Journal of Drug Targeting

ISSN: 1061-186X (Print) 1029-2330 (Online) Journal homepage: www.tandfonline.com/journals/idrt20

Combined photodynamic therapy and hollow microneedle approach for effective non-invasive delivery of hypericin for the management of imiquimod-induced psoriasis

Mariam Zewail, Haidy Abbas, Nesrine El Sayed & Heba Abd-El-Azim

To cite this article: Mariam Zewail, Haidy Abbas, Nesrine El Sayed & Heba Abd-El-Azim (2024) Combined photodynamic therapy and hollow microneedle approach for effective non-invasive delivery of hypericin for the management of imiquimod-induced psoriasis, Journal of Drug Targeting, 32:8, 941-952, DOI: 10.1080/1061186X.2024.2365930

To link to this article: https://doi.org/10.1080/1061186X.2024.2365930

JOURNAL OF DRUG TARGETING
2024, VOL. 32, NO. 8, 941–952
https://doi.org/10.1080/1061186X.2024.2365930

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Combined photodynamic therapy and hollow microneedle approach for effective non-invasive delivery of hypericin for the management of imiquimod-induced psoriasis

Mariam Zewail[a], Haidy Abbas[a], Nesrine El Sayed[b] and Heba Abd-El-Azim[a#]

[a]Department of Pharmaceutics, Faculty of Pharmacy, Damanhour University, Damanhour, Egypt; [b]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Cairo University, Cairo, Egypt

ABSTRACT

Background: Conventional topical psoriasis treatments suffer from limited delivery to affected areas and skin irritation due to high local drug concentration.

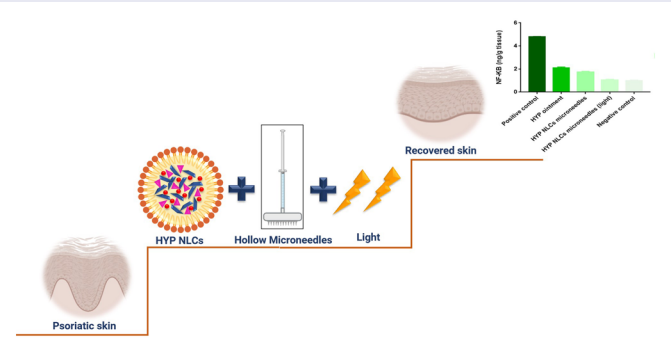
Purpose: This study aims to prepare hypericin (HYP) loaded nanostructured lipid carriers (NLCs) and their application in psoriasis treatment through intradermal administration using hollow microneedles assisted by photodynamic therapy.

Methods: The colloidal characteristics of NLCs, entrapment efficiency and morphology were evaluated. *An ex-vivo* skin distribution study was conducted along with testing the *in vivo* antipsoriatic activity in mice with the imiquimod-induced psoriasis model.

Results: The particle size and zeta potential of HYP-NLCs were 167.70 nm and −18.1, respectively. The *ex-vivo* skin distribution study demonstrated the superior distribution of HYP-NLCs to a depth of 1480 µm within the skin layers relative to only 750 µm for free HYP. *In vivo* studies revealed that the levels of NF-KB, IL 6, MMP1, GSH, and catalase in the group treated with HYP-NLCs in the presence of light were comparable to the negative control.

Conclusions: The histopathological inspection of dissected skin samples reflected the superiority of HYP-NLCs over HYP ointment. This could be ascribed to the effect of nanoencapsulation on improving HYP properties besides the ability of hollow microneedles to ensure effective HYP delivery to the affected psoriatic area.

GRAPHICAL ABSTRACT



ARTICLE HISTORY

Received 7 December 2023
Revised 18 May 2024
Accepted 3 June 2024

KEYWORDS

Intradermal delivery; Photosensitizers; Autoimmune diseases; Microneedles; Hypericin

Introduction

Psoriasis is a common autoimmune skin disease marked by increased epidermal proliferation and dermal inflammation. The most common skin lesions are red, scaly, sharply demarcated, indurated plaques. Psoriasis affects approximately 3% of the general population [1]. Even though no age group is exempt, most psoriasis patients are young or middle-aged adults [2]. Psoriasis significantly reduces patients' quality of life and life expectancy. Psoriasis patients may suffer from depression and social stigma. Also, psoriasis has been linked to heart disease and lymphoma [3]. Psoriasis pathogenesis is a multi-factorial process based on uncontrolled increased proinflammatory cytokine expression, such as IL-17, IL-21, IL-22, IL-23, and IL-26. IL-17 and IL-23 stimulate keratinocyte proliferation and increase TNF-α and chemokine secretion, which leads to dendritic cell activation inflammation [4–6].

Depending on the severity of the disease, psoriasis can be treated with topical, systemic, or phototherapy. Regarding therapeutic interventions, topical treatments are the first line of defense. Phototherapy and conventional systemic medications are often utilised when the condition is mild to severe and topical therapies are no longer helpful [7]. Topical preparations include a variety of therapeutic agents like corticosteroids, synthetic vitamin D3 analogues, retinoid derivatives or anthralin. Meanwhile, systemic

CONTACT Haidy Abbas haidy.abass@pharm.dmu.edu.eg; haidy_miu2002@yahoo.com Department of Pharmaceutics, Faculty of Pharmacy, Damanhour University, Damanhour, Egypt.
[#]Additional affiliation: Division of Engineering in Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA.

treatments may include the administration of calcineurin inhibitors, immunosuppressive medications, acitretin and isotretinoin [8,9]. Conventional topical treatments of psoriasis necessitate the administration of drugs at high doses for a certain period and this may lead to inadequate, unpredictable delivery and local toxicity due to skin irritation [8].

Despite the variability of treatment options, many psoriasis patients remain untreated, necessitating the development of new medications with superior long-term efficacy and safety. Many debates surround the use of photodynamic therapy (PDT) in psoriasis management. If topical and systemic treatments fail, phototherapy is typically prescribed for patients with moderate to severe psoriasis [8,10]. Phototherapy has been used by ancient Egyptians to treat skin disease. Also, Romans and Greeks used sunlight for therapeutic purposes. Phototherapy, especially for treating psoriasis, started in the early twentieth century. In 1925, Dr. William Goeckerman documented the benefits of treating psoriasis using UV light and unrefined coal tar. Also, Dr. John Ingram established a therapy regimen in the 1950s that used UVB radiation, coal tar, and anthralin paste. In the 1970s, broadband UVB was revealed to be beneficial in eliminating moderate types of psoriasis when administered in doses, whereas ultraviolet A (UVA) irradiation in conjunction with either oral, or topical application of psoralen was found to be useful in treating psoriasis. In the 1980s, researchers identified a more defined wavelength of UVB referred as narrowband UVB [11]. After that, psoralen ultraviolet A (PUVA) therapy or PDT, that combines a photosensitising medication (PS) and UVA radiation was utilised. This approach is now the first-line treatment for resistant psoriatic plaques [12]. When PS is triggered by light irradiation, the agent generates reactive oxygen species (ROS), which damage adjacent sick cells [13]. PDT plays an important role in inhibiting the uncontrolled production of inflammatory cytokines that cause T-lymphocyte apoptosis and inflammation during psoriasis progression [12,14,15].

Several clinical trials reported the use of 5-aminolevulinic acid (ALA) along with PDT for psoriasis management [12]. Liu et al. [16] reported the case of a 49-year-old male patient with persistent plaque psoriasis. The patient had a psoriasis lesion on his finger that included a pyogenic granuloma (PG). After one week of ALA-PDT therapy, evidence of improvement was seen. There was no evidence of recurrence after one month of therapy [16].

Other agents have been used including the study reported by Salah et al. [17] who tested the efficacy of PDT and methylene blue photoactivated with a 565 mW light emitting diode (LED) at 670 nm was assessed in patients with resistant plaque psoriasis. Results revealed the complete recovery of sixteen patients with normal skin colour and texture [17].

Several PSs have demonstrated low solubility and aggregation in water, which may cause them to be photodynamically inactive in aqueous solutions this may act as a barrier for their *in vivo* application. An optimum PS is required to show adequate water solubility and high singlet oxygen quantum yield in aqueous solution. Insoluble PS have been encapsulated in different nanocarriers to enable their utilisation in aqueous form [18].

A large group of nanocarriers have been extensively investigated in the last few years as a potential approach for improving drug delivery through the skin to treat psoriasis [19]. These nanocarriers include solid lipid nanoparticles, nanostructured lipid carriers, liposomes, niosomes, ethosomes, transfersomes, dendrimers and micelles [20]. Nanocarriers have several advantages as they can increase penetration and localisation of the loaded drug(s) in the skin thus systemic side effects are reduced and treatment outcomes are improved [19,21].

Solid lipid nanoparticles and nanostructured lipid carriers (NLCs) NLCs are both hydrophobic drug carriers that are scattered inside the centre of lipid particles. The sole difference is in structure; NLCs do not have a perfect crystal structure, therefore they allow better drug encapsulation with less drug leakage [12]. NLCs have been used for psoriasis management and have proven efficacy like the study reported by Hatem et al. who prepared luteolin loaded NLCs and reported that luteolin NLCs increased skin deposition by 3.3 folds in comparison to luteolin suspension. Furthermore, NLCs demonstrated superior anti-psoriatic effects [22].

Hypericin (HYP) is a naturally occurring photodynamic polycyclic phenanthroperylenequinone derivative extracted from Hypericum perforatum. HYP is considered one of the most effective photosensitizers [23]. It slightly reduces cell proliferation, but its performance improves significantly when it is irradiated at its excitation wavelength (590 nm) [24,25]. HYP can modulate the immune system responses in patients with mild to moderate psoriasis. It acts by regulating CD8-mediated cytotoxicity and inhibiting TNFα-induced apoptosis [26]. Rook et al. [27] reported the ability of HYP accompanied with visible light PDT to significantly improve cutaneous T-cell lymphoma and psoriasis in patients after 6 weeks of treatment suggesting its potential use in psoriasis as an alternative to psoralen [27]. Also, Najafizadeh et al. [28] reported the ability of HYP ointment to reduce psoriasis area severity (PASI) index scores in mild plaque-type psoriasis in patients after twice daily applications for four weeks [28]. Furthermore, Mansouri et al. [26] reported the ability of HYP to significantly PASI scores and TNFα levels in psoriatic tissues in twenty patients with mild to moderate plaque-type psoriasis [26].

Unfortunately, HYP suffers from poor aqueous solubility, low skin penetration capabilities and the possibility of inducing liver toxicity in high doses [23,29]. Therefore, these limitations highlight the importance of developing an innovative localised delivery system for improving HYP efficacy against psoriasis while reducing its dose.

Microneedles (MNs) are a new skin delivery device comprised of micron-sized sharp projections arranged in an array [30]. They are intended to pass through the stratum corneum without reaching the nerve endings thus providing painless drug administration [30]. They can provide sustained and controlled drug delivery in intradermal and transdermal drug delivery with high precision of drug localisation and dose reduction potential [25,31,32].

Ho-MNs can form microchannels in skin layers upon insertion that permit vertical drug diffusion and promote local drug accumulation and as a result, the pharmacological efficacy of drugs is enhanced [31]. Ho-MNs have several advantages, including the ability to deliver an accurate adjustable dose of medication and controlled drug delivery rate [25,30,33]. In the case of psoriasis, for example, resin Ho-MNs proved highly effective in successfully delivering methotrexate to psoriatic skin [34,35].

The present study's primary goal is to provide a safe and effective treatment of psoriasis that maximises treatment outcomes and increases patient compliance. This was attained by the preparation and characterisation of HYP-loaded nanostructured lipid carriers (NLCs). Their *in vivo* efficacy was evaluated in mice with an imiquimod-induced psoriasis model following intradermal administration using hollow microneedles and the application of light.

To our knowledge, this is considered the first study reporting the utilisation of HYP-loaded NLCs in PDT in psoriasis management following their intradermal administration by hollow microneedles.

Materials

Compritol 888 ATO and F 127 were provided by Gattefossé (Saint-Priest, France) and Pharco Pharmaceutical Companies

(Alexandria, Egypt), respectively. Oleic acid was bought from Nice Chemicals (Kerala, India). Hypericin (Isolated from H. perforatum, purity > 99% HPLC) was obtained from Planta Natural Products GmbH, Erlgasse 48, Austria. Commercial hypericin ointment (Bianca Rosa®, PE 0.3% hypericin) was purchased from Bianca Rosa (Canada). AdminPen™ metallic hollow microneedles were bought from NanoBioSciences LLC, USA. The rest of the chemicals and reagents are analytical grade.

Methodology

Preparation of blank and HYP-loaded NLCs

Preparation of HYP-loaded NLCs was conducted using the method reported earlier by Zewail et al. [36] as follows, a lipid phase composed of 100 mg of compritol and oleic acid in a ratio of (7: 2) was kept at 80 °C and an aqueous phase composed of 0.2 or 0.4% w/v F127 was heated to the same temperature. Then, the hot aqueous phase was added to the melted lipid phase and ultrasonicated using (SonicaR 2200 EP S3, Soltec, Milan, Italy) at 60% for 5 min. Finally, the formed primary emulsion was added to an equal volume of deionised water and sonicated for an extra 5 min at 60%.

For the preparation of HYP NLCs, Figure 1A, the HYP amount was first dissolved in a minimum amount of dimethyl sulfoxide and added to the lipid phase. The rest of the preparation procedure was carried out by the same procedure that was used for the preparation of blank NLCs. The prepared formulations were stored in the refrigerator for further investigation.

Characterisation of blank and HYP-loaded NLCs

Measurement of particle size and zeta potential

At a scattering angle of 173° at 25 °C, the average particle size, polydispersity index (PDI), and zeta potential values were determined using a Zeta sizer Nano ZS (Malvern Instrument, UK).

Measurement of entrapment efficiency (EE%) and drug loading (DL %)

Centrifugal ultrafiltration with a Centrisart®-I tube (MWCO 300 kDa, Sartorius AG, Goettingen, Germany) separated HYP-loaded NLCs from supernatant containing excess unentrapped HYP using HPLC at 590 nm was used to determine the concentration of unentrapped HYP in the supernatant.

$$\%\ EE\ (indirect) = \frac{Total\ HYP\ concentration - concentration\ of\ unencapsulated\ HYP}{Total\ HYP\ concentration} x100$$

DL was estimated by measuring HYP concentration in a given weight of freeze-dried NLCs.

$$DL\ (\%) = \frac{concentration\ of\ encapsulated\ HYP}{weight\ of\ NLCs}\ x\ 100$$

Morphological examination using transmission electron microscope (TEM)

The morphology of HYP-loaded NLCs formulation was investigated using TEM (JEM-100CX; JEOL, Japan) after staining with uranyl acetate.

Intradermal injection using AdminPen™ hollow microneedles array

Full-thickness intact human skin was kindly donated by a male volunteer after abdominal plastic surgery. Initially, subcutaneous fats were detached using suture scissors. The skin surface was cleaned with normal saline, dried, wrapped in aluminium foil and refrigerated at −20 °C. It has been reported that under these conditions, human skin could be kept up to 6 months [37].

The AdminPenTM, which consists of 43 stainless steel metallic Ho-MNs of 1200 m length located within 1 cm^2 of a circular MN array, was used to inject HYP solution and HYP-NLCs into the upper surface of washed full-thickness human skin. The AdminPenTM Ho-MNs were inserted into the skin using the thumb and index finger technique.

Assessment of hollow microneedles-assisted delivery of hypericin-loaded NLCs

Ex vivo puncture-ability of microneedles

The *ex vivo* puncture-ability of AdminPen™ Ho-MNs was evaluated as previously reported with some modifications [23]. The Ho-MNs

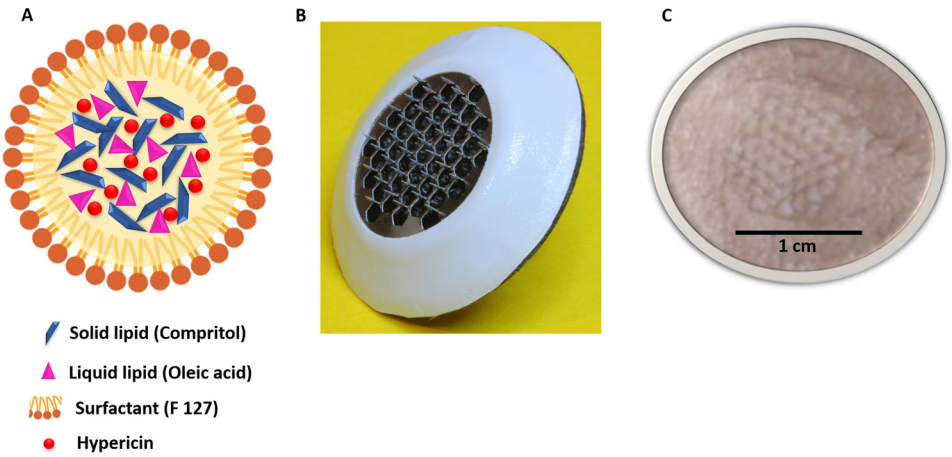


Figure 1. A) Schematic representation illustrating the prepared HYP NLCs. B) AdminPen™ hollow MNs with 43 stainless-steel MN shafts of 1200 µm in height (image was used with permission from AdminMed). C) Image captured by a standard mobile camera (Samsung Galaxy Note8, 12 MP, dual pixel, PDAF) showing excised full-thickness human skin after insertion of MNs.

were inserted into the skin according to the protocol stated in Section 3.3, kept for 30 s then removed. Following that, photographs of the skin were taken with a Samsung Galaxy Note8, 12 MP, dual pixel, PDAF camera.

The percentage of percutaneous penetration was measured by counting the number of visible holes created by Ho-MNs and using the following equation:

$$\text{The percentage of penetration} = \frac{\text{Number of created holes in skin}}{\text{Total number of hollow microneedles}} \times 100$$

Ex vivo skin distribution studies

Two female volunteers (37–45 years old) provided full-thickness human skin samples following abdominal plastic surgery, and signed informed permission forms were acquired to utilise them. Surgical scissors were used to remove fatty tissues under the skin. The skin surface was cleansed with saline solution, dried, wrapped in aluminium foil, and kept at 20 °C. Human skin may be stored in these circumstances for 3–6 months [38].

Ex vivo dermal distribution of free HYP and HYP-NLCs in full-thickness human skin was assessed using a Leica DMi8 Confocal Laser Scanning Microscope (Leica Microsystems, Wetzlar, Germany). The tested formulations were intradermally injected using the Ho-MNs and the images were recorded 2 h after treatment.

Pharmaceutical analysis of HYP

A recently developed and validated HPLC method [23, 39] was adopted in this study for HYP quantitative analysis. An Agilent UHPLC 1290 series instrument (Agilent Technologies, Santa Clara, CA, USA) was utilised for the analysis. It consisted of a quaternary pump G4204A, an automatic injector G4226A controlled at an injection volume of 20 µL, a column compartment G1316C set at 30 °C and a diode array detector (DAD) G4212A adjusted at 590 nm. The UPLC was connected to a computer loaded with Agilent OpenLAB CDS ChemStation Edition Software. The employed stationary phase was a Microsorb MV-C18 column (250 × 4.6 mm, 5 µm particle size) (Agilent Technologies, the Netherlands). The mobile phase was [18.37 mM potassium dihydrogen phosphate (pH 3.5): methanol] 5: 95%v/v. The flow rate was 1.2 ml/min and the chromatographic run was 7 min. The HYP calibration curve was constructed by diluting its standard stock dilution (400 µg/mL) with methanol to obtain concentrations within the range of 1 – 30 µg/mL.

In vivo studies

Ethical statement

The experimental procedures were accepted by the Ethical Committee for experimental and clinical studies at the Faculty of Pharmacy, Cairo University, with the permit number (PT 3272) approval were valid since 27 March 2023. The procedure was performed by the guidelines for the Care and Use of Laboratory Animals (NIH Publication, 2011, 8th Edition).

Experimental animals

Male Wistar mice weighing 150–200 gm were housed under constant temperature (25 °C), and humidity, with alternate 12 h light and dark cycles. A standard diet and free water access will be allowed throughout the experimental study. Forty male mice will be divided into five groups containing 8 mice per each; the study was carried out for 1 week. Group I: (Negative control) mice received normal saline. Psoriasis induction was carried out in Groups II, III, IV and V by the method previously reported by Elgewelly et al. [27] by the application of Imiquimod cream (ALDARA® cream 5%) daily at a dose of 62.5 mg on the shaved back and in the right ear of mice. Group II received normal saline and was kept as a positive control group. On the other hand, groups III, IV and V received HYP ointment, and HYP-NLCs in the absence and presence of light, respectively Groups IV and V received HYP-NLCs through intradermal injection by hollow MNs. HYP dose in both HYP ointment and HYP-NLCs was equivalent to 0.13 mg/kg.

After a week of treatment, mice were sacrificed by cervical dislocation under light anaesthesia and their skin was rapidly removed and weighed. Figure 2 illustrates a schematic representation of the *in vivo* experiment.

ELISA

The serum levels of granulocyte-macrophage colony-stimulating factor (GM-CSF), glutathione S transferase (GSH), interleukin 6 (IL 6), catalase, matrix metalloproteinases 1 (MMP1) and nuclear factor kappa B (NF-KB) were determined using an ELISA kit based on the stated instructions (Glory Science Co, Ltd, USA).

Histopathological examination

The skin of mice of different experimental groups was fixed in 10% (v/v) formalin for 72 h to perform histopathological examination. Samples were trimmed and processed in successive grades of alcohol, then cleared in xylene before being infiltrated and embedded in Paraplast tissue embedding media. Haematoxylin and eosin-stained tissue sections were examined under a light microscope.

Statistical analysis

Data are represented as mean ± SD. All statistical analyses were carried out using Prism 7 software. The results of ELISA were analysed by One-way ANOVA ($p < 0.0001$), and the rest of the results were analysed by student's t-test.

Results and discussion

Preparation, particle size and zeta potential measurements of NLCs

Nanotechnology has emerged as one of the most promising medicinal areas in recent years. This field has led to the creation of innovative nanostructured drug release devices, as well as the use of novel psoriasis therapeutics. While nanotechnology paired with PDT has been widely explored in other skin illnesses, its use in psoriasis therapy has received little study attention [12].

Lipid-based colloidal carriers are considered a promising approach to improve the solubility of hydrophobic drugs [40,41]. NLCs possess superior properties compared with solid lipid nanoparticles including higher stability with less drug expulsion, higher drug loading ability, reduced burst effect and their ability to provide a sustained drug release profile [42–44]. HYP, being a poorly soluble drug NLCs is considered a good choice for its nanoencapsulation and loading. To our knowledge, this is the first report for the preparation of HYP-NLCs for intradermal treatment of psoriasis by hollow microneedles. Even though HYP NLCs have been previously prepared by Sato

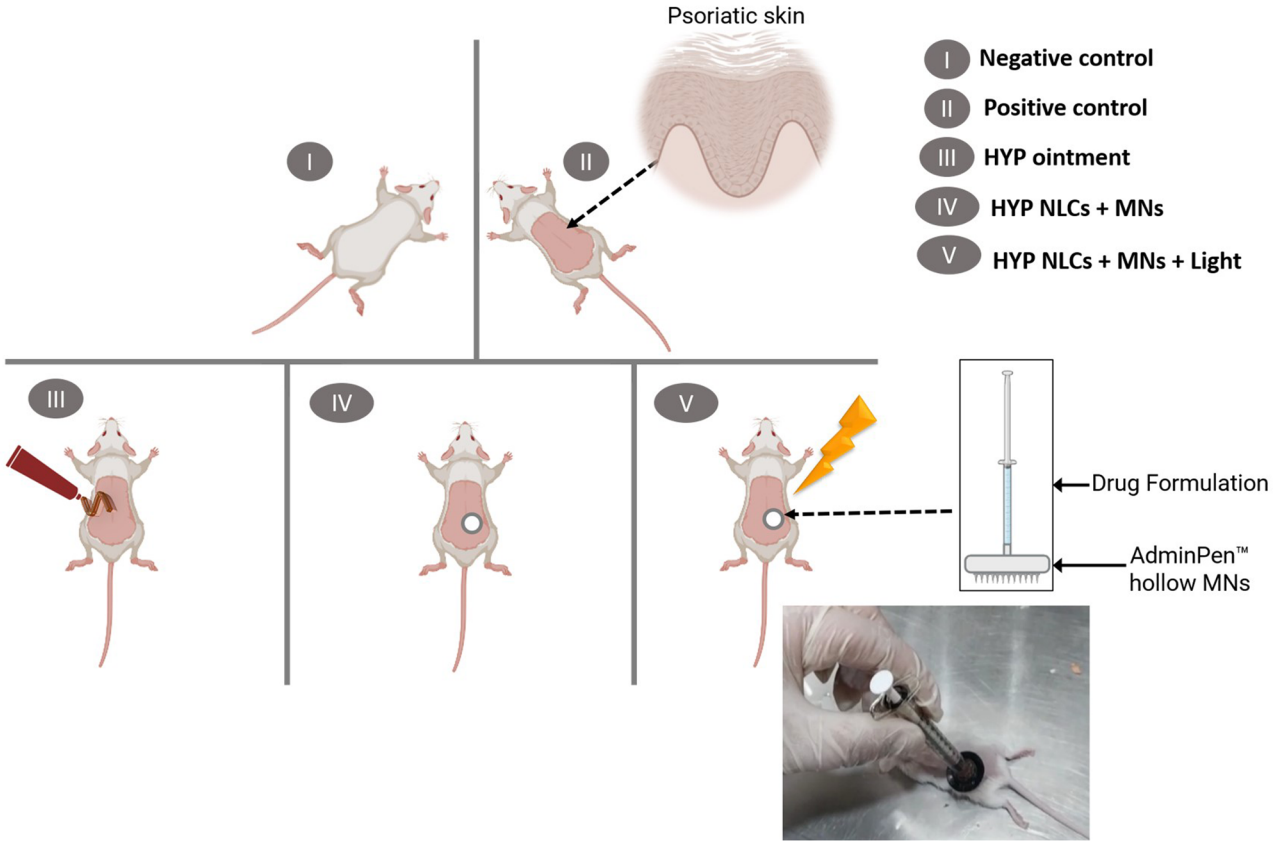


Figure 2. A) Schematic representation showing the different treatment groups investigated in the *in vivo* study and further characterisation techniques. B) Intradermal administration of drug formulations using AdminPen™ hollow MNs into the psoriatic lesion in mice.

et al. [45] and evaluated their effect combined with PDT for the treatment of vulvovaginal candidiasis, our formulation has a completely different composition compared with theirs and this study is considered the first one reporting the use of HYP NLCs assisted with Ho-MNs for psoriasis management. NLCs were formulated using melt emulsification method that has proven efficacy in the preparation of lipophilic drugs with high entrapment efficiency and without the incorporation of organic solvents [35,36,44]. Compritol 888 ATO was utilised as solid lipid due to its effectiveness in drug entrapment due to its complicated structure and less precise orientation, providing more area for drug loading. The extended chain length of behenic acid in Compritol 888 ATO increases drug trapping by interchain intercalation [46]. Oleic acid was selected as a liquid lipid as it is known to increase the percutaneous absorption of drugs [47]. Also, it acts as a permeability enhancer by acting selectively on the extracellular lipids [48].

Surfactants play an important role in reducing surface tension and surface energy of particles [49]. F127 was used in the preparation of NLCs. It is made up of non-linear hydrophobic chains that can arrange themselves within the lipid layer, causing particle compaction and, ultimately, particle size [49]. The increase in surfactant concentration can increase the stability and decrease the particle's surface tension and as a result, particle size is reduced [50]. Also, F127 is known as a stabiliser for lipid nanoparticles [51]. Muller et al. [52] reported that SLNs composed of compritol and pluronic had low toxicity in differentiated HL60 cells suggesting their high safety profile [52]. Also, Chen et al. reported that oleic acid loaded NLCs had no cytotoxic effects on neutrophils [53]. Furthermore, it was reported that NLCs composed of oleic acid and palmitic acid had no detrimental effects on the cell membrane of TR 146 cells [54].

As shown in Table 1 increasing F127 concentration from 0.2% to 0.4% resulted in a significant decrease in particle size (Student t-test $p < 0.05$). The particle size of F1 (composed of 0.2% F127) and F2 (composed of 0.4% F127) decreased from 166 nm to 123.6 nm, respectively. The addition of HYP resulted in a significant increase in NLCs' particle size compared to their corresponding blank formulation (Student t-test $p < 0.05$). The particle size of HYP-NLCs (F3) was 167.7 nm. PDI is a measure of dispersion or the standard deviation of size distributions [55]. PDI of the prepared formulation has values less than 0.4 indicating nanodispersion homogeneity (Table 1). PDI values reflect the degree of nanodispersion homogeneity, values closer to zero indicate a more uniform dispersion [56–58].

Zeta potential has a pivotal role in imparting the stability of emulsion as it controls particle surface charges. High surface charges generate repulsive forces, which prevent particles from coalescing and flocculating. Zeta potential values are less than ± 30 mV so they are considered stable [57,59–62]. As Table 1 illustrates, F1, F2 and F3 carried negative surface charges that ranged from −16.4 to −18.1 confirming the stability of the prepared NLCs. The addition of HYP resulted in a slight increase in zeta potential from − 17 to −18.1.

Measurement of efficiency entrapment (EE%) and drug loading (DL %)

HYP analytical determination was performed using a previously published HPLC method [23,25]. The obtained analytical linear range was 1 – 30 µg/mL and the corresponding regression was: Y = −43.76 + 53.83 X (R2 = 0.999).

Table 1. Colloidal characteristics of HYP NLCs.

Formulation	HYP conc. (µg/ml)	F 127 conc. (%, w/v)	Particle size (nm)	PDI	Zeta potential (meV)	EE (%)	DL (%)
F1	---------	0.2	166.00 ± 1.23	0.360 ± 0.12	−16.40 ± 0.56	---------	---------
F2	---------	0.4	123.60 ± 1.34	0.320 ± 0.45	− 17.00 ± 0.01	---------	---------
F3	50	0.4	167.70 ± 2.45	0.349 ± 0.89	− 18.10 ± 0.12	98.24 ± 0.06	0.39 ± 0.02

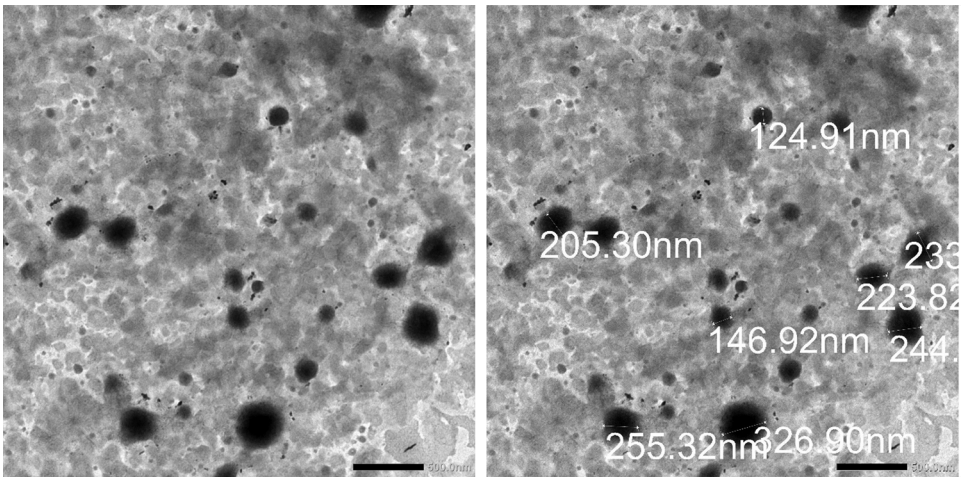


Figure 3. TEM micrograph of HYP NLCs.

HYP had a high EE % in NLCs. EE % in F3 was about 98.24% ± 0.06. This is along with previous reports of high EE% of poorly soluble drugs in NLCs [35,36]. This may be attributed to the imperfect core structure of NLCs that permit greater accommodation of lipophilic drugs like HYP and prevent its abrupt storage [63]. The concentration of HYP in the formulation was 50 µg/ml as shown in Table 1 by substituting in the equation mentioned in section 3.2.2., DL % of F3 was about 0.39%. This may be attributed to the fact that HYP has a potent effect and is used in very small amounts [23].

TEM

As shown in Figure 3, morphological examination of HYP-NLCs revealed that the prepared nanocarriers had spherical shapes with smooth outlines and uniform distribution. The absence of drug crystals in the examined fields revealed the good encapsulation of HYP within the prepared NLCs. The particle size of NLCs ranged from 124.9 to 326 nm. The largest particle size is around 300 nm suggesting its capability to infiltrate the skin layers as well as attain great skin deposition [64].

Ex vivo puncture-ability of microneedles

Conventional topical psoriasis treatments suffer from poor skin permeation, low skin retention of drug formulation and lack of controlled release. On the other hand, systemic drug administration has numerous side effects and poor patient compliance. In this study we investigated the role of nanoencapsulation assisted with using Ho-MNs to overcome poor skin permeation to deliver HYP locally to effectively treat psoriasis. Ho-MNs can increase the bioavailability, along with achieving the desired therapeutic effect with a lower dosage. Nonetheless, MNs might provide long-term drug delivery, resulting in lower dosage frequency. All of these benefits contribute significantly to lowering total treatment costs, as well as mitigating the disadvantages of alternative formulations [65].

Successful dermal piercing is crucial for MNs to employ their intended action [66]. To further explore, the AdminPen™ Ho-MNs were used to puncture the excised human skin. As illustrated in Figure 1C, evident 43 holes were observed after MNs removal indicating that AdminPen™ Ho-MNs had successfully and entirely punctured the stratum corneum with a dermal penetration percentage of 100%. Thus, the proposed MN-NLCs combined delivery system demonstrated a promising approach for the effective transdermal delivery of HYP to psoriatic lesions.

Ex vivo skin distribution studies

Excised human skin is considered the "gold standard" for evaluating *in vitro* permeation investigations [38]. Although mice, rats and humans have the same skin structure in epidermis, dermis and hypodermis, stratum corneum thickness differs among them. In mice, it is about 5 µm while in humans it ranges between 10 and 20 µm [67]. In this study, we wanted to test the penetration depth of HYP-loaded NLCs in human skin in an attempt to closely mimic clinical conditions.

According to a preliminary study that we conducted and previously published research articles like the studies reported by Rosita et al. [68] and Ghazwani et al. [69]. NLCs can reach skin depth lower than that could be attained using HoMNs alone. Rosita et al. reported that NLCs reached a depth of 412 µm and 1079 µm after 2 and 4.5 h of skin application [68]. Also, Ghazwani et al. reported that NLCs could only reach 30 µm [69]. The application of MNs alone could lead to skin penetration depth that reaches 1200 µm [13,23,25]. Therefore, there was an unmet necessity for the use of ID delivery system to overcome the skin barrier properties and enable deep skin delivery.

To implement the main aim of the current study of delivering HYP NLCs deeply into the psoriatic lesion through the skin, the loaded NLCs were injected into full-thickness excised human skin using AdminPen™ Ho-MNs where HYP liquid formulations were freely seeped into the deeper skin layers through the created

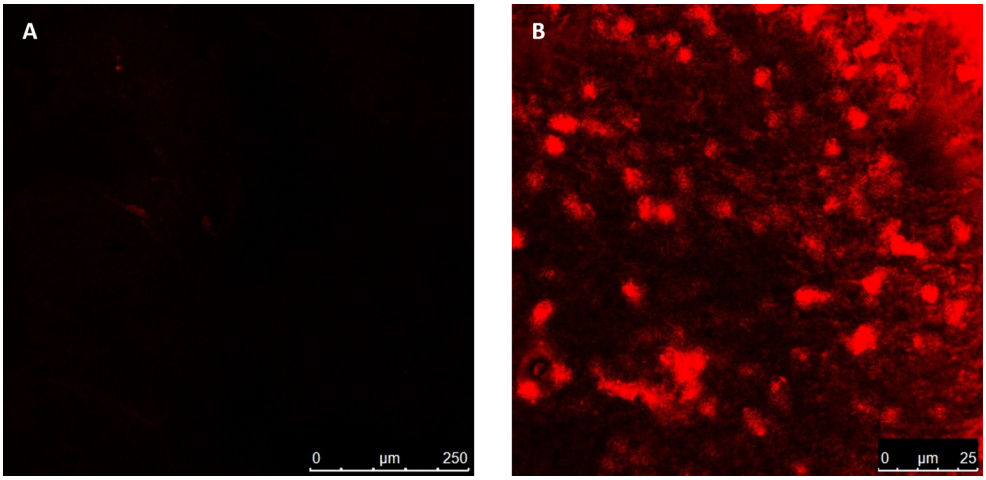


Figure 4. Confocal fluorescence images of excised full thickness human skin after injection of, A) HYP solution as a control and B) HYP NLCs using AdminPen™1200 µm Ho-MNs.

microchannels without any observed needle blockage or formulation leakage.

Confocal images were reproduced to assess the extent of intradermal distribution of the fluorescent HYP from loaded NLCs versus control HYP solution following AdminPen™ Ho-MNs application. It is worth mentioning that HYP solubility in biological samples could be correlated to the fluorescence signal recorded by confocal microscopy [23,70]. The reproduced images, Figure 4, showed a higher fluorescence signal for HYP-NLCs compared to hardly detected fluorescent intensity for free HYP solution reflecting higher solubility for the encapsulated HYP. Moreover, results showed the distribution of HYP-NLCs to a depth of 1480 µm within the skin layers relative to only 750 µm for free HYP. These findings revealed that HYP-NLCs were able to move within the skin layers and distribute themselves to a depth beyond the MN length (1200 µm).

The lipophilic nature of the free HYP contributes to the formation of stable non-fluorescent aggregates in biological tissues that strongly interact with the stratum corneum hindering the intradermal distribution of free HYP [71]. Besides, free HYP leans towards forming hydrogen bonding with the surrounding biological components. Both phenomena result in hindering skin penetration of free HYP [70]. Interestingly, encapsulation of HYP into NLCs masked its ability to form unwanted hydrogen bonding, prevented aggregate formation and improved its solubility in biological conditions. Accordingly, NLCs enhanced the depth and extent of intradermal distribution for HYP. Collectively, the presented Ho-MN system combined with NLCs could hypothetically amplify the localised availability of HYP in psoriatic lesions leading to enhanced therapeutic outcomes.

In vivo study

Psoriasis was induced using an imiquimod-induced mouse model that has been reported as an effective model for psoriasis induction [9,72–74]. This model was chosen because of its ability to mimic many human plaque-type psoriatic inflammatory features such as skin erythema, acanthosis, thickening, parakeratosis, scaling and neoangiogenesis, as well as the inflammatory presence of T cells, neutrophils, and dendritic cells [72]. Mice were divided into five groups; negative control, positive control, HYP ointment, and HYP-NLCs using AdminPen™ Ho-MNs in the absence and presence of light.

ELISA

Psoriasis is a multi-factorial skin disease with a complex pathogenesis. It is thought that several factors contribute to psoriasis pathogenesis [75]. MMPs are thought to be involved in epidermal structural changes through changing the intracellular connections and extracellular matrix composition, increasing angiogenesis in cutaneous blood vessels and immune cell infiltration [76].

NF-KB is a transcription factor that belongs to the Re1 family and regulates the activity of many proinflammatory genes that possess a significant role in psoriasis. Many of the psoriasis-causing factors have been discovered to cause inflammation by activating NF-KB [77]. Activation of NF-KB has been associated with increased levels of different inflammatory mediators and genes like MMP1 and MMP 9 genes [78]. Also, IL 6 is thought to have a direct contribution to the epidermal hyperplasia observed in psoriasis also IL 6 affects the function of dermal inflammatory cells [79].

In addition, GS-CSF can modulate inflammatory reactions and activate T cells. It was detected in most of the scale extracts from psoriasis and its level was significantly higher than that from the controls [80]. The activity of antioxidant enzymes like CAT is through to plays an important role in the pathogenesis of psoriasis. Drewa et al. [81] reported that catalase level was 27% lower in psoriasis patients compared with the control subjects [81]. Furthermore, GSH plays an anti-inflammatory role in controlling the proliferation of keratinocytes [82].

Levels of IL6, MMP1, NF-KB, GS-CSF, catalase and GSH were evaluated at the end of the experiment as Figure 5 illustrates. Generally, levels of IL6, GM-CSF, MMP1, and NF-KB were elevated compared with the negative control group. On the contrary, levels of catalase and GSH were depressed in all groups in comparison with the negative control group. These results are along with previous reports of levels of these biomarkers in psoriasis [76,77,80–82]. Levels of catalase were reduced by 2.94, 1.7, 1.57 and 1.01 folds in the positive control group, HYP ointment group, and the group treated with HYP-NLCs in the absence and presence of light, respectively compared with the negative control group.

On the other hand, levels of IL6 have increased by 4.48, 2.5, 1.7, and 0.9 folds in the positive control group, HYP ointment group, the group treated with HYP-NLCs in the absence and presence of light, respectively compared with the negative control group. Levels of GSH were 0.95, 2.4, 2.76, 4.23 and 4.29 in the positive control group, the HYP ointment group, the group treated with HYP-NLCs in the absence and presence of light and the negative control group, respectively. Also, levels of MMP1 were

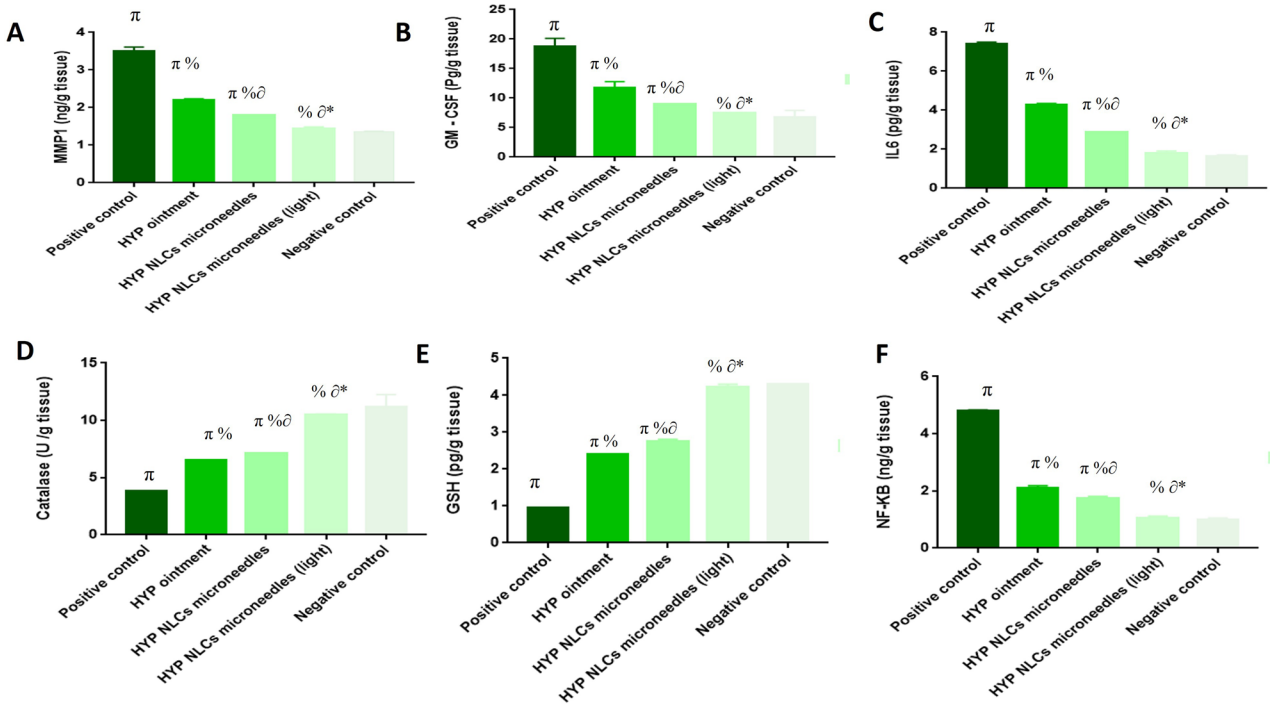


Figure 5. Levels of different biomarkers at the end of the experiment. A) MMP1 level, B) GM-CSF level, C) IL 6, D) Catalase level, E) GSH level and F) NF-KB level. Statistical analysis were carried out using one-way ANOVA followed by Tukey's test. π significant difference from negative control. % significant difference from positive control. * significant difference from HYP NLCs in absence of light. ∂ significant difference from HYP ointment.

3.5, 2.2, 1.8, 1.54 and 1.3 ng/g tissue in the aforementioned groups in the same order. The superior effect of groups treated with HYP compared with the positive control may be attributed to the effect of HYP on reducing immunological responses from IL-17 A-producing γδ T cells and associated cytokines through MAPK/STAT3 pathways [83]. Also, It can inhibit cyclooxygenase-1 and 5-lipoxygenase, key enzyme-inflammatory pathways [28]. Furthermore, HYP can reduce edoema and inflammation, prevent the generation of lipid inflammatory mediators (which enhance vascular permeability) and lower TNFα and IL-6 levels [26].

Statistical analysis of the results using One WAY ANOVA ($p < 0.0001$) revealed that there were statistically significant differences between the positive control group and the rest of the experimental groups. Also, statistically significant differences were observed among different treatment groups and statistically significant differences were noted between the negative control group and HYP-NLCs group in the absence of light. On the other hand, no statistically significant differences in the levels of different biomarkers between the negative control groups and the group treated with HYP-NLCs in the presence of light.

Histopathological examination

As observed, Figure 6A represented the negative control group and showed the normal histological structure of the skin. The positive control group was illustrated in Figure 6B and demonstrated ulcer formation covered by serocellular crust (black arrow) with increasing epidermal layer thickness (star) and infiltration of the dermis by mononuclear inflammatory cells (red arrow). Group treated with HYP ointment, Figure 6C, demonstrated an epidermal layer covered by serocellular crust (star) with infiltration of the dermis by mononuclear inflammatory cells (arrow).

On the other hand, the group treated with HYP-NLCs in MNs in the absence of light showed parakeratosis formation (star) with infiltration of the dermis by mononuclear inflammatory cells (arrow), Figure 6D. Furthermore, the group treated with HYP-NLCs in MNs in the presence of light showed infiltration of the dermis by low numbers of mononuclear inflammatory cells (arrow), Figure 6E.

Strikingly, the results of the histopathological examination are in line with the ELISA results. Collectively, the recorded *in vivo* therapeutic effectiveness against psoriasis between the tested groups could be arranged as HYP ointment < HYP-NLCs MNs in dark < irradiated HYP-NLCs MNs. First, the observed improvement in mice treated with HYP ointment be attributed to the phenomenon called the "Dark side effect of HYP" which reported a weaker level of antiproliferative effect on inflammatory cells in the absence of light [84]. However, this improvement was limited due to the absence of light activation and the high hydrophobicity of HYP that strongly hindered its dermal penetration. Second, mice treated with HYP-NLCs without light showed stronger improvement than those treated with HYP ointment providing further proof for the dark anti-inflammatory effect of HYP. Nanoencapsulation of HYP improved its solubility within the biological fluids. Besides, the application of MNs in this group enabled the HYP-NLCs to bypass the protective stratum corneum. Both factors enhanced the pharmacological effects of HYP-NLCs in this group. Third and most importantly, mice treated with HYP-NLCs in MNs in the presence of light showed the highest superiority among all the groups. This may be ascribed to the effect of light on the generation of singlet oxygen and mediating cell apoptosis predominantly *via* the intrinsic (mitochondrial) pathway. in a mechanism similar to ALA and other porphyrins used in PDT [27]. Also, the synergistic effect of nanoencapsulation, MNs and light played a significant role in improving HYP therapeutic outcomes. HYP has a tendency to distribute as monomers in lipids, encapsulation of HYP into NLCs prevented its aggregation and improved its solubility and intradermal distribution [23,61,85]. Ho-MNs succeeded in deep depositing the developed HYP-NLCs into the deeper layers of the psoriatic skin. Light significantly potentiated the antiproliferative activity of HYP as reported

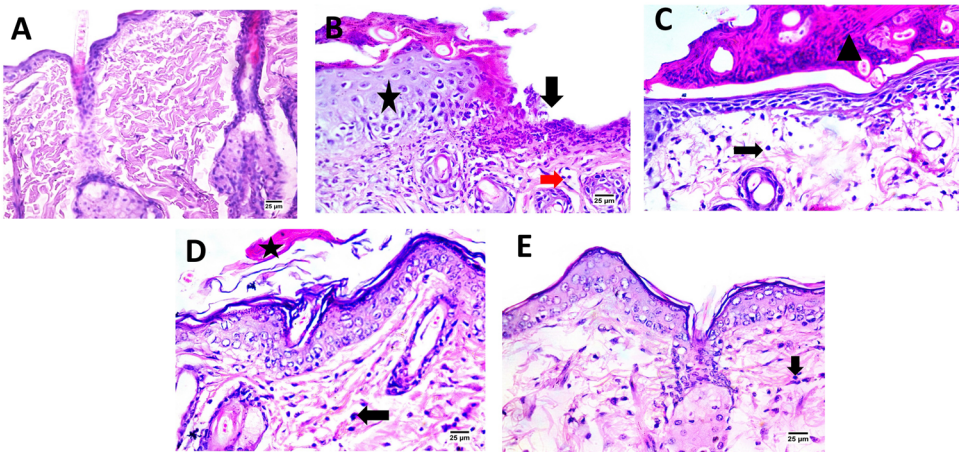


Figure 6. Histopathological examination of different experimental groups at the end of the experiment. A) Negative control group, B) positive control group, C) group treated with hypericin ointment, D) group treated with HYP NLCs in microneedles in absence of light and E) group treated with HYP NLCs in presence of light.

in previous studies [23,24,86]. In specific, photoactivated HYP was found to be a potent inhibitor of casein kinase II and tyrosine kinase, thus interrupting the aetiology of psoriasis [87]. Therefore, the presented triple system of NLCs, MNs and PDT enhanced the pharmacological effect of HYP and provided a promising platform to improve psoriasis manifestations.

Conclusion

This study unleashes new capabilities in the innovative dermal approaches for the treatment of psoriasis. Uniform spherical HYP-NLCs were developed with homogenous size distribution and high entrapment efficiency. According to the *ex vivo* puncture-ability testing, AdminPen™ Ho-MNs entirely penetrated the excised human skin to successfully deliver the injected formulation. Moreover, *ex vivo* skin distribution studies showed that AdminPen™ Ho-MNs deposited the HYP-NLCs into the deeper layers of the skin. Furthermore, when combined with MNs, the incorporation of HYP into NLCs significantly enhanced its solubility and subsequent intradermal distribution. *The in vivo* study in an imiquimod-induced mouse model confirmed the superiority of irradiated HYP-NLCs after intradermal injection using AdminPen™ Ho-MNs as proved by ELISA and histopathological examination. Collectively, photoactivated HYP-NLCs combined with Ho-MNs exhibited a potent antiproliferative effect as a potential non-invasive antipsoriatic platformmice. Further studies should be done to test the efficacy of the HYP-NLCs combined with Ho-MNs in psoriasis patients.

Future directions

More work could be conducted for loading anti-psoriatic agents along with HYP in nanocarriers to provide a synergistic effect and inject them using Ho-MNs. Furthermore, different types of MNs should be prepared and explored to test their efficacy and compare them with Ho-MNs.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The author(s) reported there is no funding associated with the work featured in this article.

ORCID

Haidy Abbas http://orcid.org/0000-0003-4243-1442

References

[1] Pujari VM, Ireddy S, Itagi I. The serum levels of malondialdehyde, vitamin e and erythrocyte catalase activity in psoriasis patients. JCDR. 2014;8(11):CC14. doi: 10.7860/JCDR/2014/10912.5085.
[2] Dobrică E-C, Cozma M-A, Găman M-A, et al. The involvement of oxidative stress in psoriasis: a systematic review. Antioxidants. 2022;11(2):282. doi: 10.3390/antiox11020282.
[3] Weigle N, McBane S. Psoriasis. Am Fam Phys. 2013;87(9):626–633.
[4] Fitch E, Harper E, Skorcheva I, et al. Pathophysiology of psoriasis: recent advances on IL-23 and Th17 cytokines. Curr Rheumatol Rep. 2007;9(6):461–467. doi: 10.1007/s11926-007-0075-1.
[5] Wang A, Bai Y. Dendritic cells: the driver of psoriasis. J Dermatol. 2020;47(2):104–113. doi: 10.1111/1346-8138.15184.
[6] Ogawa E, Sato Y, Minagawa A, et al. Pathogenesis of psoriasis and development of treatment. J Dermatol. 2018;45(3):264–272. doi: 10.1111/1346-8138.14139.
[7] Mascarenhas-Melo F, Carvalho A, Gonçalves MBS, et al. Nanocarriers for the topical treatment of psoriasis-pathophysiology, conventional treatments, nanotechnology, regulatory and toxicology. Eur J Pharm Biopharm. 2022;176:95–107. doi: 10.1016/j.ejpb.2022.05.012.
[8] Nordin UUM, et al. Lipid-based nanoparticles for psoriasis treatment: a review on conventional treatments, recent works, and future prospects. RSC Adv. 2021;11(46):29080–29101.
[9] Elgewelly MA, Elmasry SM, Sayed NSE, et al. Resveratrol-loaded vesicular elastic nanocarriers gel in imiquimod-induced psoriasis treatment: in vitro and in vivo

evaluation. J Pharm Sci. 2022;111(2):417–431. doi: 10.1016/j.xphs.2021.08.023.
[10] Elsheikh MA, Gaafar PME, Khattab MA, et al. Dual-effects of caffeinated hyalurosomes as a nano-cosmeceutical gel counteracting UV-induced skin ageing. Int J Pharm X. 2023;5:100170. doi: 10.1016/j.ijpx.2023.100170.
[11] Wong T, Hsu L, Liao W. Phototherapy in psoriasis: a review of mechanisms of action. J Cutan Med Surg. 2013;17(1):6–12. doi: 10.2310/7750.2012.11124.
[12] Makuch S, Dróżdż M, Makarec A, et al. An update on photodynamic therapy of psoriasis—current strategies and nanotechnology as a future perspective. Int J Mol Sci. 2022;23(17):9845. doi: 10.3390/ijms23179845.
[13] Abd-El-Azim H, Abbas H, El Sayed N, et al. Hypericin emulsomes combined with hollow microneedles as a non-invasive photodynamic platform for rheumatoid arthritis treatment. Int J Pharm. 2024;653:123876. doi: 10.1016/j.ijpharm.2024.123876.
[14] Byun JY, Lee GY, Choi HY, et al. The expressions of TGF-β1 and IL-10 in cultured fibroblasts after ALA-IPL photodynamic treatment. Ann Dermatol. 2011;23(1):19–22. doi: 10.5021/ad.2011.23.1.19.
[15] Tandon YK, Yang MF, Baron ED. Role of photodynamic therapy in psoriasis: a brief review. Photodermatol Photoimmunol Photomed. 2008;24(5):222–230. doi: 10.1111/j.1600-0781.2008.00376.x.
[16] Liu J, Zhou B-R, Yi F, et al. Pyogenic granuloma in a patient with psoriasis successfully treated by 5-aminolevulinic acid photodynamic therapy: a case report. Exp Ther Med. 2016;11(1):345–347. doi: 10.3892/etm.2015.2899.
[17] Salah M, Samy N, Fadel M. Methylene blue mediated photodynamic therapy for resistant plaque psoriasis. J Drugs in Dermatol: JDD. 2009;8(1):42–49.
[18] Gunaydin G, Gedik ME, Ayan S. Photodynamic therapy—current limitations and novel approaches. Front Chem. 2021;9:691697. doi: 10.3389/fchem.2021.691697.
[19] Shaif M, Kushwaha P, Usmani S, et al. Exploring the potential of nanocarriers in antipsoriatic therapeutics. J Dermatol Treatment. 2022;33(7):2919–2930. doi: 10.1080/09546634.2022.2089616.
[20] Gungor S, Rezigue M. Nanocarriers mediated topical drug delivery for psoriasis treatment. Curr Drug Metab. 2017;18(5):454–468. doi: 10.2174/1389200218666170222145240.
[21] Gad HA, Abbas H, El Sayed NS, et al. Berberine loaded thermosensitive lipid nanoparticles: in vitro characterization, in silico study, and in vivo anti-arthritic effect. J Liposome Res. 2024;34(2):303–315.
[22] Hatem S, El-Kayal M. Novel anti-psoriatic nanostructured lipid carriers for the cutaneous delivery of luteolin: a comprehensive in-vitro and in-vivo evaluation. Eur J Pharm Sci. 2023;191:106612. doi: 10.1016/j.ejps.2023.106612.
[23] Abd-El-Azim H, Tekko IA, Ali A, et al. Hollow microneedle assisted intradermal delivery of hypericin lipid nanocapsules with light enabled photodynamic therapy against skin cancer. J Control Release. 2022;348:849–869. doi: 10.1016/j.jconrel.2022.06.027.
[24] Gallardo-Villagrán M, Leger DY, Liagre B, et al. Photosensitizers used in the photodynamic therapy of rheumatoid arthritis. Int J Mol Sci. 2019;20(13):3339. doi: 10.3390/ijms20133339.
[25] Abd-El-Azim H, Abbas H, El Sayed NS, et al. Non-invasive management of rheumatoid arthritis using hollow microneedles as a tool for transdermal delivery of teriflunomide loaded solid lipid nanoparticles. Int J Pharm. 2023;644:123334. doi: 10.1016/j.ijpharm.2023.123334.
[26] Mansouri P, Mirafzal S, Najafizadeh P, et al. The impact of topical Saint John's Wort (Hypericum perforatum) treatment on tissue tumor necrosis factor-alpha levels in plaque-type psoriasis: a pilot study. J Postgrad Med. 2017;63(4):215–220. doi: 10.4103/0022-3859.201423.
[27] Rook AH, Wood GS, Duvic M, et al. A phase II placebo-controlled study of photodynamic therapy with topical hypericin and visible light irradiation in the treatment of cutaneous T-cell lymphoma and psoriasis. J Am Acad Dermatol. 2010;63(6):984–990. doi: 10.1016/j.jaad.2010.02.039.
[28] Najafizadeh P, Hashemian F, Mansouri P, et al. The evaluation of the clinical effect of topical St Johns wort (Hypericum perforatum L.) in plaque type psoriasis vulgaris: a pilot study. Australas J Dermatol. 2012;53(2):131–135. doi: 10.1111/j.1440-0960.2012.00877.x.
[29] Shih C-M, et al. Hypericin induced malformation, mortality and hepatotoxicity during zebrafish development. Curr Topics in Nutraceut Res. 2019;17(4):432.
[30] Zhang W, Zhang W, Li C, et al. Recent advances of microneedles and their application in disease treatment. Int J Mol Sci. 2022;23(5):2401. doi: 10.3390/ijms23052401.
[31] Wang J, Zeng J, Liu Z, et al. Promising strategies for transdermal delivery of arthritis drugs: microneedle systems. Pharmaceutics. 2022;14(8):1736. doi: 10.3390/pharmaceutics14081736.
[32] Larrañeta E, Lutton REM, Woolfson AD, et al. Microneedle arrays as transdermal and intradermal drug delivery systems: materials science, manufacture and commercial development. Mater Sci Engin: R: Reports. 2016;104:1–32. doi: 10.1016/j.mser.2016.03.001.
[33] Yang J, Liu X, Fu Y, et al. Recent advances of microneedles for biomedical applications: drug delivery and beyond. Acta Pharmaceutica Sinica B. 2019;9(3):469–483. doi: 10.1016/j.apsb.2019.03.007.
[34] Ren Y, Li J, Chen Y, et al. Customized flexible hollow microneedles for psoriasis treatment with reduced-dose drug. Bioeng Transl Med. 2023;8(4):e10530. doi: 10.1002/btm2.10530.
[35] Zewail M, El-Deeb NM, Mousa MR, et al. Hyaluronic acid coated teriflunomide (A771726) loaded lipid carriers for the oral management of rheumatoid arthritis. Int J Pharm. 2022;623:121939. doi: 10.1016/j.ijpharm.2022.121939.
[36] Zewail M, Nafee N, Helmy MW, et al. Coated nanostructured lipid carriers targeting the joints–an effective and safe approach for the oral management of rheumatoid arthritis. Int J Pharm. 2019;567:118447. doi: 10.1016/j.ijpharm.2019.118447.
[37] Dragicevic-Curic N, Gräfe S, Gitter B, et al. Surface charged temoporfin-loaded flexible vesicles: in vitro skin penetration studies and stability. Int J Pharm. 2010;384(1-2):100–108. doi: 10.1016/j.ijpharm.2009.10.006.
[38] Elhalmoushy PM, Elsheikh MA, Matar NA, et al. Novel berberine-loaded hyalurosomes as a promising nanodermatological treatment for vitiligo: biochemical, biological and gene expression studies. Int J Pharm. 2022;615:121523. doi: 10.1016/j.ijpharm.2022.121523.
[39] Wang X, et al. Optimization of extraction process of hypericin from St. John's Wort by central composite design-response surface methodology. J Chem Pharmaceutical Res. 2014;6(7):1667–1675.

[40] Hou D, Xie C, Huang K, et al. The production and characteristics of solid lipid nanoparticles (SLNs). Biomaterials. 2003;24(10):1781–1785. doi: 10.1016/s0142-9612(02)00578-1.
[41] Abd-El-Azim H, Ramadan A, Nafee N, et al. Entrapment efficiency of pyridoxine hydrochloride in unilamellar liposomes: experimental versus model-generated data. J Liposome Res. 2018;28(2):112–116. doi: 10.1080/08982104.2016.1275679.
[42] Saedi A, Rostamizadeh K, Parsa M, et al. Preparation and characterization of nanostructured lipid carriers as drug delivery system: influence of liquid lipid types on loading and cytotoxicity. Chem Phys Lipids. 2018;216:65–72. doi: 10.1016/j.chemphyslip.2018.09.007.
[43] Ng WK, Saiful Yazan L, Yap LH, et al. Thymoquinone-loaded nanostructured lipid carrier exhibited cytotoxicity towards breast cancer cell lines (MDA-MB-231 and MCF-7) and cervical cancer cell lines (HeLa and SiHa). Biomed Res Int. 2015;2015:263131–263110. doi: 10.1155/2015/263131.
[44] Zewail M, Nafee N, Helmy MW, et al. Synergistic and receptor-mediated targeting of arthritic joints via intra-articular injectable smart hydrogels containing leflunomide-loaded lipid nanocarriers. Drug Deliv Transl Res. 2021;11(6):2496–2519. doi: 10.1007/s13346-021-00992-9.
[45] Sato MR, Oshiro-Junior JA, Rodero CF, et al. Photodynamic therapy-mediated hypericin-loaded nanostructured lipid carriers against vulvovaginal candidiasis. J Med Mycol. 2022;32(4):101296. doi: 10.1016/j.mycmed.2022.101296.
[46] Aburahma MH, Badr-Eldin SM. Compritol 888 ATO: a multifunctional lipid excipient in drug delivery systems and nanopharmaceuticals. Expert Opin Drug Deliv. 2014;11(12):1865–1883. doi: 10.1517/17425247.2014.935335.
[47] Moreira TSA, Pereira de Sousa V, Pierre MBR. A novel transdermal delivery system for the anti-inflammatory lumiracoxib: influence of oleic acid on in vitro percutaneous absorption and in vivo potential cutaneous irritation. AAPS PharmSciTech. 2010;11(2):621–629. doi: 10.1208/s12249-010-9420-1.
[48] Naik A, Pechtold LA, Potts RO, et al. Mechanism of oleic acid-induced skin penetration enhancement in vivo in humans. J Controlled Release. 1995;37(3):299–306. doi: 10.1016/0168-3659(95)00088-7.
[49] Mistry K, Sarker D. SLNs can serve as the new brachytherapy seed: determining influence of surfactants on particle size of solid lipid microparticles and development of hydrophobised copper nanoparticles for potential insertion. J Chem Eng Process Technol. 2016;7(3):302. doi: 10.4172/2157-7048.1000302.
[50] Zirak MB, Pezeshki A. Effect of surfactant concentration on the particle size, stability and potential zeta of beta carotene nano lipid carrier. Int J Curr Microbiol App Sci. 2015;4(9):924–932.
[51] Iglič A, Rappolt M, Perez PL. Advances in biomembranes and lipid self-assembly. Vol. 27. United States: Academic Press; 2018.
[52] Müller RH, Rühl D, Runge S, et al. Cytotoxicity of solid lipid nanoparticles as a function of the lipid matrix and the surfactant. Pharm Res. 1997;14(4):458–462. doi: 10.1023/a:1012043315093.
[53] Chen C-Y, Lee Y-H, Chang S-H, et al. Oleic acid-loaded nanostructured lipid carrier inhibit neutrophil activities in the presence of albumin and alleviates skin inflammation. Int J Nanomedicine. 2019;14:6539–6553. doi: 10.2147/IJN.S208489.
[54] Jeitler R, Glader C, Tetyczka C, et al. Investigation of cellular interactions of lipid-structured nanoparticles with oral mucosal epithelial cells. Front Mol Biosci. 2022;9:917921. doi: 10.3389/fmolb.2022.917921.
[55] Karmakar S. Particle size distribution and zeta potential based on dynamic light scattering: techniques to characterize stability and surface charge distribution of charged colloids. Recent Trends Mater Phys Chem. 2019;28:117–159.
[56] Mehanna MM, Sarieddine R, Alwattar JK, et al. Anticancer activity of thymoquinone cubic phase nanoparticles against human breast cancer: formulation, cytotoxicity and subcellular localization. Int J Nanomedicine. 2020;15:9557–9570. doi: 10.2147/IJN.S263797.
[57] Zewail M, E Gaafar PM, Ali MM, et al. Lipidic cubic-phase leflunomide nanoparticles (cubosomes) as a potential tool for breast cancer management. Drug Deliv. 2022;29(1):1663–1674. doi: 10.1080/10717544.2022.2079770.
[58] Abd El Azim H, Nafee N, Ramadan A, et al. Liposomal buccal mucoadhesive film for improved delivery and permeation of water-soluble vitamins. Int J Pharm. 2015;488(1–2):78–85. doi: 10.1016/j.ijpharm.2015.04.052.
[59] Rahman HS, Rasedee A, How CW, et al. Zerumbone-loaded nanostructured lipid carriers: preparation, characterization, and antileukemic effect. Int J Nanomedicine. 2013;8:2769–2781. doi: 10.2147/IJN.S45313.
[60] Abbas H, El-Deeb NM, Zewail M. PLA-coated Imwitor® 900 K-based herbal colloidal carriers as novel candidates for the intra-articular treatment of arthritis. Pharm Dev Technol. 2021;26(6):682–692. doi: 10.1080/10837450.2021.1920617.
[61] Zewail M. Folic acid decorated chitosan-coated solid lipid nanoparticles for the oral treatment of rheumatoid arthritis. Ther Deliv. 2021;12(4):297–310. doi: 10.4155/tde-2020-0123.
[62] Zewail M, Gaafar PME, Youssef NAHA, et al. Novel siprulina platensis bilosomes for combating UVB induced skin damage. Pharmaceuticals. 2022;16(1):36. doi: 10.3390/ph16010036.
[63] Ortiz AC, Yañez O, Salas-Huenuleo E, et al. Development of a nanostructured lipid carrier (NLC) by a low-energy method, comparison of release kinetics and molecular dynamics simulation. Pharmaceutics. 2021;13(4):531. doi: 10.3390/pharmaceutics13040531.
[64] Elmowafy M. Skin penetration/permeation success determinants of nanocarriers: pursuit of a perfect formulation. Colloids Surf, B. 2021;203:111748. doi: 10.1016/j.colsurfb.2021.111748.
[65] Gowda BHJ, Ahmed MG, Hani U, et al. Microneedles as a momentous platform for psoriasis therapy and diagnosis: a state-of-the-art review. Int J Pharm. 2023;632:122591. doi: 10.1016/j.ijpharm.2023.122591.
[66] Permana AD, Tekko IA, McCrudden MTC, et al. Solid lipid nanoparticle-based dissolving microneedles: a promising intradermal lymph targeting drug delivery system with potential for enhanced treatment of lymphatic filariasis. J Controlled Release. 2019;316:34–52. doi: 10.1016/j.jconrel.2019.10.004.
[67] Wei JCJ, Edwards GA, Martin DJ, et al. Allometric scaling of skin thickness, elasticity, viscoelasticity to mass for micro-medical device translation: from mice, rats, rabbits, pigs to humans. Sci Rep. 2017;7(1):15885. doi: 10.1038/s41598-017-15830-7.
[68] Rosita N, Sultani AA, Hariyadi DM. Penetration study of p-methoxycinnamic acid (PMCA) in nanostructured lipid

carrier, solid lipid nanoparticles, and simple cream into the rat skin. Sci Rep. 2022;12(1):19365. doi: 10.1038/s41598-022-23514-0.

[69] Ghazwani M, Hani U, Alqarni MH, et al. Beta caryophyllene-loaded nanostructured lipid carriers for topical management of skin disorders: statistical optimization, in vitro and dermatokinetic evaluation. Gels. 2023;9(7):550. doi: 10.3390/gels9070550.

[70] Boiy A, Roelandts R, Roskams T, et al. Effect of vehicles and esterification on the penetration and distribution of hypericin in the skin of hairless mice. Photodiagnosis Photodyn Ther. 2007;4(2):130–139. doi: 10.1016/j.pdpdt.2007.02.002.

[71] Barras A, Boussekey L, Courtade E, et al. Hypericin-loaded lipid nanocapsules for photodynamic cancer therapy in vitro. Nanoscale. 2013;5(21):10562–10572. doi: 10.1039/c3nr02724d.

[72] Jabeen M, Boisgard A-S, Danoy A, et al. Advanced characterization of imiquimod-induced psoriasis-like mouse model. Pharmaceutics. 2020;12(9):789. doi: 10.3390/pharmaceutics12090789.

[73] Lin Y-K, Yang S-H, Chen C-C, et al. Using imiquimod-induced psoriasis-like skin as a model to measure the skin penetration of anti-psoriatic drugs. PLoS One. 2015;10(9):e0137890. doi: 10.1371/journal.pone.0137890.

[74] Chen T, Fu L-X, Guo Z-P, et al. Involvement of high mobility group box-1 in imiquimod-induced psoriasis-like mice model. J Dermatol. 2017;44(5):573–581. doi: 10.1111/1346-8138.13695.

[75] Das RP, Jain AK, Ramesh V. Current concepts in the pathogenesis of psoriasis. Indian J Dermatol. 2009;54(1):7–12. doi: 10.4103/0019-5154.48977.

[76] Mezentsev A, Nikolaev A, Bruskin S. Matrix metalloproteinases and their role in psoriasis. Gene. 2014;540(1):1–10. doi: 10.1016/j.gene.2014.01.068.

[77] Moorchung N, Kulaar JS, Chatterjee M, et al. Role of NF-κB in the pathogenesis of psoriasis elucidated by its staining in skin biopsy specimens. Int J Dermatol. 2014;53(5):570–574. doi: 10.1111/ijd.12050.

[78] Starodubtseva NL, Sobolev VV, Soboleva AG, et al. Genes expression of metalloproteinases (MMP-1, MMP-2, MMP-9, and MMP-12) associated with psoriasis. Russ J Genet. 2011;47(9):1117–1123. doi: 10.1134/S102279541109016X.

[79] Grossman RM, Krueger J, Yourish D, et al. Interleukin 6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. Proc Natl Acad Sci USA. 1989;86(16):6367–6371. doi: 10.1073/pnas.86.16.6367.

[80] Takematsu H, Tagami H. Granulocyte-macrophage colony-stimulating factor in psoriasis. Dermatology. 1990;181(1):16–20. doi: 10.1159/000247852.

[81] Drewa G, et al. Activity of superoxide dismutase and catalase and the level of lipid peroxidation products reactive with TBA in patients with psoriasis. Med Sci Monitor: Int Med J Exper Clin Res. 2002;8(8):BR338–43.

[82] Campione E, Mazzilli S, Di Prete M, et al. The role of Glutathione-S transferase in psoriasis and associated comorbidities and the effect of dimethyl fumarate in this pathway. Front Med (Lausanne). 2022;9:760852. doi: 10.3389/fmed.2022.760852.

[83] Zhang S, Zhang J, Yu J, et al. Hyperforin ameliorates imiquimod-induced psoriasis-like murine skin inflammation by modulating IL-17A–producing γδ T cells. Front Immunol. 2021;12:635076. doi: 10.3389/fimmu.2021.635076.

[84] Jendželovská Z, Jendželovský R, Kuchárová B, et al. Hypericin in the light and in the dark: two sides of the same coin. Front Plant Sci. 2016;7:560. doi: 10.3389/fpls.2016.00560.

[85] Ho Y-F, Wu M-H, Cheng B-H, et al. Lipid-mediated preferential localization of hypericin in lipid membranes. Biochim Biophys Acta. 2009;1788(6):1287–1295. doi: 10.1016/j.bbamem.2009.01.017.

[86] Barras A, Skandrani N, Gonzalez Pisfil M, et al. Improved photodynamic effect through encapsulation of two photosensitizers in lipid nanocapsules. J Mater Chem B. 2018;6(37):5949–5963. doi: 10.1039/C8TB01759J.

[87] Kamuhabwa AR, Roelandts R, de Witte PA. Skin photosensitization with topical hypericin in hairless mice. J Photochem Photobiol B. 1999;53(1–3):110–114. doi: 10.1016/S1011-1344(99)00135-9.