distilled water with 0.1 percent Ortho phosphoric acid (OPA) in the ratio of 70:30 as the mobile phase, at a flow rate of 1.0 mL/min. Using the mobile phases mentioned above, the resolution and peak symmetry obtained were excellent, as was the symmetry of the peaks. The quantification of peak area was performed at the determined wavelength based on the peak area. The proposed method's suitability for the proposed system was assessed. The system suitability test was performed on a freshly prepared standard stock solution of Guggulsterone Z (Gz) to ensure that it was as effective as possible. To determine the suitability of the system, various parameters such as resolution, peak tailing, and HETP were investigated.

Method Validation

The validation of the developed HPLC method was carried out in accordance with ICH guidelines. The linearity was analysed for concentration ranging from 5 to 45 µg/ml of Guggulsterone Z by using Least-square regression analysis where, peak areas were plotted against the corresponding concentrations. The repeatability of the device was evaluated by repeatedly injecting a solution of a drug with a concentration of 25µg/ml. The intra- day and inter- day precision was evaluated by triplicates of three different concentrations of each Guggulsterone Z was spotted and analyzed on same day for intra-day study and two different days for inter-day study with respective chromatographic conditions.

Recovery study method was employed to evaluate accuracy. The samples were spiked with 80, 100 and 120 % of median concentrations of standards.

$$\text{Accuracy} = \frac{\text{spiked concentration} - \text{mean concentration}}{\text{spiked concentration}} \times 100$$

Robustness was carried out by making deliberate changes in the mobile phase, detection wavelength, flow rate and quantitative analysis was determined. The estimation of LOD and LOQ were done by standard deviation method. Detection limit =3.3σ /S and quantitation limit=10 σ /S (σ is residual standard deviation of a regression line and S is the slope of the calibration curve).

Formulation of Guggul extract- loaded nanoliposomes

A. Formulation by ethanol injection method

The formulations were prepared by dissolving free GE with Sunflower lecithin and varying amounts of cholesterol in specified ratios. The mixture was dissolved in a combination of ethanol and chloroform (1:1), as indicated in Table 1. The ethanol and chloroform volumes were increased in direct proportion to the lipid content in the drug:lipid ratio. The combinations were subjected to ultrasonic treatment in a water bath for a duration of 15 minutes. The homogeneous solutions were rapidly injected using a syringe into 25ml of deionized water that had been stirred at 500 RPM on a magnetic stirrer with a teflon-coated bead. The stirring was continued until the chloroform and ethanol evaporated. The composition was agitated for a duration of 4-8 hours. The liposomes were stabilized by refrigerating them for a minimum of 6 hours.

Table 1: Composition of the nano-liposome (NLs)

Sr. No.	Name of Ingredients	GENL 1	GENL 2	GENL 3	GENL4	GENL5	GENL6
1.	GE	5 mg	5 mg	5 mg	5 mg	5 mg	5 mg
2.	Sunflower lecithin	7.5 mg	5 mg	2.5 mg	7.5 mg	5 mg	2.5 mg
3.	Cholesterol	3.75 mg	2.5 mg	1.25 mg	7.5 mg	5 mg	2.5 mg
4.	Ethanol and Chloroform (1:1)	2.5 ml 2.5 ml	3.75 ml 3.75 ml	5 ml 5 ml	7.5 ml 7.5 ml	8.75 ml 8.75 ml	10 ml 10 ml
5.	Deionised water	25 ml	25 ml	25 ml	25 ml	25 ml	25 ml

Characterization of GE-loaded Nanoliposomes

Particle Size analysis and poly dispersibility Indices

The measurement of particle size and PDI were done on a Zetasizer instrument at 25°C. This technique produces the mean particle diameter and particle size distribution. The analysis was done by the software provided by Malvern Instruments. Before analysis samples were placed in a refrigerator maintained at 4°C.

Zeta potential

The zeta potential of the nano cochleate formulation was determined with the use of a Malvern Zetasizer device. The zeta potential was determined with the use of a zeta potentiometer. The material was poured into the cell, and an electrode was attached, which was then placed under the microscope and linked to the zeta metre for analysis. The experiment was conducted out at a temperature of 25°C.

% Drug Loading

The drug loading was determined using high-performance liquid chromatography (HPLC). One millilitre of nanoliposomes formulation was dissolved in one millilitre of ethanol and EDTA (1:1), and the volume was increased to ten millilitres by adding deionized water. After that, the solution was sonicated for 5 minutes. Filters with a 0.45micron opening are used to filter the resultant solution. The filtrate was subsequently subjected to high-performance liquid chromatography (HPLC) analysis.

Entrapment Efficiency

The nanoliposomes formulation (10 mcg/ml) was centrifuged at 4000 rpm for 18 min at 4°C temperature by using a Remi cooling centrifuge to separate the free drug. A supernatant contains the GE-NL in the suspending stage and free drug on the wall of the centrifuge tube. The supernatant was again centrifuged for half an hour at 15000 rpm (4°C). As a result, a transparent solution of supernatant and nanoliposomes pellet was attained. The pellet consisting of nanoliposomes were disrupted using EDTA into nanoliposomes and then disrupted in ethanol (100 ml) to discharge the drug. The discharged drug was determined for the drug entrapment. The amount of GEwas estimated by using HPLC system. Percentage entrapment efficiency was determined as

$$\text{Percentage Entrapment Efficiency} = \frac{Wc}{Wt} \times 100$$

Where amount of drug content (entrapped) in the nanoliposomes is denoted as W_c and total amount of drug in the dispersion is denoted as W_t.

HRTEM

To obtain an interference image, high-resolution transmission electron microscopy (HRTEM) employs both transmitted and scattered beams. It's a phase contrast image that can be as small as a crystal unit cell. In this situation, the outgoing modulated electron waves interfere with themselves during propagation through the objective lens at very low angles. At a point in the picture plane, all electrons emanating from the object are merged. HRTEM has been utilised extensively and successfully for investigating crystal structures and lattice defects in a variety of sophisticated materials at the atomic level. Point defects,

stacking faults, dislocations, precipitates grain boundaries, and surface features can all be characterised with it.

Animal study

Anti-Psoriatic: Oxazolone mice model

After the approval from the Institutional Animal Ethics Committee (Protocol No. PESRTBCOP/IAEC, 2022R-96). The experimental work was performed at animal house of Rajaram and Tarabai Bandekar college of Pharmacy, Ponda, where Albino Wistar rats of 200-250 g weight were used to determined psoriatic action. The animals were divided in five groups. Group I as normal, group II as control, group III as GU extract, group IV as GU liposome.

Sensitization and Elicitation (Challenge Application) Procedure: The animals were sensitized for six days by applying 100 µl of 1.5% oxazolone in ethanol to their abdomen area. On days 7, 10, 13, and 16, 20 µl of % oxazolone in a combination of acetone and olive oil (4:1) was applied to both sides of the mouse ear after 7days of sensitization.

Measurements: Ear thickness was estimated using digital Vernier Calipers at several time periods during the investigation. To determine swelling ear responses, ear thickness was evaluated before the sensitization phase (Day 7) and after each elicitation on days 10, 13, 16, and 19. Mouse ears were removed, fixed in 10% buffered formalin solution, embedded in paraffin, cut into 5 µm slices, and stained with hematoxylin-eosin 72 hours after the last administration of oxazolone using conventional techniques. The epidermal thickness was evaluated as the distance between the bottom of the stratum corneum and the foundation membrane in the interfollicular epidermis during the histological examination, after the microscopic fields were photographed. Inhibition of epidermal thickness was calculated.

In-vitro study of inflammatory Cytokines by ELISA method of extracts and their Nanoliposomes

Psoriasis is a chronic inflammatory skin disease that affects 2% to 3% of the population worldwide and causes significant morbidity. Its etiology is unknown, but it is generally believed to be a complex autoimmune inflammatory disease with a genetic basis. In psoriasis, TGF-β1 is elevated in plasma and in scales of lesion. This release combined with activation of dendritic cells through their pattern-recognition receptors, is likely sufficient to generate Th17 cells in skin-draining lymph nodes capable of inducing capable of inducing expression of IL-23R on developing Th17 cells. IL-23R expression promotes responsiveness to IL-23, the key cytokine in the survival and proliferation of Th17 cells.

Macrophages play major roles in innate immunity and they recognize the pathogen-associated molecules such as LPS (a bacterial endotoxin) and trigger innate immune response through toll like receptor (TLR) signaling. Activation through Toll-like receptor (TLR) 4 signaling induced by lipopolysaccharide can trigger IL-23 production by dendritic cells. Thus, IL-23 pathways are identified as playing the key roles in the immunopathogenesis of psoriasis, but numerous other cytokines are also involved in the pathway.

IL-10 is a type 2 cytokine with numerous immunosuppressive and anti-inflammatory capacities. IL-10 inhibited antigen-presenting cells including dendritic cells, monocytes and macrophages. Evidence also showed that ultraviolet light, a confirmed therapy for psoriasis, modulated inflammation by inducing expression of IL-10 in keratinocytes. In psoriatic skin, the diminished level of IL-10 was once considered a critical way to induce disease flare up.

The inhibition of adverse macrophage activation or selectively neutralizing the overproduction of macrophage products was recommended as a promising therapeutic route against diverse inflammatory conditions.

A) Cell line, Reagents and kits

RAW 264.7 cell line was obtained by the National centre for cell science (NCCS) Pune, India. Dulbecco's Modified Eagle's medium (DMEM), fetal bovine serum (FBS), and phosphate buffer saline were purchase from Invitrogen (Carlsbad, USA). LPS purified from Escherichia (serotype 026:B6) was purchase from Sigma-Aldrich Co. (St. Louis, MO, USA). All other chemicals and reagents used in this study were of analytical grade. Enzyme-linked immunosorbent assay (ELISA) kits were purchase from Ray Biotech Inc, USA.

Cell culture and culture conditions

RAW 264.7 cells were cultured in DMEM supplemented with 10% heat-inactivated FBS, 100 µg/ml streptomycin and 50 units/ml penicillin. The cells were incubated at 37°C in the presence of 5% CO_2 and sub-cultured every 2 days.

Cell viability assay

The viability of the cell was evaluated by MTT assay. The samples were prepared by sonicating 1gm with 100 ml of methanol for 1 hr resulting in 10 mg/ml of solution. The 1 ml of solution further diluted with DMSO to produced 1000 µg/ml concentration. The RAW 264.7 cells were seeded into 96-well cultured plate at a density of 5×10^4 cells/well and incubated overnight at 37°C and 5% CO_2 for attachment. The cells were then treated with sample of concentrations range of 0.625 – 1000 µg/ml with (1 µg/ml) or without LPS and incubated for 24 hrs. After incubation, the culture medium was removed and 100 µl of fresh DMEM and 20 µl of MTT (5 mg/ml in PBS) solution was added to each well. Following 4 hrs incubation in

the dark, the media was discarded again and 100 µl of DMSO was added to each well for the solubilization of formazan deposits. The optical density of the cells at 570 nm were measured using an ELISA plate reader (Bio-Rad Laboratories, CA, USA) and the experiment was carried out in triplicate.

Measurement of pro-inflammatory and anti-inflammatory cytokines (IL-23 and IL-10) production:

The RAW 264.7 cells were seeded at a density of 2×10^4 cells/well in 24 well-cultured plates and incubated for 24 hrs at 37°C and 5% CO_2 for adherence. The adhered cells were incubated for 24 hrs, with the indicated concentrations of test samples (500 mcg/ml) in the absence or presence of LPS (1µg/ml). The cell culture supernatant was harvested after 24 hrs of incubation of cells with LPS and samples. These supernatants were tested for quantitation of pro- and anti-inflammatory cytokines (IL-23 and IL-10) using mouse-specific enzyme immune-assay kit (RayBiotech Inc, USA) according to manufacturer's instructions.IL-23 is a key cytokine for promoting inflammatory responses in a variety of target organs. The most important function ascribed to IL-23 is its role in the development and differentiation of effector Th17 cells via activation of STAT3. Interleukin 10 (IL-10) is also potent anti-inflammatory cytokine that plays a central role in limiting host immune response to pathogens, thereby preventing damage to the host and maintaining normal tissue homeostasis.

B) ELISA

Briefly, the ELISA plates (96 well) were coated with specific mouse IL- 23, IL-10 antibodies (100 µl/well) and incubated at 4°C for overnight. The assay diluents (200 µl/well) were used to block the non-specific protein-binding sites present in the plate. Immediately, 100 µl of culture supernatant or respective standard was added into the suitably coated wells and incubated at room temperature for 2 hrs. After incubation, the plates were washed 5 times thoroughly with wash buffer [phosphate-buffered saline (PBS) containing 0.05% Tween-20]. About 100 µl of detecting solution (detection antibody and streptavidin horse-radish peroxidase) was added into each well.

The plates were properly covered with a plate sealer and incubated for 1 hr at room temperature and again wash 5 times thoroughly using wash buffer. Substrate solution of 100 µl, tetramethylbenzidine (TMB) was added to each well and the plate was further incubated (without plate sealer) for 30 min in the dark at room temperature. Finally, 50 µl of stop solution (2N H_2SO_4) was added to each well. ELISA results were recorded at 450 and 570 nm with an ELISA reader (Bio-Rad Laboratories, CA, USA). The absorbance at instruction. The concentration determined for three wells for each cytokine and values were derive from the standard curve and express as pg/ml.

5. RESULTS AND DISCUSSION

Optimization of RP- HPLC method

The essential parameters that determine the optimization of any chromatographic method for analysis are good resolution, peak shape, theoretical plates, retention time, and asymmetry. Several circumstances of chromatographic procedures, such as varied compositions of mobile phase, flow rate, and various stationary phases, were optimized and evaluated for the assessment of Guggulsterone Z in order to achieve all of these characteristics. With mobile phase, the produced peak was observed to be excellent, crisp, symmetrical, and well defined. Acetonitrile: water (0.10 %OPA) in a 70:30 v/v ratio, with a flow rate of 1 ml/minute at 255 nm. The retention time of Guggulsterone Z was observed at 11.73 min (Figure 1). Optimized characteristics are given in table below.

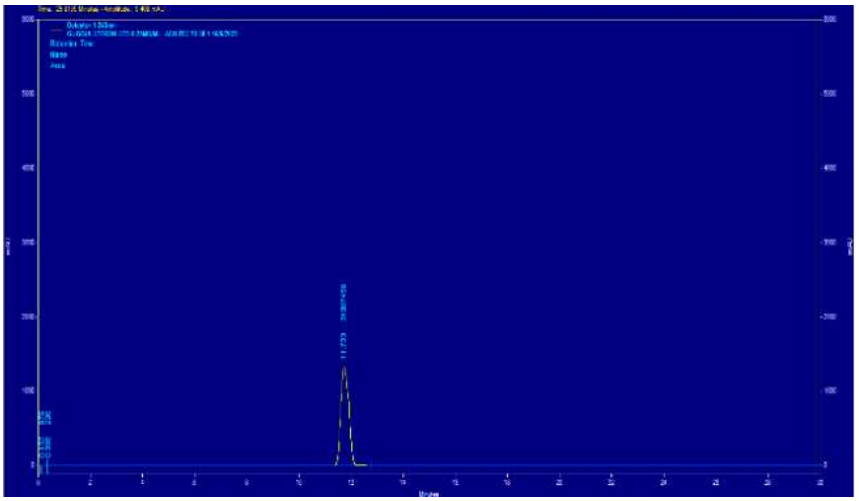


Figure 1: Chromatogram of Guggulsterone Z

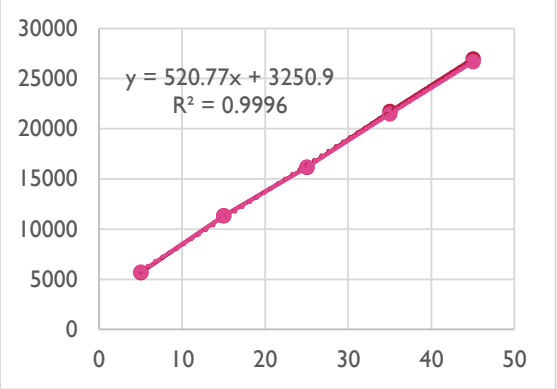


Figure 2: Linearity curve of Guggulsterone Z

Table 2: HPLC method Optimized Parameters

Sr. No.	Parameters	Specifications
1.	Composition of Mobile phase	Acetonitrile: water (0.1% of OPA) in a ratio 70:30 v/v
2.	Column Specifications	FortisC18 (100 x 4.6 mm id with 2.5 µm)
3.	Flow Rate	1.0 ml/min
4.	Retention Time	11.73 min

Evaluation of Liposomes:

% Entrapment Efficiency and Drug loading

The entrapment efficiencies of prepared formulations were determined by finding the concentration of free drug in the dispersion medium. The percentage entrapment efficiency and drug loading of nanoliposomes is shown below. The percentage drug encapsulation was calculated according to the standard procedure. The percentage entrapment efficiency of formulations prepared by ethanol injection method were varied between 45.12 to 78.20%. The entrapment efficiency was affected by nature and amount of lecithin used. The values of percent drug loading of formulations prepared by ethanol injection method were ranges from 53.35 to 85.37%. The formulation formulated by ethanol injection method (GENL3) showed highest entrapment efficiency i.e. 78.20% compared with all the formulations. Considering the stability of formulation based on better entrapment efficiency GENL3 was used for further studied.

Table 3: Evaluation of Nanoliposomes (GENL 1 to GENL 6)

Formulation	Average % EE	% Drug Loading
GENL 1	66.66±1.55	66.96±1.00
GENL 2	56.01±0.98	74.65±1.61
GENL 3	78.20±1.43	85.37±1.49
GENL 4	53.68±0.67	62.97±0.35
GENL 5	52.77±1.10	53.53±0.17
GENL 6	45.12±0.88	64.22±

Particle Size, Zeta Potential, PDI

GENL3 was selected based on their EL and DL percentage. The value for the determination of particles size, PDI and Zeta potential of formulation GENL3 is given in table below. Polydispersity index (PDI) is an indication of the size distribution with values ranging from 0 to 9.

This evaluation of GENL3 the nanoliposomes clearly indicated that GENL3 formulation is superior as the particle size minimum is 125.7 ±0.11 and poly-dispersibility indices 0.238 ± 0.0252 and zeta potential -31.2 ±0.31. As smaller particle size indicates better diffusion and permeability and lesser value of polydispersity indicates narrow size distribution of particles in the dispersion.

Zeta potential measurement the physical stability of nano dispersed system and predicted for long term stability. The maximum standard limit for zeta potential is ± 30 mV. Formulation GENL3 showed good zeta potential value, indicated the better stability of formulation with no agglomeration. The results indicate the repulsive force of attraction between particles. Hence prevents aggregation. Overall, GENL3 formulation is proved to be better formulation based on all evaluation parameters.

Table 3: Evaluation of Nanoliposomes (GENL 3)

Formulation	Particle size	PDI	Zeta potential
GENL 3	123.4	0.218	-30.2

HRTEM

The particle size and morphology of GENL3 nanoliposome was examined by high resolution transmission electron microscope (HRTEM) (MAKE: JEOL, MODEL: JEM 2100 plus, Japan). Sample was prepared by taking a drop of sample on parafilm. Then, a drop of 2% phosphotungstic acid solution were kept over sample drop and left it for 30 sec.The copper grid was placed on sample. Then air drying of copper grids were performed for 1 h and observed under TEM and photomicrographs were captured.

The morphology and structure of nanoliposomes was validated by high resolution transmission electron microscopy. Figure showed the nanoliposomes was formed as spherical with smooth surface, mono-dispersed pattern confirmed the slow release of the drug.

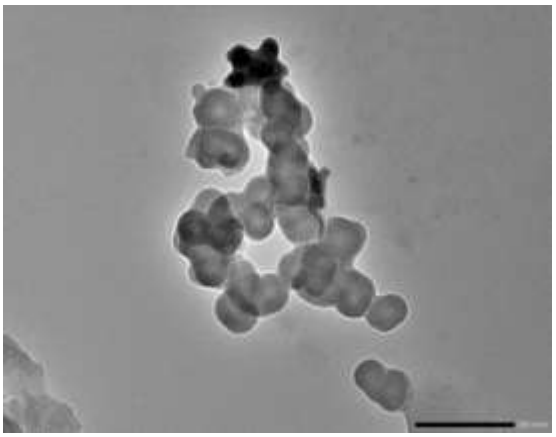


Figure 3: HRTEM of GENL3

Animal Study

The effect of extract (GU) and formulation (GU liposomes) was evaluated by topical application of formulations to oxazolone induced psoriasis in mice for 16 days. The animals were sensitized by applying oxazolone to the abdominal region of the mice. The formulations were applied after the challenge. The evaluation was performed by measuring the thickness of ear. After the completion of treatment with both the formulations showed significant decreased in ear thickness, indicating the anti-inflammatory potential of the extracts and formulations.

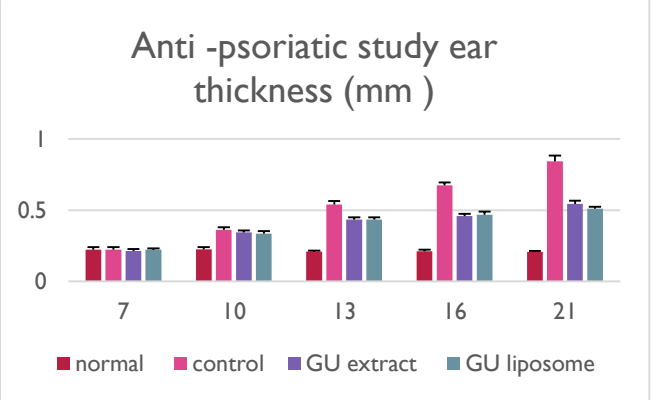


Figure 4: Measurement of ear thickness

Histopathology Study

The histopathology study revealed that, developed formulations of the active has increased the efficacy with decrease number of inflammatory cells, improved skin surface and reduction in the thickening of the skin when compared with respective extracts.

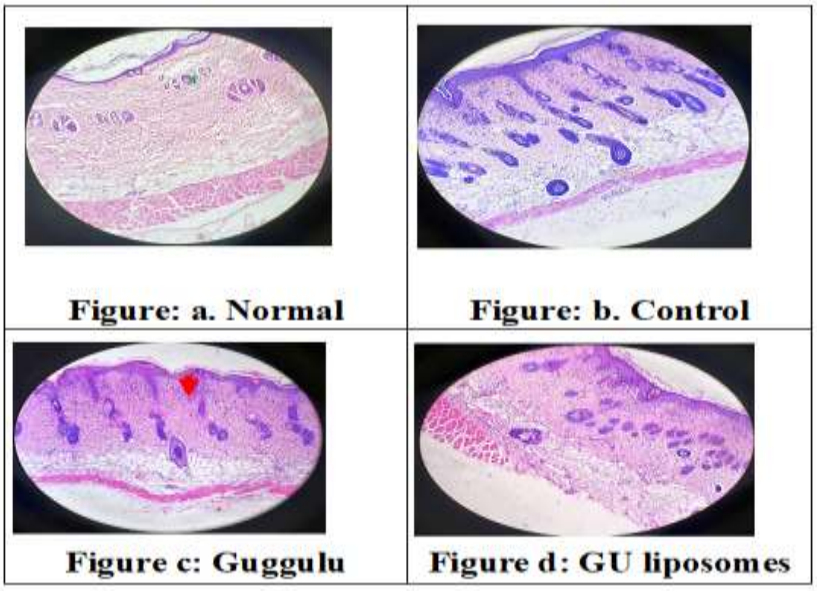


Figure 5: Histopathology Study

In-vitro study of inflammatory Cytokines by ELISA method of extracts and their Nanoliposomes

The comparative effect of extracts and their respective liposomes on production on these cytokines (IL-23 and IL-10) were analyzed by ELISA. To investigate the effects of samples on psoriasis, a Raw 264.7 cell macrophagic cell inflammatory model was established, wherein cells were treated with LPS for 1 h. The anti-inflammatory cytokine IL- 10 was elevated by liposomes at 500 mcg/ml (48.51% and 46%). The maximum suppressive effect of liposomes on IL- 23 evaluated was approx 22% at 500 mcg/ml. As the formulation regulated the production of cytokines which are related to psoriasis etiology or treatment. It can be concluded that the formulation has potent anti-psoriatic action.

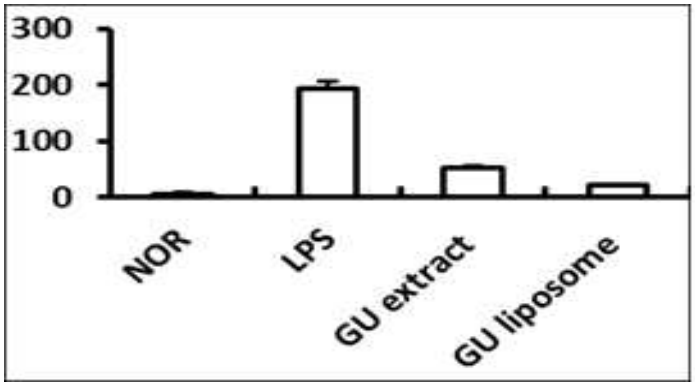
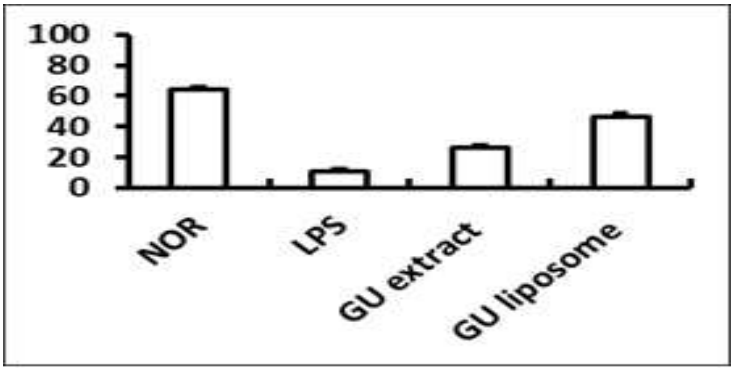


Fig. 6: Cytokine study (a): IL-23 cytokines levels with various samples



(b): IL-10 cytokines levels with various samples

6. DISCUSSION:

The optimization of any chromatographic analytical method depends on resolution, peak shape, theoretical plates, retention duration, and asymmetry. To analyze Guggulsterone, several chromatographic techniques, such as mobile phase composition, flow rate, and stationary phases, were optimized and evaluated. The mobile phase peak was clear, symmetrical, and well defined. Acetonitrile: water (0.10 %OPA) 70:30 v/v, 1 ml/minute at 255 nm. The Guggulsterone Z retention time was 11.73 min.

The method was validated for linearity, accuracy, repeatability, interday and intraday precision, LOD, and LOQ per ICH criteria.The calibration curve was linear from 5 to 45 micro g/ml Gz. Peak area vs Gz concentration was plotted to estimate the regression equation and correlation coefficient (Figure 1). The recovery rates at 80, 100, and 120% were 103.31, 105.70, and 101.58. Since the recovery rate was 100%, the procedure was accurate. We calculated and compared the recovery and RSD rates at each drug dose. Three levels of responses were evaluated in each level.

Repeatedly injecting a 25-micro g/ml drug solution tested the device's constancy. RSD should not exceed 2%. The performance of intermediate precision was measured intraday and interday. Three medication concentrations were tested for intraday and interday precision. % RSD of intra-day precision at 15, 25, and 35 µg/ml concentrations was 1.34, 1.01, and 0.06, respectively. The % RSD of interday precision at 15, 25, and 35 µg/ml concentrations was 0.85, 0.22, and 0.25. Modest chromatographic alterations like mobile phase flow rate and wavelength provided robustness. No significant chromatogram alterations were found. The devised approach proved robust, with RSD values <2%. Both LOD and LOQ were found to be 0.002 and 0.123 µg/ml, respectively.

By measuring free drug concentration in the dispersion medium, formulation entrapment efficiencies were found. Nanoliposome entrapment and drug loading percentages are below. We calculated drug encapsulation % using established methods. Ethanol injection formulations had 45.12–78.20% entrapment efficiency. Nature and amount of lecithin affects entrapment efficiency. Formulations developed by ethanol injection had 53.35 to 85.37% drug loading. The formulation formulated by ethanol injection (GENL3) had the highest entrapment efficiency (78.20%). For formulation stability and higher entrapment efficiency, GENL3 was examined.

GENL3 was chosen for EL and DL %. The GENL3 nanoliposome formulation was found to be superior, with a minimum particle size of 125.7 ±0.11, poly-dispersibility indices of 0.238 ± 0.0252, and zeta potential of -31.2 ±0.31. Smaller particles have better diffusion and permeability, and lower polydispersity means narrower particle size distribution.

Zeta potential measures nanodispersed system physical stability and predicts long-term stability. Maximum zeta potential standard limit is ± 30 mV. Formulation GENL3 had an excellent zeta potential, indicating stability without agglomeration. Results show particle repellence. Prevents aggregation. All evaluation factors show that GENL3 formulation is better. High-resolution transmission electron microscope (HRTEM) (JEOL, JEM 2100 plus, Japan) studied GENL3 nanoliposome particle size and shape. Sample was prepared by dropping it on parafilm. A drop of 2% phosphotungstic acid solution was placed over the sample drop for 30 seconds.Copper grid was on sample. After 1 h of air drying, copper grids were examined under TEM and photomicrographed.

High-resolution transmission electron microscopy confirmed nanoliposome structure and shape. Figure revealed round, smooth, monodispersed nanoliposomes that released the medication slowly.

Topical use of extract (GU) and formulation (GU liposomes) to mice with oxazolone-induced psoriasis for 16 days assessed their effects. Mice were sensitized with oxazolone on their abdomens. The formulations were used following the challenge. Ear thickness was measured for examination. Both formulations reduced ear thickness after treatment, demonstrating their anti-inflammatory properties.

The histopathology analysis showed that created active formulations reduced inflammatory cells, enhanced skin surface, and reduced skin thickness compared to extracts. ELISA was used to compare the effects of extracts and liposomes on IL-23 and IL-10 production. In a Raw 264.7 cell macrophagic cell inflammatory model, LPS was applied for 1 h to test samples' effects on psoriasis. Liposomes at 500 mcg/ml increased IL-10 (48.51% and 46%). IL-23 suppression by liposomes peaked at 22% at 500 mcg/ml. The formulation-controlled psoriasis-related cytokines. Anti-psoriatic activity is strong in the formulation.

7. CONCLUSION

Psoriasis is a disorder in which up to 78% of cases have a strong underlying stress connection. Several herbal nano formulations, including those containing curcumin, aloe vera, and neem, have been shown to exhibit anti-inflammatory and immunomodulatory impacts, which will be useful within the treatment of psoriasis. However, more study is required to decide the efficacy and safety of these details, as well as the optimal dosing, duration of treatment, and potential side effects. This research paper involves the development and evaluations of guggul extract loaded nanoliposomes prepared by using sunflower lecithin to improve the stability and efficacy of the formulation. The study focused on the various parameters including particle size, zeta potential, entrapment study, % drug loading invitro study as well as cytokine study indicate that formulation GENL3 found to be effective as well as stable.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

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