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Development of Herbal Liposomal gels for Enhanced Anti-Psoriatic Therapy: Incoporting Isolated antipsoriatic compound from Methanol extract of Mirabilis jalapa

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ABSTRACT

Introduction: Psoriasis, a chronic immune-mediated skin disorder, affects patients' quality of life significantly due to its associated physical, psychological, and economic burden. Conventional treatments often pose challenges, including limited efficacy and adverse effects. The present study aimed to formulate Isolated antipsoriatic compound from methanol extract of roots of Mirabilis jalapa, DTDO into a liposomal gel (DTDO-L) and investigate the therapeutic potential of DTDO-L in UV-B-induced psoriasis model.

Materials and Methods: The study employed a UV-B-induced psoriasis model to mimic human pathology, determining erythema, thickness, and scaling scores. Key biomarkers of inflammation and oxidative stress, including IL-6, TNF-α, IL-1β, and antioxidant parameters (SOD, CAT, GPx, GSH, and MDA), were quantified. The DTDO-L gel was compared against a standard tretinoin gel and other formulations, including a non-liposomal DTDO gel and MEM extract-loaded gel.

Results and Discussion: DTDO-L demonstrated superior efficacy in reducing psoriatic markers compared to standard treatments. It significantly normalized keratinocyte proliferation and reduced inflammatory cytokines (TNF-α, IL-6, and IL-1β), similar with previous studies on liposomal drug delivery systems. Oxidative stress parameters improved significantly in the DTDO-L group, emphasizing its antioxidative potential. The liposomal formulation's nanoscale size enhanced skin penetration and drug retention, addressing key limitations of traditional treatments. Comparative analysis with standard and non-liposomal formulations underscored the superior anti-inflammatory and antioxidant effects of DTDO-L.

Conclusion: The findings confirm the potential of DTDO-L liposomes as an effective therapeutic intervention for psoriasis. By improving drug delivery, reducing systemic side effects, and targeting multiple inflammatory pathways, DTDO-L offers a promising alternative to conventional therapies. Further clinical studies are warranted to establish its long-term safety and efficacy.

Keywords: Mirabilis jalapa, Psoriasis, Anti-inflammatory, Anti-proliferative, HaCaT cell lines

1. INTRODUCTION

Psoriasis is one of the most effective chronic autoimmune dermatological disorders that involves uncontrolled proliferation of keratinocytes, activation of immune cells that are circulated in the body. This results in the scales and

drying of skin and hardening of vascular edothelium. Often psoriasis is triggered by genetic, drugs or any external factor (Elkordy et al., 2024). There are various kinds of therapies for psoriasis depending on the degree of severity where mild psoriasis if managed using corticosteroids, anti-inflammatory agents in the form of topical creams, ointments. Moderate psoriasis is treated using combination of biological agents combined with phototherapy and systemic formulations that are adminsitered via oral/parenteral route (Rendon and Schakel, 2019; Gisondi et al., 2017).

In practical observation, though the existing treatments are effective in oral or parenteral routes, they reach the blood stream and cause unwanted effects. Topical drug delivery is limited by skin permeation and also drug bioavailability. In addition, low retention of formulation at the site of administration, improper release kinetics of the drug results in the reduced efficacy at the psoriatic lesions. On the other hand, patient compliance to certain anti-psoriatic formulations with dianthranol and coal-tar due to resulting in discoloration are evident (Ávalos-Viveros et al., 2023). In order to overcome these problems with drug delivery, herbal drugs with known low to lack of side effects which are effective to treat psoriasis can be selected. The choice of effect drug delivery system that is efficient in maintaining the proper release of the drug through transdermal route can also bypass the deficiencies of conventional drug delivery via transdermal formulations.

Among the available drug carrier technologies, lipid-based drug delivery systems are considered most advantageous. They are efficient in delivering drugs of various solubility and effective to deliver in topical route (Jahanfar et al., 2021). Liposomes are colloidal drug delivery systems that is composed of bi layered phospholipid membranes that incorporates aqueous layers thereby carrying most drugs efficiently (Li et al., 2021). Especially herbal drugs, entrapped in the liposomal drug delivery system have enhanced stability and sustained release over longer durations (Bummer et al., 2004; *Dehkharghanian et al., 2009*). Also better biocompatility, moisture retention, diodegradability are acheived with liposomal formulations making it efficient for topical drug delivery most suited for conditions like psoriasis. As the diphospholipid membranes in the liposomes are similar to the cell walls, biological membranes allow them to pass through the barrier (Rahimpour et al., 2012). The liposomes interact with keratinocytes in the epidermal lipids and result in the enhanced absorption of the drug through the dermal layers (Khan et al., 2021).

Mirabilis jalapa is well known to treat various kinds of disorders that include kidney and digestive tract problems like constipation traditionally (Sharma et al., 2001; Chetty et al., 2008). Roots of the plant are used to control the symptoms of inflammation and lymph node edema by discharging the excess lymph. Seeds and flowers of the plant are employed to treat skin disorders (Khurian et al., 2003). Various potent compounds have been isolated from the plant like rotenoids, Mirabijolone A, B, C and D (Saha et al., 2020). Literature suggests that the plant was proven to exhibit Antidiabetic, Antioxidant, cytotoxic, Antibacterial, Anti-inflammatory and Antiviral activities (Akintobi et al., 2011; Nath et al., 2010; Eneji et al., 2011; Zhou et al., 2012; Aher et al., 2016; Sadiq et al., 2018). Our recent study proved the cytotoxicity of isolated compound mirabijolone-B from the methanol extract of roots of plant on the keratinocyte, HaCaT cell lines. The invivo antipsoriatic activity was investigated on the IMQ induced psoriasis in Balb/C mice which showed similar activity compared to the standard drug, Clobetasol Tenovate (Manasa and Harikrishnan, 2024).

2. MATERIALS AND METHODS

Collection and extraction of plant material

Fresh roots of Mirabilis jalapa were collected from Hills of Tirumala, Tirupati in the month of December 2021 and authenticated by Prof. P. Jayaraman, Plant Anatomy Research Centre, Chennai (PARC/2022/168). The roots are cleaned out of sand and debris. The washed roots were cut into small pieces and dried under shade for 10-14 days in a well ventilated area. The dried crumbs of roots were powdered using a rotary blender and sifted to get a smooth powder which was stored in an air tight container till use. The powder (50g) was packed in a soxhlet apparatus and extracted using various solvents like Methanol and the obtained extract was filtered using whatman filter paper #4. The filtrate was collected and evaporated using a rotary vacuum evaporator and a dried extract (25.43%w/w) (MEM-Methanol Extract

of Mirabilis) in the form of paste was obtained.

Isolation of antipsoriatic compound from Methanol extract (MEM) of Mirabilis jalapa roots

Freshly cut pieces of roots of Mirabilis jalapa were collected from Seshachala hills of Tirumala, Tirupathi in December 2021 that were duly authenticated by Prof Jayaraman, PARC, Chennai. The roots were washed, dried under shade and made into a fine powder for extraction. 50g of powder was extracted with methanol using soxhlet apparatus and filtrate (whatman filter paper #4) (25.43%w/w). The extract (MEM) was subjected to coloumn chormatography (Silica gel #100-200) using various ratios of Dichloromethane and ethanol (90:10-10:90) and resultant fraction 8 was further subjected to recoloumn using various of Dichloromethane, Acetone and Ethanol. This resulted in 5 isolated fractions out of which Isolate 2 was subjected to further isolation using Methanol and chloroform (7:3 and 5:5) which gave DTDO. The solvent in the fraction was evaporated using rotary evaporator and the structural elucidation was performed using FTIR, C^{13} and H^{1} NMR and GC.

Formulation of DTDO loaded liposomes

Chemicals and reagents

All the chemicals and reagents used in this work were procured from Sigma-Aldrich, India and SD Fine Chem LTd. India. The reagents were of analytical grade and chemicals and drugs were of highest quality as stated by the company broucher.

Construction of standard graph of DTDO

10 mg of DTDO was dissolved in 10 ml of Methanol and diluted further to obtain 20µg/ml. solution The prepared solution was scanned on a UV scanner between 200-400 nm. The absorption maximum obtained in the graph was considered as λmax for the isolated drug, DTDO.

Required amount of DTDO was dissolved in Phosphate buffer (7.2pH) solution to achieve 100µg/ml stock solution. Further form this stock, sample solution of concentrations between 5-25µg/ml were prepared by serial dilutions. the absorbance was measured at 262nm using UV spectrophotometer. The standard curve was obtained by plotting absorbance v/s. concentration.

FTIR Analysis

The FTIR spectra of Pure drug DTDO and the physical co-mixture of excipients and DTDO were generated and anlysed to check the characteristic peaks for corresponding functional groups indicating any reaction, degradation or transformation within the formulation.

Formulation design of DTDO loaded liposomes

The liposomes loaded with DTDO were prepared using thin-film hydration method with Cholesterol, Soyalecithin and temperature of preparation as variables (Kim et al., 1997). Various concentrations (as per table 1) of cholesterol, soya lecithin and DTDO (50mg in all formulations) were solubilised in solution of 5ml of chloroform and methanol (2:1) in a round bottomed flask. Under reduced pressure in a rotary evaporator (100rpm), the film formation was observed at temperatures 40±2^{O}C. The film was collected and dried overnight. The dried films were rehydrated using distilled water (10ml) under controlled temperatures of hydration as per table. the suspension of the vesicles was sonicated (Cleaner 30A) for 30min at room temperature. The vesicles were allowed to settle at room temperature overnight and re-sonicated for 30mins and used in further experiments.

Table 1: Formulation design of the DTDO loaded liposomes

Formulation code	Cholesterol (mg)	Soya lecithin (mg)	Temp (°C)
DL1	200	400	50
DL2	200	400	60
DL3	400	400	50
DL4	400	400	60

DL5	200	800	50
DL6	200	800	60
DL7	400	800	50
DL8	400	800	60

Evaluation of the DTDO loaded liposomes

Determination of Entrapment Efficiency (EE%)

The liposomal suspension containing drug equivalent to 50mg was centrifuged at 10000rpm for 60min and the supernantant was separated. The solution was filtered using 0.45µm nylon membrane syringe filter. The amount of unentrapped DTDO was analysed using UV spectroscopy at 262nm using standard graph of DTDO. The drug entrapment efficiency (EE) was determined using the formula

$EE\% = (Wt - Wf)/Wt * 100$

Where, EE% = % entrapment efficiency of DTDO,

Wt = Loading weight of DTDO,

Wf = weight of unentrapped DTDO.

Determination of Stability of liposomes

The stability of the DTDO loaded liposomes was evaluated by determining the EE% by storing the liposomes at −25°C, 4°C and 25°C for 45days.

Invitro Drug Release studies

The release of DTDO from the liposomes was determined using dialysis method. The preservatives on the dialysis sacs (Sigma-Aldrich) were removed by soaking them in distilled water overnight at room temperature. The drug release study was performed in 150ml Phosphate buffered saline (PBS) (pH-5.6) with propylene glycol (7%v/v) and methanol (25%v/v) at 37°C. DTDO loaded liposomes equivalent to 50mg were dispersed in 1ml of bicarbonate buffer (pH-9) solution are placed in dialysis sac. Both ends of the sac were bound with threads and were hung inside a conical flask using a glass rod so that a portion of the sac lies inside the buffer solution. The solution was constantly stirred using a thermostat controlled magnetic stirrer at 100rpm at 37^{O}C. The aliquots of samples were withdrawn at regular intervals form 30min to 12hr and were assayed at 262nm using UV spectroscopy (Panwar et al., 2010; Zhang et al., 2005). The formulation showing best release of DTDO was chosen for further analysis.

TEM of DTDO-L Liposomes

The optimum liposome formulation DTDO-L was placed on the TEM grids and were dehydrated. TEM analysis was performed on TEM microscope (JEOL JEM 2100 HR-TEM) at 80 KV. The size measurements of the liposomes were obtained with TEM images.

Determination of vesicle size and charge

The mean vesicular size of liposomes was determined using PCS in tandem with Zetasizer Nano ZS-90 (Malvern Instruments Ltd., UK) at 90^{0} inclination to the incident radiation. Laser doppler anemometer was conjugated at base line ±150mV to determine the zeta potential of the prepared liposomes (Shabouri, 2003).

Formulation of DTDO-L liposomal gels

The prepare DTDO-L liposomal suspension was formulated into a topical carbopol gel for easy application of the formulation. Accurately weighed carbopol 640 was dissolved in distilled water in a beaker and stirred for 15min using magnetic stirrer to achieve 5%w/v carbopol gel. DTDO-L suspension was incorporated into the gel to achieve 5%w/w DTDO gel. Similarly 5%w/w gels were prepared using pure DTDO and methanol extract of Mirabilis (MEM) (Rasheed et al., 2011). These gels were used directly in further studies.

Determination of In-vivo Skin penetration

DTDO-L gel was mixed with Coumarin 6 as a fluorescent dye. The abdominal hair of the rats was shaved off carefully and 50mg/cm^2 of the prepared gel was applied on the surface of skin. 2hr after application of the gel, skin was excised under anesthesia and washed with distilled water to clear off the debris. The tissue was frozen and 100µm thick sections were prepared using a crytome for confocal mcroscopic analysis (Schindelin et al., 2019). The micrograpahic images were procesed using Fiji software (version 2.9.0/1.54f) using the Z-project function.

Invivo antipsoriatic activity

Experimental Animals

Wistar albino rats of both the sex (160-190g) were procured from local supplier from Bengaluru and were kept it polyacrylic cages with access to water and standard pellet feed. They were maintained at 22 ± 2 °C/ 50 ± 5 % RH and care was taken to prevent autophagy and suffering. The IAEC and CPCSEA approval was obtained before the starting of experiments.

Determination of dermal toxicity

Healthy animals were randomly selected for the acute dermal toxicity study. 24hr before study, fur on the dorsal surface of the rats was depilated and gels with DTDO-L, DTDO and MEM (targeted dose 2000mg/kg) were applied uniformly over the surface. The gels were held intact with the skin for 24 hr using dressing guage bound with non-irritable tape. The animals were carefully observed for any signs of irritation or inflammation for 24hr through 1hr intervals with continuous monitoring till 4 hr of administration (Pai et al., 2020; Vijayalakshmi and Geetha, 2014).

Invivo antipsoriatic activity

Anti-psoriasis activity of the prepared DTDO-L formulation was investigated using UV-B irradiation method for 14 days. The animals were mounted on a wooden board wiht its legs tied so as to prevent the movement of animal during UV exposure. an area of 1.5x2cm skin on the dorsal surface was depilated leaving which the entire animal was covered with UV protective film. UV-B (385nm) was 20cm above the animal and exposed for 20min daily (Vijayalakshmi and Geetha, 2014; Lakshmi et al., 2020). The rats were divided into 6 groups with 6animals each. Normal control and induced groups received 10ml/kg, p.o. normal saline solution, Standard group received 0.05% tretinoin gel for 14 days topical, Group IV, V and VI received formulations prepared from DTDO-L liposomal suspension, Pure Drug DTDO and Methanol extract (MEM) (5% in carbopol gel) topically for 14 days. The drugs were applied 12 hr after UV irradiation. A faint eythema and dryness was observed on the skin in 30min after UV-B exposure which turned into brownish red spot after 6hr and peaked by 24-30hr. significant erythematous sclaes and silvery white shredding plaques were noticed after 72hr (*Schon & Boehncke, 2005*). On the last day 2hr after application of gels, rats were sacrificed using anesthesia and indicators like epidermial thickness, scales, erythema, itching and overall PASI scores were determined on by scoring (0-none; 1-slight; 2-moderate; 3-marked; 4-severe) (Sun et al., 2013). The longitudinal sections of the skin were prepared for histological observations by staining with hematoxylin-eosin. The skin tissue of each rat was collected and homogenized using a tissue homogenizer with PBS at pH 7.4 and centrifuged for 15min at 5000rpm. The supernatant was collected and anlyzed for inflammatory paramters like IL-6, TNF and IL-1β using ELISA technique with available kits. The solution was also analyzed for the antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx), and MDA activity (at 532 nm) (Paglia et al., 1967).

RESULTS AND DISCUSSION

Preformulation studies

The roots of Mirabilis japala were extracted using methanol and the extract (MEM) was utilized to isolate an antipsoriatic compound using coloumn chromatography. after isolation, the compound was subjected to FTIR, NMR and MS analysis to determine the structure of the compound. It was identified similar to mirabijolone B which was isolated from the plant (Yi-Fen et al., 2002). The IUPAC name of the compound was derived as 4,6,9,11-tetrahydroxy-8,10-dimethyl-6,12-dihydro-5,7-dioxatetraphen-12-one and the compound was coded as DTDO (Manasa et al., 2023).

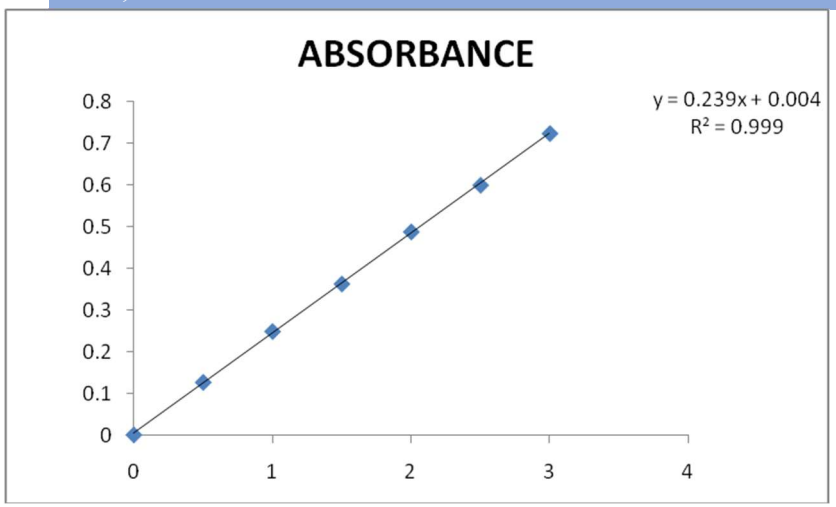

Figure 1: Standard graph of DTDO-L

FTIR analysis

The FTIR analysis were conducted to assess potential compatibility issues and interactions among the ingredients used in the DTDO-loaded liposomal formulations. The FTIR spectra confirmed that the characteristic peaks of the drug remained intact in the formulations, indicating no chemical interactions or incompatibilities between the ingredients. This suggests that the chemical composition of the drug was preserved during formulation development and that the excipients were chemically inert under the conditions employed. These findings validate the selection of the excipients and ensure the stability and efficacy of the formulations, supporting their suitability for further development and application.

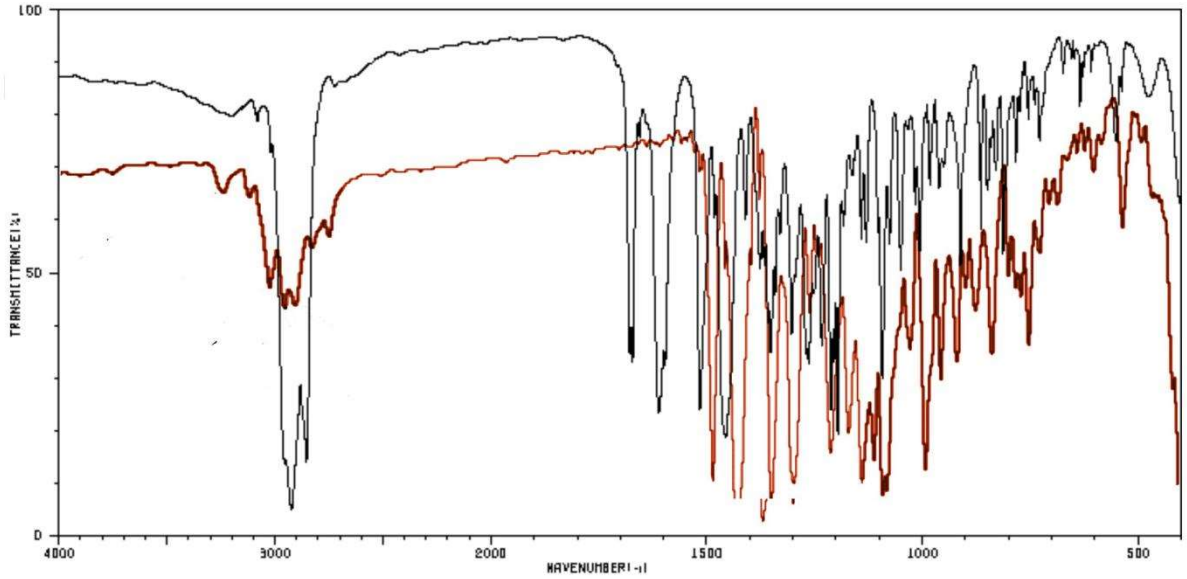

Figure 2: Overlapped FTIR spectra of DTDO and DTDO admixture with excipients

Drug entrapment of DTDO in liposomes

The results of the drug entrapment and stability studies for DTDO-loaded liposomes (Table 2) revealed notable differences among the formulations. Drug loading values ranged from **17.08 mg (DL3)** to **31.05 mg (DL6)**, indicating variations in the encapsulation capacity of the liposomal formulations. The highest drug entrapment efficiency was

observed in **DL6 (62.11%)**, highlighting its superior encapsulation potential.

Stability study

Stability assessments across storage conditions (-25°C, 4°C, and 25°C) suggested minimal variations in entrapment efficiency, confirming the stability of all formulations. Notably, DL6 retained its high entrapment efficiency across all temperatures, underscoring its suitability for long-term storage. Similarly, **DL5**, with efficiencies ranging between **55.99% and 56.37%**, exhibited comparable stability. In contrast, DL1 and DL3 showed lower entrapment efficiencies, which may be attributed to less optimized formulation parameters, suggesting room for improvement in their compositions.

Table 2: Drug entrapment and Stability studies of DTDO loaded liposomes

Formulation	Free DTDO (mg)	Drug loading (mg)	Entrapment Efficiency (%) RT	Entrapment Efficiency (%) RT		
				-25^{O}C	4^{O}C	25^{O}C
DL1	26.09±0.57	23.90±0.57	47.81±1.14	45.35±0.21	45.64±2.06	45.69±0.89
DL2	24.75±0.56	25.25±0.56	50.5±1.13	51.80±2.07	52.36±1.35	51.44±1.58
DL3	32.92±0.33	17.08±0.33	34.16±0.67	34.27±1.03	33.40±1.52	34.63±0.66
DL4	29.16±0.55	20.83±0.55	41.66±1.11	41.55±1.23	41.86±1.33	42.79±1.05
DL5	21.96±0.18	28.04±0.18	56.08±0.36	55.99±0.34	56.37±1.82	54.87±0.70
DL6	18.94±0.55	31.05±0.55	62.11±1.10	62.94±0.45	60.95±1.26	61.14±0.62
DL7	25.71±0.52	24.29±0.52	48.58±1.04	48.64±0.43	50.01±1.20	51.55±0.45
DL8	23.72±0.81	26.28±0.81	52.56±1.62	53.72±1.23	53.19±0.85	52.73±0.33

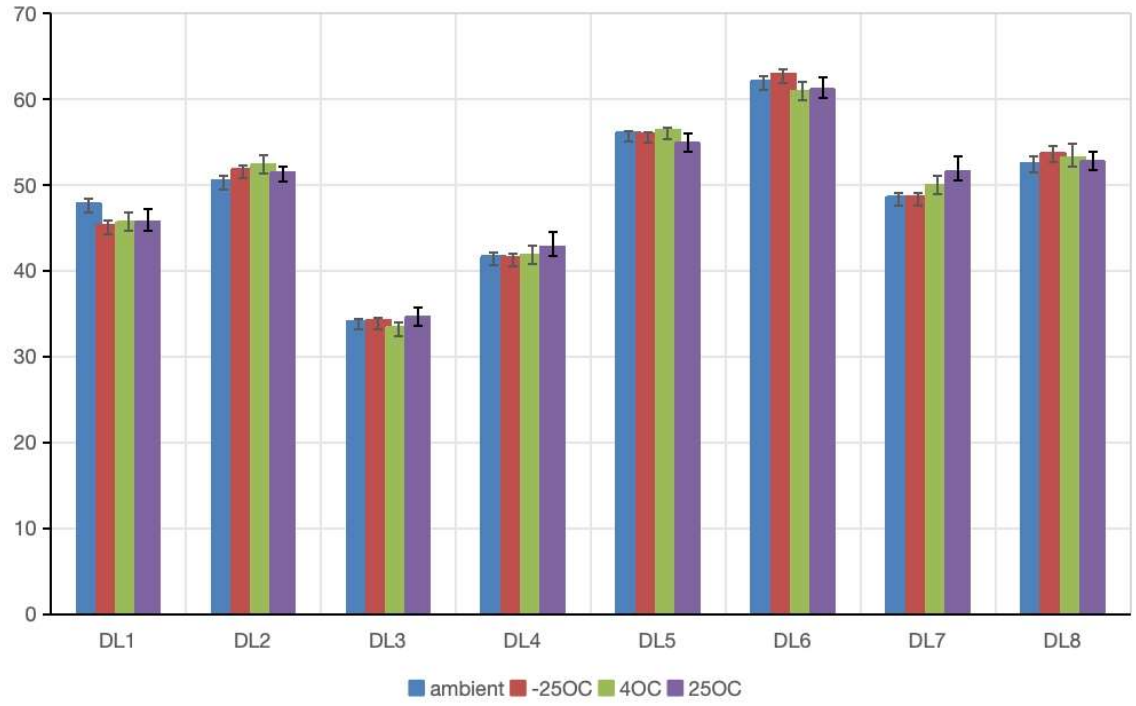

Figure 3: Drug entrapment and stability studies of the formulations.

Drug release studies

The drug release studies (Table 3) provided further evidence for the performance of these liposomal formulations. All formulations exhibited time-dependent release, with several achieving near-complete drug release by 12 hours. Among

these, DL6 was concluded as the most promising formulation, achieving 99.85% release at 12 hours with a consistent and sustained release profile. Similarly, DL7 and DL8 demonstrated efficient release profiles, with drug release exceeding 90% within 12 hours. These formulations also displayed a moderate burst release in the initial phases, providing a rapid onset of action. On the other hand, DL3 and DL4 exhibited slower release kinetics.

Table 3: Drug release studies of DTDO loaded liposomes

Formulation	0.5	1	2	4	6	8	12
DL1	20.06±0.96	26.55±2.24	38.71±1.56	56.41±2.96	62.18±0.40	70.66±1.99	79.11±0.85
DL2	21.54±1.82	29.56±0.53	48.25±0.75	67.34±0.23	80.23±1.91	87.11±0.90	91.32±3.98
DL3	11.05±1.33	15.66±1.83	31.25±2.62	52.25±4.28	65.24±3.27	71.06±1.64	82.19±1.25
DL4	15.42±0.75	19.06±0.60	29.55±0.38	53.82±0.82	69.03±2.84	75.41±4.21	82.11±4.80
DL5	25.24±2.25	29.65±0.99	42.36±1.60	63.82±0.07	79.03±1.88	85.14±1.48	92.21±2.78
DL6	38.77±0.39	62.19±0.65	82.18±1.37	95.55±1.87	99.51±3.39	99.64±0.30	99.85±3.77
DL7	27.55±0.36	32.19±0.83	62.22±2.81	82.36±0.14	90.05±3.05	94.06±2.51	97.62±5.07
DL8	33.28±0.11	55.49±0.77	72.41±3.72	85.88±1.09	93.09±0.02	97.61±3.89	99.06±4.20

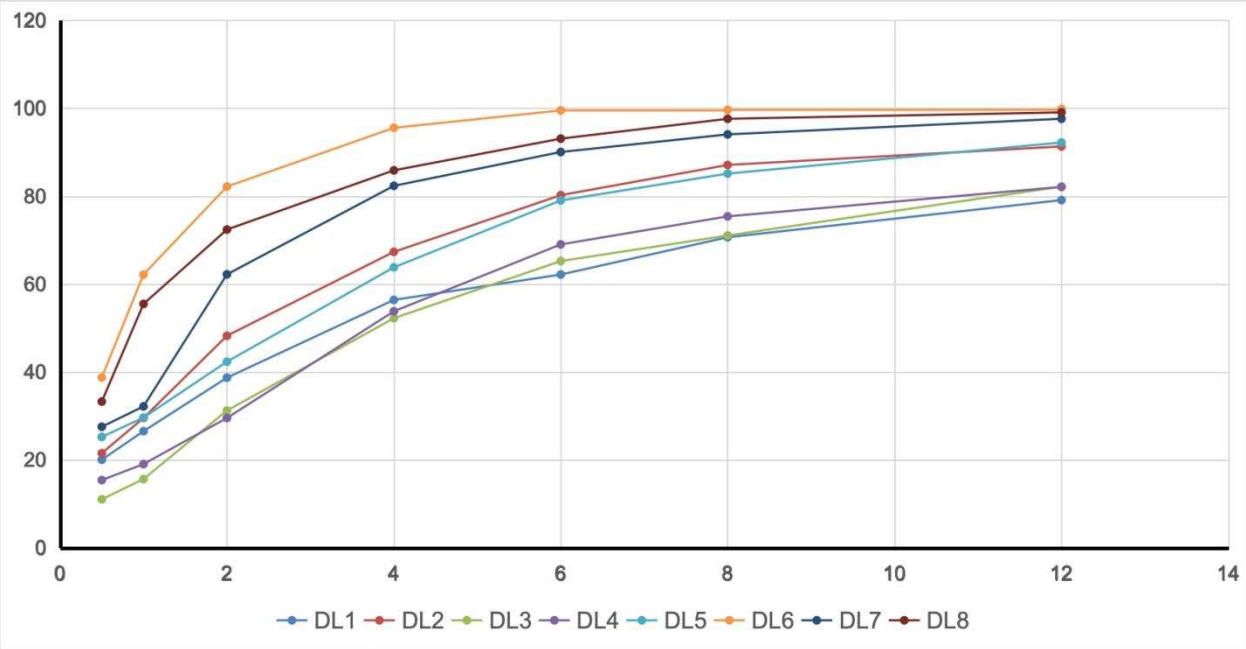

Figure 4: Drug Release studies from various DTDO liposomal formulations

Selection of optimal formulation

The data collectively highlight DL6 as the optimal formulation, combining high drug loading, superior entrapment efficiency, stability across storage conditions, and a balanced release profile. This makes DL6 an ideal candidate for applications requiring prolonged therapeutic effects with minimal degradation over time. DL5, DL7, and DL8 also demonstrated favorable characteristics, making them viable alternatives, though with slightly lower entrapment efficiencies. These findings provide a strong basis for further exploration of DL6 coded as DTDO-L in preclinical and clinical settings.

TEM analysis

TEM images provided clear evidence of the liposomal structure, as well as the deposition and dispersal of the liposomes within the sample. After observing the TEM images, the morphology of the DTDO-L is smooth and even. Notably, the liposomes exhibit a distorted sphere shape, characterized by quasi-spherical structure with a remarkably smooth surface. This observation indicates the effectiveness of the DTDO-L with well-defined and uniform shapes. This variability in size and shape could arise from the inherent complexity of biological processes and the dynamic difference between biological molecules and the synthesis pathways (Jaidev and Narasimha, 2010). Despite their spherical nature, there is observable slight agglomeration, indicating a tendency of the liposomes to cluster. This agglomeration phenomenon is likely attributed to the presence of bio-molecules within the sample, which may contribute to the cohesive forces leading to the clustering of particles (Polte, 2015).

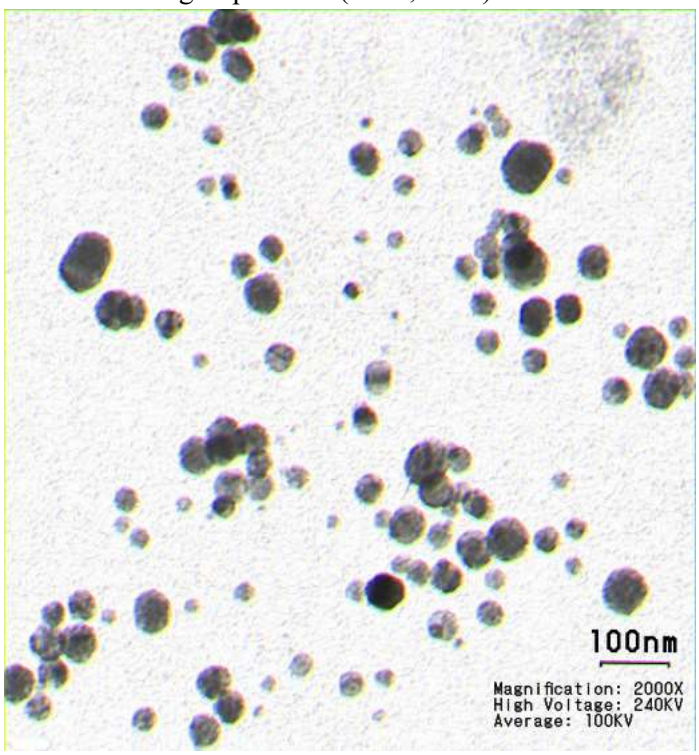

Figure 5: TEM analysis of DTDO-L

Particle size and Zeta potential analysis

The Zeta potential of the nanoparticles was found to be -15.42mV showing the charge on the surface of liposomes is not neutral thus showing stability. As the charge is less than -30mV it can be suggested that there may be slight agglomeration of the particles (Faried et al., 2016; Paosen et al., 2017). This negative potential indicates that the surface charge might be due to the presence of charged groups from DTDO such as phenolic and carbonyl groups. The particle size of liposomes was found to be 169.84±43.8nm.

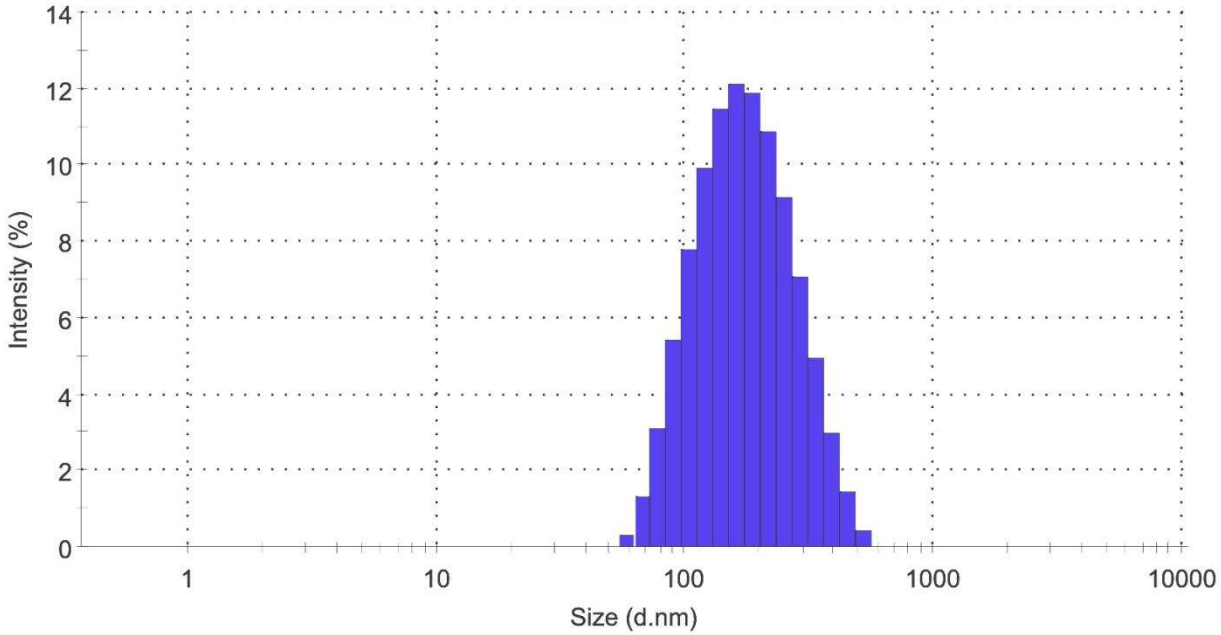

Figure 6: Particle size analysis of DTDO-L

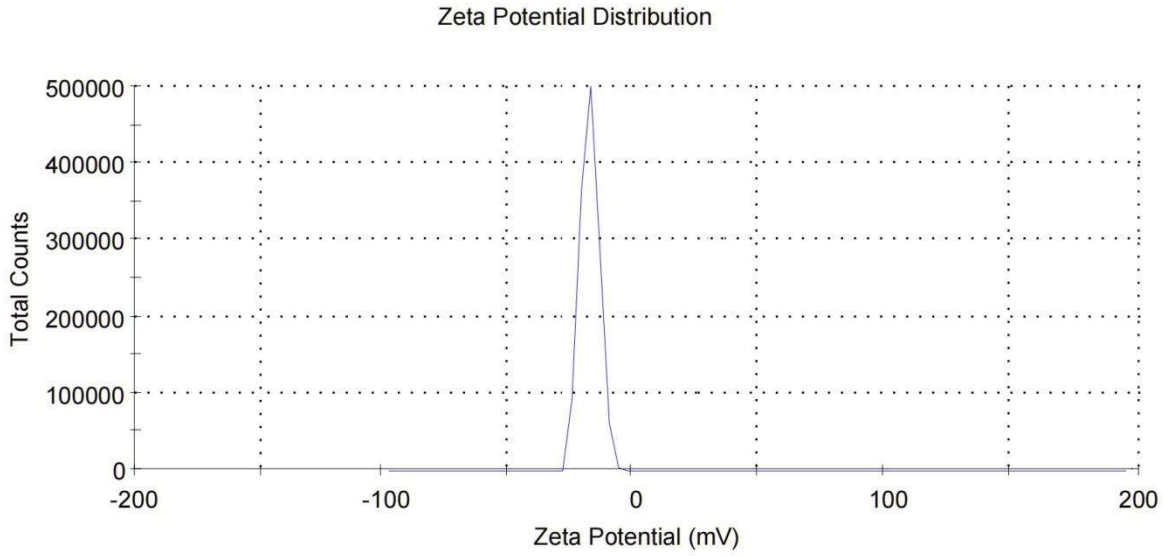

Figure 7: Zeta potential analysis of DTDO-L

Skin penetration and deposition of DTDO-L

The penetration study revealed that the liposomal gel formulation (DTDO-L) successfully penetrated the dermis to a depth of approximately 100 microns. The success of this penetration can be attributed to the nanoscale size of liposomes, which facilitates their movement through intercellular lipid pathways and enables bypassing the skin's barrier function.

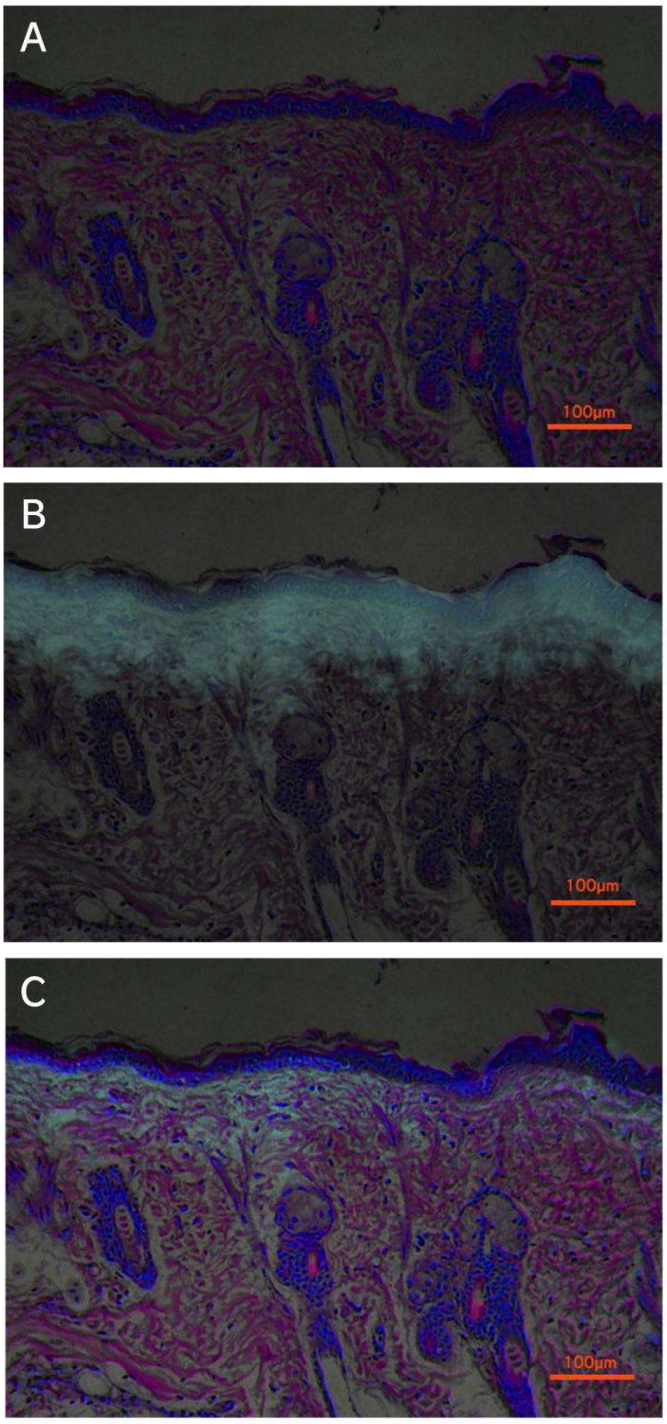

Figure 8: Invivo Skin penetration images of DTDO-L

Effect of DTDO Liposomal Gel on Erythema, Thickness, and Scale Scores

The effect of DTDO liposomal gel (DTDO-L) was evaluated on erythema, thickness, and scale scores in UV-B-induced psoriasis. In the induced group, the erythema (3.57±0.74), thickness (3.86±0.94), and scales scores (3.86±0.39) were significantly elevated compared to the normal control (0.17±0.37, 0.52±0.85, and 0.34±0.48, respectively, $P<0.001$). Treatment with the standard 0.05% tretinoin gel markedly reduced these scores to 0.58±0.44, 0.68±0.47, and 0.81±0.85 ($P<0.001$) respectively. DTDO-L showed comparable efficacy to the standard, with erythema (0.35±0.61), thickness

(0.51±0.29), and scale scores (0.37±0.62) being significantly reduced. In contrast, the 5% DTDO gel and MEM gel exhibited a lesser reduction, indicating the superior efficacy of the liposomal formulation in controlling the psoriasis symptoms.

Table 4: Effect of Liposomal gel (DTDO-L) on Erythema, thickness and scales scores in UV-B induced psoriasis

Group	Erythma Score	Thickness Score	Scales scores
Control-Normal saline 10ml/kg	0.17±0.37	0.52±0.85	0.34±0.48
Induced-Normal saline 10ml/kg	3.57 ± 0.74^{A}	3.86 ± 0.94^{A}	3.86 ± 0.39^{A}
Standard-0.05% w/w tretinoin gel	0.58 ± 0.44^{B}	0.68 ± 0.47^{B}	0.81 ± 0.85^{B}
DTDO-L-5%w/w DTDO liposomal gel	0.35 ± 0.61^{B}	0.51 ± 0.29^{B}	0.37 ± 0.62^{B}
DTDO-5%w/w DTDO gel	2.05 ± 0.52^{BD}	2.39 ± 076^{bd}	1.65 ± 0.8^{Bd}
MEM-5%w/w extract loaded gel	2.54 ± 0.48^{bD}	2.85 ± 0.55^{D}	2.39 ± 0.38^{bD}

The values were expressed as mean±SEM; A, B, D-indicates significant at P<0.001 compared to control, UV-B and Standard groups respectively; a,b and d indicates significant at P<0.01 compared to control, UV-B and Standard groups respectively

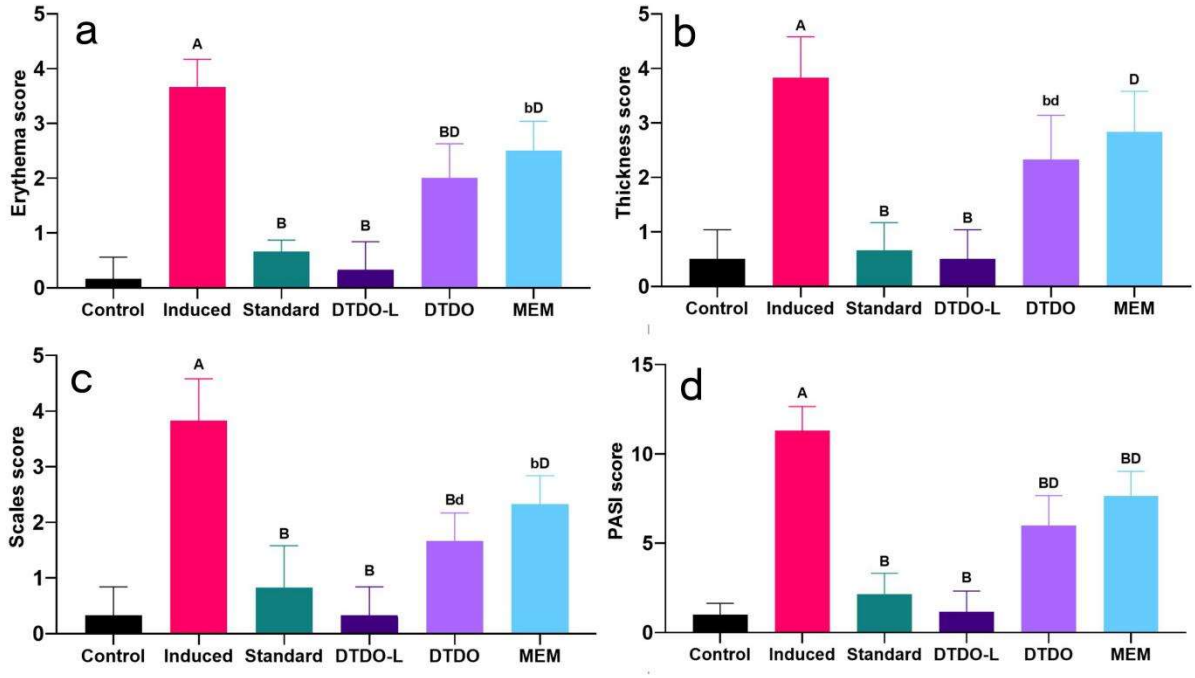

Figure 9: Effect of DTDO-L gel on the posriatic paramters. a.Erythema scores, b. Thickness score, c. Scales score and d.PASI score

Effect of DTDO Liposomal Gel on Psoriasis scores

The Psoriasis Area and Severity Index (PASI) and epidermal thickness were significantly increased in the induced group (11.43±2.17 and 107.86±9.44 µm, respectively, P<0.001). Treatment with DTDO-L significantly decreased PASI (1.22±1.06) and epidermal thickness (49.53±5.02 µm), showing values comparable to the standard tretinoin gel group (2.28±1.25, and 59.21±4.86 µm, respectively). The DTDO gel and MEM gel treatments showed partial improvement but were less effective than DTDO-L, highlighting the enhanced therapeutic potential of the liposomal formulation.

Table 5: Effect of Liposomal gel (DTDO-L) on the PASI score, ear and epidermal thickness in UV-B induced psoriasis

Group	PASI Score	Epidermal thickness (µm)
Control-Normal saline 10ml/kg	1.06±0.43	41.05±4.39
Induced-Normal saline 10ml/kg	11.43±2.17^{A}	107.86±9.44^{A}
Standard-0.05% w/w tretinoin gel	2.28±1.25^{B}	59.21±4.86^{B}
DTDO-L-5%w/w DTDO liposomal gel	1.22±1.06^{B}	49.53±5.02^{Bd}
DTDO-5%w/w DTDO gel	6.06±1.99^{Bd}	63.66±4.28^{d}
MEM-5%w/w extract loaded gel	7.48±1.64^{bD}	88.76±4.55^{D}

The values were expressed as mean±SEM; A, B, D-indicates significant at P<0.001 compared to control, UV-B and Standard groups respectively; a,b and d indicates significant at P<0.01 compared to control, UV-B and Standard groups respectively

Effect of DTDO Liposomal Gel on Inflammatory Cytokines

UV-B-induced psoriasis resulted in significant elevations of inflammatory cytokines IL-6 (9.78±0.54 pg/ml), TNF-α (20.02±0.74 pg/ml), and IL-1β (23.49±3.45 pg/ml) in the induced group compared to the normal control (2.54±0.35, 2.64±0.62, and 7.75±0.47 pg/ml, respectively, P<0.001). Treatment with DTDO-L significantly reduced cytokine levels (IL-6: 2.86±0.23 pg/ml, TNF-α: 3.38±0.48 pg/ml, IL-1β: 8.33±0.54 pg/ml) to values close to those achieved by the standard tretinoin gel (3.87±0.74, 4.64±0.44, and 9.46±0.38 pg/ml, respectively). DTDO gel and MEM gel exhibited moderate reductions in cytokine levels, further reinforcing the superior anti-inflammatory activity of the DTDO liposomal gel.

Table 6: Effect of Liposomal gel (DTDO-L) on the inflammatory cytokines in UV-B induced psoriasis

Group	IL-6 (pg/ml)	TNF-α (pg/ml)	IL-1β (pg/ml)
Control-Normal saline 10ml/kg	2.54±0.35	2.64±0.62	7.75±0.47
Induced-Normal saline 10ml/kg	9.78±0.54^{A}	20.02±0.74^{A}	23.49±3.45^{A}
Standard-0.05% w/w tretinoin gel	3.87±0.74^{B}	4.64±0.44^{B}	9.46±0.38^{B}
DTDO-L-	2.86±0.23^{Bd}	3.38±0.48^{B}	8.33±0.54^{B}

5%w/w DTDO liposomal gel			
DTDO-5%w/w DTDO gel	4.47 ± 0.84^{B}	7.32 ± 0.58^{BD}	10.51 ± 0.66^{B}
MEM-5%w/w extract loaded gel	6.62 ± 1.33^{BD}	10.55 ± 0.56^{BD}	15.84 ± 1.37^{BD}

The values were expressed as mean±SEM; A, B, D-indicates significant at P<0.001 compared to control, UV-B and Standard groups respectively; a,b and d indicates significant at P<0.01 compared to control, UV-B and Standard groups respectively

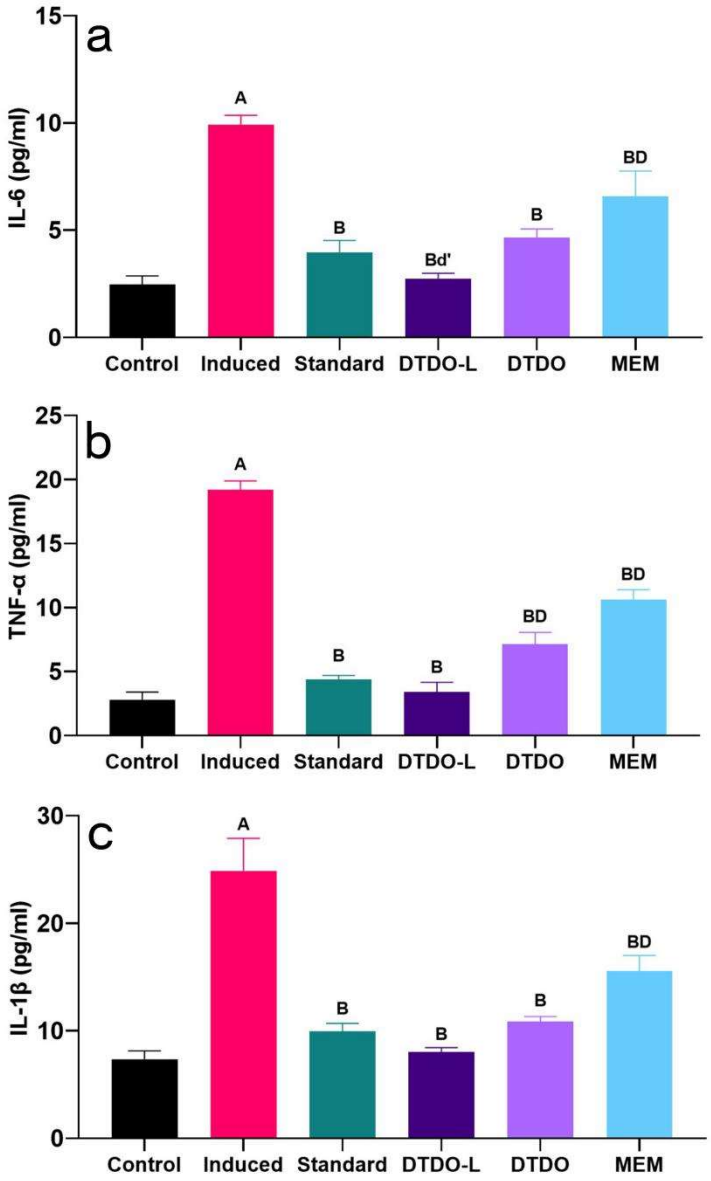

Figure 10: Effect of DTDO-L on the inflammatory markers.

Effect of DTDO Liposomal Gel on Antioxidant Parameters

UV-B-induced psoriasis caused significant oxidative stress, as evidenced by decreased antioxidant parameters in the induced group: SOD (4.38±0.33 u/mg protein), CAT (43.57±2.28 µM H2O2/mg protein), GPx (5.38±0.44 µg/mg protein), and GSH (23.22±1.36 µg/mg protein), along with an increase in MDA levels (13.39±0.74 nM/mg protein, P<0.001). DTDO-L treatment restored antioxidant levels, bringing SOD (8.53±0.86), CAT (82.39±1.38), GPx (9.95±0.28), and GSH (53.06±1.47) near control levels, with a significant reduction in MDA (2.88±0.94). These results paralleled the effects of the standard tretinoin gel. In summary, DTDO liposomal gel (DTDO-L) demonstrated remarkable efficacy in reducing psoriatic symptoms, inflammatory cytokines, and oxidative stress markers, with performance comparable to or surpassing the standard tretinoin gel. These results underline the potential of DTDO-L as a superior therapeutic option for managing UV-B-induced psoriasis.

Table 7: Effect of Liposomal gel (DTDO-L) on the antioxidant parameters in UV-B induced psoriasis

Group	SOD u/mg protein	CAT µM H_2O_2 /mg protein	Gpx µg/mg protein	GSH µg/mg protein	MDA nM/mg protein
Control-Normal saline 10ml/kg	8.65±0.64	93.64±1.45	9.67±0.62	56.64±1.52	7.04±0.47
Induced-Normal saline 10ml/kg	$4.38±0.33^{A}$	$43.57±2.28^{A}$	$5.38±0.44^{A}$	$23.22±1.36^{A}$	$13.39±0.74^{A}$
Standard-0.05% w/w tretinoin gel	$8.67±0.29^{B}$	$84.38±1.41^{B}$	$9.55±0.64^{B}$	$54.05±1.29^{B}$	$2.83±0.48^{B}$
DTDO-L-5%w/w DTDO liposomal gel	$8.53±0.86^{B}$	$82.39±1.38^{B}$	$9.95±0.28^{B}$	$53.06±1.47^{B}$	$2.88±0.94^{B}$
DTDO-5%w/w DTDO gel	$7.77±0.81^{Bd}$	$65.28±3.56^{Bd}$	$8.58±0.63^{Bd}$	$47.32±1.27^{Bd}$	$5.19±0.28^{Bd}$
MEM-5%w/w extract loaded gel	$6.36±0.45^{BD}$	$52.11±1.38^{BD}$	$8.35±0.41^{Bd}$	$39.27±0.84^{BD}$	$6.56±0.39^{BD}$

The values were expressed as mean±SEM; A, B, D-indicates significant at P<0.001 compared to control, UV-B and Standard groups respectively; a,b and d indicates significant at P<0.01 compared to control, UV-B and Standard groups respectively

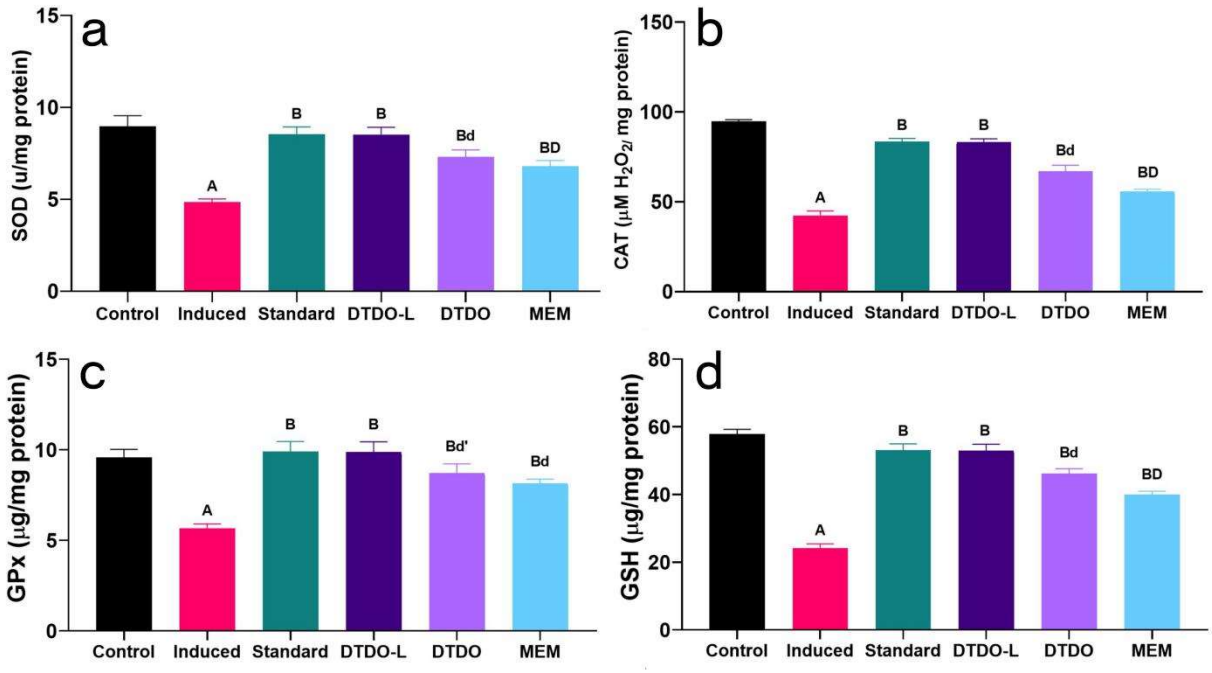

Figure 11: Effect of DTDO-L on the antioxidant enzymes

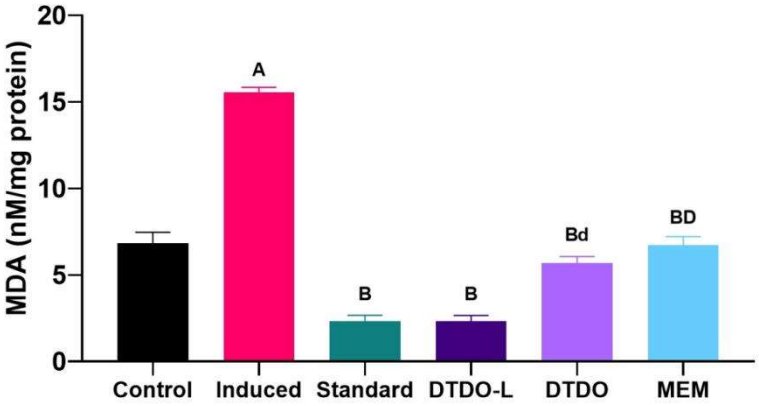

Figure 12: Effect of DTDO-L on the lipid peroxidation

DISCUSSION

The skin, being the largest exposed organ of the body, is highly susceptible to immune-mediated chronic inflammatory disorders, including psoriasis, dermatitis, urticaria, and angioedema (Meeuwis et al., 2011). Psoriasis, in particular, significantly affects patients' quality of life and poses a substantial economic burden due to its chronic nature and high treatment costs (Krueger et al., 2001). Traditional medicinal plants, known for their safety and diverse bioactive compounds like quercetin, have garnered considerable attention as potential sources for novel psoriasis treatments (Kaur et al., 2012; Chen et al., 2017).

In this study DTDO, was isolated from Mirabilis jalapa roots was fomrulated into liposomal gels and investigated for the antipsoriatic activity in UV-B method. This study highlighted the superior therapeutic efficacy of DTDO-L liposomes

in the treatment of UV-B-induced psoriasis. DTDO-L liposomes demonstrated significant reductions in inflammation, keratinocyte hyperproliferation, and epidermal abnormalities, indicative of their potential as an advanced drug delivery system. Liposomes enhance drug bioavailability by enabling controlled release and targeted delivery to the stratum corneum, overcoming the barrier posed by this outermost skin layer. The findings corroborate previous studies demonstrating the advantages of lipid-based carriers in managing skin disorders. Liposomal formulations containing methotrexate and calcipotriol have shown enhanced skin penetration and reduced systemic side effects, although challenges in sustained drug release and penetration into deeper skin layers persisted (Bahramizadeh et al., 2019; Knudsen et al., 2012). The DTDO-L formulation appears to address these limitations effectively, likely due to optimized encapsulation and its ability to modulate inflammatory pathways.

The UV-B-induced psoriasis model employed in this study is a well-established tool for replicating human psoriasis pathology. UV-B irradiation causes keratinocyte hyperproliferation and an inflammatory cascade marked by elevated cytokines such as TNF-α, IL-6, and IL-1β, which contribute to psoriatic plaques and epidermal thickening (Sun et al., 2013). In line with previous literature, the untreated psoriasis-induced group exhibited heightened levels of these cytokines (Kaur et al., 2017). The DTDO-L-treated group showed significant reductions in these markers, emphasizing the anti-inflammatory potential of its bioactive compounds. The enhanced efficacy of DTDO-L liposomes over conventional formulations stems from their unique structural properties. Liposomes provide a dual hydrophilic and lipophilic environment, which enables encapsulation of bioactive agents targeting multiple inflammatory pathways, such as TNF-α and IL-17. This dual action not only suppresses inflammation but also stabilizes keratinocyte turnover, thereby addressing two critical aspects of psoriasis pathology (Piao et al., 2023). This mechanism aligns with findings from other studies on nanocarrier-based psoriasis treatments that highlight improved drug stability and efficacy through liposomal encapsulation.

Compared to standard treatments like tretinoin gel, DTDO-L exhibited better outcomes in terms of inflammation control and skin normalization. The nanoscale size of the liposomes facilitates deeper penetration into psoriatic plaques, ensuring localized drug action while minimizing systemic exposure and associated adverse effects. These observations are consistent with research emphasizing the pharmacokinetic advantages of nanocarriers in delivering psoriasis drugs effectively (Niehues et al., 2022). In conclusion, DTDO-L liposomes emerge as a promising strategy for psoriasis management, offering enhanced therapeutic outcomes through improved skin penetration, localized drug delivery, and modulation of inflammatory and oxidative pathways. Future studies should explore their long-term safety and comparative effectiveness in clinical settings to validate their potential as an alternative or adjunct to existing therapies.

CONCLUSION

Though psoriasis is debilating and challenging chronic skin condition, this study highlights the therapeutic efficacy of DTDO-L liposomal gel in managing psoriasis. The liposomal formulation demonstrated significant superiority in mitigating psoriatic symptoms such as erythema, scaling, and epidermal thickness, with remarkable reductions in pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) and oxidative stress markers. These findings are in par with already confirmed advantages of liposomal systems, which enhance drug bioavailability, skin retention, and targeted delivery. The enhanced efficacy of DTDO-L in normalizing keratinocyte proliferation and modulating inflammatory pathways highlights its potential as an alternative to conventional treatments, which often present limitations such as systemic side effects and improper penetration thourgh skin layers. By addressing critical challenges in psoriasis management, DTDO-L offers a novel therapeutic approach with the potential to improve patient outcomes significantly. Future clinical studies are essential to validate these findings and translate this promising therapeutic innovation into clinical practice.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest between the authors

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