Mangiferin glycethosomes as a new potential adjuvant for the treatment of psoriasis

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A B S T R A C T

Mangiferin, a natural compound isolated from Mangifera indica L, was incorporated in glycerosomes, ethosomes and alternatively in glycerol-ethanol phospholipid vesicles (glycethosomes). Actually, only glycethosomes were able to stably incorporate the mangiferin that was loaded at increasing concentrations (2, 4, 6, 8 mg/mL). The morphology, size distribution, rheological properties, surface charge and entrapment efficiency of prepared vesi- cles were deeply measured. All vesicles were mainly spherical, oligolamellar, small in size (~145 nm) and neg- atively charged (~−40 mV), as confirmed by cryo-TEM observation and dynamic laser light scattering measure- ments. The higher concentration of mangiferin (8 mg/mL) allowed an increase of vesicle mean diameter up to ~288 nm. The entrapment efficiency was inversely proportional to the amount of loaded mangiferin.In vitro stud- ies performed by using human abdominal skin, underlined that, the dose-dependent ability of vesicles to promote mangiferin retention in epidermis. In addition, glycethosomes were highly biocompatible and showed a strong ability to protect in vitro the fibroblasts against damages induced by hydrogen peroxide. In vivo results under- lined the superior ability of mangiferin loaded glycethosomes respect to the mangiferin dispersion to promote the heal of the wound induced by TPA, confirming their potential application for the treatment of psoriasis or other skin disorders.

1. Introduction

Inflammatory skin diseases are cause of important adverse events and disability for a large number of patients, thus negatively affecting their quality of life. The incidence of these skin diseases, which include atopic dermatitis and psoriasis acute, recurrent or chronic, is increasing in recent years, especially in developed countries (Owczarek and Ja- worski, 2016).

Currently, the treatment of both atopic dermatitis and psoriasis in- cludes the use of different drugs such as corticoids, calcineurin in- hibitors, antihistamines, for topical application and immunosuppres- sants and biological drugs for systemic treatments (Rodríguez-Luna et al., 2017). However, many patients, especially those with generalized psoriasis, are not adequately treated and long-term therapies are often combined with various side effects of different degrees.

The role of oxidative stress in these diseases has been previously demonstrated in preclinical and clinical studies (Body-Malapel et al., 2018; Tanaka et al., 2018) and the use of antioxidants may represent an ideal strategy to reduce and control the damages caused by pso- riasis and atopic dermatitis.

During the last decades, the use of phytodrugs for the treatment of different skin disorders has haroused a great scientific interest (Mar- tinez et al., 2016; Furue et al., 2017; Janeczek et al., 2018). Among the different natural antioxidant molecules, curcumin and quercetin have generated great attention since their large number of im- portant biological and beneficial activities (Abrahams et al., 2019; Rauf et al., 2018).

Considering the promising properties of these, new alternative nat- ural antioxidant molecules such as baicalin, berberin and mangiferin have been isolated and their beneficial properties evaluated (Mir-Palomo et al., 2019; Manca et al., 2019). Mangiferin or 1,3,6,7-tetrahydroxyxanthone-C2-β-d-glucoside (C-glucosyl xanthone) is an antioxidant molecule isolated from Mangifera indica L, a member of the anacardiaceae family. It possesses strong free radical-scavenging
(Saha et al., 2016) and anti-inflammatory activities (Szandruk et al., 2018) and it is effective in a variety of diseases, including tumorigenesis (Zou et al., 2017), hypersensitivity (Guo et al., 2014) and tis- sue repair (Imran et al., 2017). Like other natural antioxidants, it is in vivo biological effectiveness is limited by low bioavailability (1.71%) (Gu et al., 2018; Du et al., 2018) especially when applied on the skin. To overcome these limitations, the loading of natural antioxidants into nanocarriers has been proposed aiming at increasing their bioavailabil- ity and reducing possible side effects (Pimentel-Moral et al., 2018). Many studies have demonstrated an improvement of the effectiveness of these molecules when topically applied. In particular, liposomes or mod- ified phospholipid vesicles disclosed optimal performances in the skin delivery providing an accumulation up to the lower tissues (Manconi et al., 2018; Fang et al., 2018; Doppalapudi et al., 2017). The analy- sis of research studies demonstrated that each molecule requires a spe- cific ad hoc tailored phospholipid vesicle formulation to maximizes the efficacy.

Taking into account these results, in this work, specific phospho- lipid vesicles were tailored to stably deliver mangiferin in the skin. Af- ter a pre-formulation study, a special kind of phospholipid vesicles load- ing mangiferin were prepared hydrating phospholipid with a mixture of water, glycerol and ethanol and so called glycethosomes. Mangiferin was loaded at increasing concentrations (2, 4, 6, 8 mg/mL). The choice of glycerol was mainly related to its moisturizing and cosolvent prop- erties (Angelova-Fischer et al., 2018), which are requested for the treatment of atopic dermatitis, as reported by the therapeutic guidelines (Ring et al., 2012; Eichenfield et al., 2014). While the addition of ethanol was linked to their penetration ability.

Glycethosomes loaded with mangiferin were fully characterized measuring size, size distribution, surface charge, stability on storage and rheological properties. Their ability to promote the accumulation and distribution of mangiferin into and through the skin was evaluated us- ing human abdominal skin. In addition, their potential therapeutic ap- plication for the treatment of psoriasis was evaluated in vivo using a 12-O-tetradecanoylphorbol-13-acetate (TPA)-treated mice model.

2. Material and methods

2.1. Materials

Lipoid® S75 a mixture of soybean lecithin containing lysophos- phatidylcholine (3% maximum), phosphatidylcholine (70%), phos-phatidylethanolamine (10%), fatty acids (0.5% maximum), triglycerides (3% maximum) was purchased from Lipoid GmbH (Ludwigshafen, Ger- many). Tween 80 were purchased from Scharlab S.L. (Barcelona, Spain). Mangiferin was purchased to Carbosynth Limited (UK). Glycerol was purchased from Guinama S.L.U. (Valencia, Spain), Ethanol was pur- chased from VWR chemicals S.A. (France). Phorbol 1, 2-myristate 1, 3-acetate was purchased from Sigma-Aldrich (Madrid, Spain). Cell medium, foetal bovine serum, penicillin and streptomycin and all the other reagents for cells studies, were purchased from Thermo Fisher Sci- entific Inc. (Waltham, MA, US).

2.2. Analytical method

Mangiferin content was determined by high-performance liquid chromatography (HPLC) using a Perkin Elmer® Series 200 equipped with a photodiode array UV detector and a C18 reverse-phase column (Teknokroma®Brisa "LC2" 5.0 μm, 150 mm × 4.6 mm). The isocratic mobile phase consisted of a mixture of hydrochloric acid (pH 4.0) and methanol (60:40, v/v), the flow rate was 1.2 mL/min. The detection wavelength was set at 254 nm.

2.3. Preparation of vesicles

A preformulation study was performed to evaluate the best com- position able to incorporate and retain high amount of mangiferin and promote its efficacy for the topical treatment of psoriasis and atopic dermatitis. Various amounts of different phospholipids were tested, tween 80 was added as excipient aiming at ameliorating the physico-chemical properties of vesicles, Table 1. Among all formulation tested, those obtained using S75 as phospholipid and a mixture of glyc- erol, ethanol and water (50:25:25) seemed to be the ideal vesicle as it was the smaller and homogeneous system. The glycerol: ethanol: water blend was used to hydrate S75 (100 mg/mL) and tween 80 (5 mg/mL). The mixture was slightly heated (40 °C) in a water bath and then differ- ent amounts of mangiferin (2, 4, 6 and 8 mg/mL) were added to evalu- ate the higher quantity of mangiferin which could be stably incorporated and retained in these systems. The dispersions were sonicated for 4 min with a CY-500 ultrasonic disintegrator (Optic Ivymen system, Barcelona, Spain) and then extruded through a 0.20 μm membrane (Whatman, GE Healthcare, Fairfield, Connecticut, US) by using Avanti® Mini

Extruder (Avanti Polar Lipids, Alabaster, Alabama). Mangiferin dispersion (8 mg/ mL) and empty glycethosomes were also prepared as control. The com- position of vesicles is given in Table 2.

2.4. Characterization of vesicles

Vesicle formation and morphology were checked by cryogenic Trans- mission Electron Microscopy (cryo-TEM). A thin aqueous film was formed by placing a sample drop on a glow-discharged holey carbon grid and then blotting the grid against filter paper. The film was vitri- fied by plunging the grid in ethane maintained at its melting point with liquid nitrogen, using a Vitrobot (FEI Company, Eindhoven, The Nether- lands). The vitreous films were transferred to a Tecnai F20 TEM (FEI Company) and the samples were observed in a low-dose mode. Images were acquired at 200 kV at a temperature between −170–175 °C, using

low-dose imaging conditions not exceeding 20 e-/Å2, with a CCD Eagle camera (FEI Company).

The average diameter and polydispersity index (PI) of glycethosomes were measured by Photon Correlation Spectroscopy (PCS) using a Zeta- sizer nano-ZS® (Malvern Instruments, Worcestershire, UK). Zeta poten- tial was estimated using the Zetasizer nano-ZS by means of the M3-PALS (Mixed Mode Measurement-Phase Analysis Light Scattering) technique, which measures the particle electrophoretic mobility.

Table 1
Composition of hydrating medium, mean diameter (MD), polydispersity index (PI) and zeta potential (ZP) of liposomes, glycerosomes, ethosomes and glycethosomes loading mangiferin (2 mg/mL). Each value represents the mean ± standard deviation of at least three determinations.

Formulation	Hydrating medium	MD (nm)	PI	ZP (mV)
Liposomes	Water	452 ± 3	0.40	−37 ± 2
Glycerosomes	Glycerol:water	391 ± 2	0.50	−31 ± 1
Ethosomes	Ethanol: water	643 ± 3	0.40	−39 ± 1
Glycethosomes	Glycerol:ethanol:water	140 ± 1	0.32	−42 ± 1

Table 2
Mangiferin concentration (MC), mean diameter (MD), polydispersity index (PI), zeta po- tential (ZP) and entrapment efficiency of 2-glycethosomes, 4-glycethosomes, 6-glycetho- somes, 8-glycethosomes. Each value represents the mean ± standard deviation of at least three determinations.

Formulation	MC (mg/mL)	MD (nm)	PI	ZP (mV)	EE (%)
	0	141 ± 2	0.32	−40 ± 1	
2-Glycethosomes	2	140 ± 2	0.31	−43 ± 2	78 ± 1
4-Glycethosomes	4	149 ± 2	0.33	−40 ± 1	70 ± 1
6-Glycethosomes	6	151 ± 3	0.29	−38 ± 1	65 ± 1
8-Glycethosomes	8	288 ± 2	0.32	−39 ± 2	62 ± 1

The stability on storage was evaluated for 90 days at 4 °C, by mea- suring the particle size, PI and Zeta potential of glycethosomes every 30 days, which provided useful information about changes in size distri- bution and surface charge.

The entrapment efficiency (EE) of the glycethosomes was determined as the percentage of the amount of mangiferin recovered after dialy- sis versus the amount initially used. Each sample (1 mL) was loaded into Spectra/Por®tubing (12–14 kDa MW cut-off; Spectrum Laborato- ries Inc., DG Breda, The Netherlands) and dialyzed against 1L of water at room temperature for 8 h. The mangiferin content was measured by HPLC after disruption of non-dialysed and dialysed vesicles with Triton
X-100 (10%).

2.5. Rheological measurements of mangiferin loaded vesicles

Rheological measurements were carried out at 25 ± 1 °C, using a controlled stress rheometer (RheoStress 1, Thermo Haake, Germany) equipped with a Haake *K*10 thermostatic bath control and data log- ging software (RheoWin 4.0.1). Samples were allowed to rest for at least 300 s for temperature equilibrium and stress relaxation. Cone-plate sen- sor (2°, 35 mm diameter) were used for the analyses.

Step flow curves were obtained in a controlled stress mode (30 s each step in logarithmic distribution). The shear stress range was cho- sen to evaluate viscosities corresponding to very low shear rates (up to ~100 s^{-1}). All measurements were performed in triplicate, at 25 °C. The viscosities results can be calculated by using the simplified Carreau model.

$$\eta = \frac{\eta_0}{\left(1 + \left(\frac{\dot{\gamma}}{\dot{\gamma}_c}\right)^2\right)^s} \quad (1)$$

where η_0 is the zero-shear viscosity, $\dot{\gamma}_c$ is the critical shear rate, and s the shear thinning index.

2.6. Evaluation of in vitro skin delivery of mangiferin loaded vesicles

Experiments were performed using human abdominal skin obtained from donors aged 40–50 years who undergone cosmetic surgical pro- cedures. The skin was given by Hospital Clinic Universitario (Valencia, Spain) after informed consent obtained from patients. The skin was pre- pared within the first 3 h after excision, removing all subcutaneous fatty tissue, and was stored at −80 °C until use (Alves et al., 2007). The full-thickness human epidermis was placed between the donor and re- ceptor compartments of Franz diffusion vertical cells (effective diffusion area of 0.784 cm^2). The receptor compartment (6 mL) was filled with an aqueous solution containing 1% of tween 80, thermostated at 37 ± 1 °C, and continuously stirred. The different formulations (200 μL) were ap- plied onto the surface of epidermis. At regular time intervals, the receiv- ing solution was withdrawn, replaced with the same volume of pre-ther-
mostated fresh aqueous solution up to 24 h and analysed by HPLC for mangiferin content. At the end of the permeation experiments, to verify the integrity of the epidermis, 1 mL of phenol red solution (0.5 mg/mL) was applied on the skin surface, which was considered intact when the amount of phenol red in the receptor compartment was lower than 1%. After 24 h the skin surface was removed and gently washed and dried with filter paper, then it was putted in glass vials and methanol (5 mL) was added to allow the extraction of mangiferin accumulated in the epidermis. The extraction procedure was performed for 24 h under constant stirring (350 rpm) at room temperature (25 ± 1 °C), then the extractive solution was analysed by HPLC for mangiferin content.

2.7. Evaluation of in vitro biocompatibility of mangiferin loaded vesicles

3T3 mouse fibroblasts (ATCC, Manassas, VA, USA) were grown as monolayers in 75-cm^2 flasks, incubated at 37 °C in 5% CO_2 and 100% humidity. Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% (V/V) fetal bovine serum, penicillin/streptomicin (100 U/ mL), and 0.1% fungizone was used as culture medium. For biocom- patibility experiments, 3T3 cells were seeded in 96-well plates at den- sity of 1×10^4 cell/well. After 24 h of incubation, 3T3 cells were ex-
posed for 48 h to mangiferin in dispersion (8 mg/mL) or loaded in vesi- cles (2,4,6,8 mg/mL) at different dilutions (1:1000, 1:10000; 1:100000 and 1:1000000). Cell viability was evaluated by means of MTT [3 (4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] colorimet- ric assay. MTT (100 μL, 0.5 mg/mL final concentration) was added to each well, and then, after 3 h the formazan crystals formed were dis- solved in DMSO (100 μL). The reaction was spectrophotometrically mea- sured at 570 nm using a microplate reader (Multiskan EX, Thermo Fisher Scientific, Inc., Waltham, MA, US). The experiments were re- peated at least three times. The results are presented as a percentage of untreated cells (100% viability).

2.8. Protection of cells against oxidative stress by using mangiferin loaded vesicles

3T3 cells were seeded into 96-well plates and exposed to hydrogen peroxide (1:40000 dilution) and simultaneously treated with mangiferin in dispersion or loaded in vesicles (8 mg/mL and 2, 4, 6 and 8 mg/ mL for dispersion and vesicles, respectively). After 4 h, the cells were washed with PBS and cell viability was evaluated by MTT assay. Un- treated cells were used as negative control, while cells treated with hy- drogen peroxide only were used as positive control. Final results are re- ported as the percentage of untreated cells (100% viability).

2.9. Study of effectiveness of mangiferin loaded vesicles in inflammatory mice models

Mice were supplied by Envigo laboratories (Barcelona, Spain), and were acclimated for one week before their use. All studies were per- formed in accordance with the European Union regulations for the han- dling and use of laboratory animals. The protocols were approved by the Institutional Animal Care and Use Committee of the University of Valen- cia (code 2018/VSC/PEA/0032 type 2).

Inflammation and ulceration were induced by applying topically TPA, dissolved in acetone (3 μg/20 μL), to the shaved dorsal area (1 cm^2) of female CD-1 mice (5–6 weeks old, 25–35 g). All glycetho- somes (200 μL) were topically applied in the same dorsal site 3 h af- ter TPA application. The protocol/experimental plan was repeated for 3 days. Mice (n = 5, per group) were sacrificed on day fourth by cervi- cal dislocation. Visual inspection of the skin after the treatment, along

with myeloperoxidase activity (MPO) and oedema formation were mea- sured. Inhibition of MPO activity was evaluated as previously reported (Pleguezuelos-Villa et al. 2019). The treated dorsal skin was excised, weighed and used to measure oedema formation. For histological assays, tissue samples were stored in formaldehyde (0.4%, V/V) and longitudi- nal sections (5 mm) of the skin, obtained using a rotary microtome, were mounted on slides and marked with haematoxylin and eosin according to standard protocols. Tissues were observed and analysed using an opti- cal microscope (DMD 108 Digital Micro-Imaging Device, Leica, Wetzlar, Germany).

2.10. Statistical analysis of data

Statistical differences were determined by one-way ANOVA test and Tukey's test for multiple comparisons with a significance level of $p < 0.05$. All statistical analyses were performed using IBM SPSS statis- tics 22 for Windows (Valencia, Spain). Data are shown as mean ± stan- dard deviation.

3. Results and discussion

3.1. Preparation and characterization of vesicles

In this study, aiming at promoting the effectiveness of mangiferin in the treatment of psoriasis or atopic dermatitis, new phospholipid vesi- cles were designed. To achieve versatile and stable formulations, capa- ble of incorporating high amount of mangiferin, a preformulation study was carried out. Lipoid S75 were use as phospholipid (100 mg/mL) and combined with tween 80 (5 mg/mL). The last acts as edge activator and improve the flexibility of the bilayer (Perez et al., 2016). Mangiferin were loaded at concentration of 2 mg/mL. Lipids, surfactant and pay- load were mixed and hydrate with water to obtain liposomes, water and glycerol (50:50 v/v) to obtain glycerosomes, water and ethanol (50:50 v/v) to obtain ethosomes, glycerol, ethanol and water (50:25:25 v/v) to obtain glycethosomes. Composition of hydrating medium as well mean diameter, polydispersity index and zeta potential of vesicles load- ing mangiferin are reported in Table 1. Glycethosomes was the smallest vesicles (~140 nm), most homogeneously dispersed (PI 0.32) and highly negatively charged (−42 mV). The mean diameter of other vesicles was 2–3 times higher. Additionally, the dispersion was very instable and af-
ter few days formed two phases. As a consequence, the further studies were focuses on glycethosomes. The same formulation was prepared us- ing the same amount of phospholipid, tween and increasing concentra- tions of mangiferin (2, 4, 6 and 8 mg/mL), in order to find the high- est amount of phytodrug which should be loaded. Empty glycethosomes were also prepared and used as reference. The mean physico-chemi- cal characteristics of vesicles were measured (Table 2). Empty vesicles and those loading 2, 4 and 6 mg/mL showed the same mean diameter (~140 nm), polydispersity index and zeta potential without differences statistically significant among the group ($p > 0.05$). The highest con- centrations of mangiferin seem to be critic and the mean diameter of these vesicles was double (~288 nm) compared with the other samples. Probably, using this concentration, the mangiferin which preferentially located in the vesicle bilayer, saturated it modifying the its assembling and curvature radius leading to the increase of particle size (Huang et al. 2017). However, according to other authors, vesicles sized between 100 and 1000 nm are suitable for transdermal administration (Hussain et al. 2017).

The entrapment efficiency (EE%) of mangiferin in glycethosomes slightly decrease as the amount of mangiferin loaded in the formulation increased (Table 2). Vesicle morphology was evaluated by using Cryo-TEM, which un- derline the formation of uni- and oligolamellar, spherical and regularly shaped vesicles. These results are in agreement with those obtained by using dynamic light scattering technique, as particle size was ~ 140 nm for 2-, 4- and 6-glycethosomes and bigger for 8-glycethosomes, (Fig. 1). The prepared vesicles were stored at 4 °C for 3 mounts and their physico-chemical characteristics (size, PI and zeta potential) were mea- sured at scheduled times. All vesicle desperations were highly stable as any significant variation ($p < 0.05$) of the measured parameters were
detected during the storage (Fig. 2).

3.2. Rheological measurements

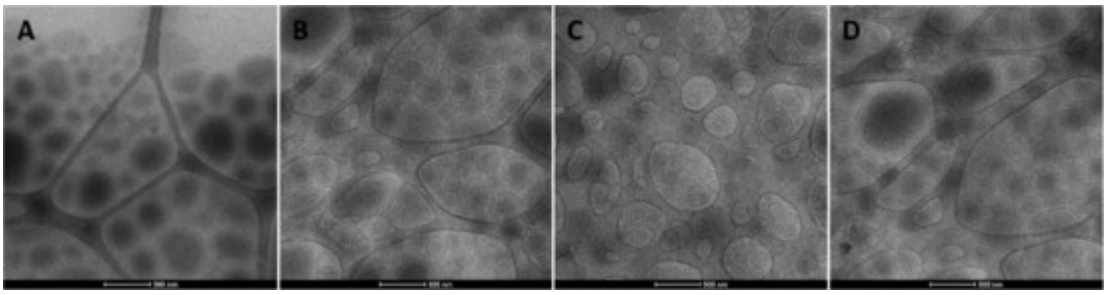
The physical state of dispersions and the possible interactions be- tween the vesicles were evaluated by rheological analyses. Viscoelastic properties of the different glycethosomes were evaluated by measuring their viscosity (Fig. 3).

As expected, 8-glycethosomes were the most viscous (2584 ± 46 Pa s) as the viscosity increased as the mangiferin concen- tration increased: 2-glycethosomes (279 ± 4 Pa s); 4-glycethosomes (730 ± 12 Pa s) and 6-glycethosomes (1364 ± 33 Pa s). Besides, all formulations had a similar pseudoplastic behavior (s ~0.42). The high- est value of viscosity measured for 8-glycethosomes was clearly depen- dent to the increased mean diameter of vesicles which can encapsulated a large amount of aqueous medium reducing that free in intervesicle spaces. However, a viscosity increase was observed as a function of other mangiferin concentrations too (2, 4 and 6 mg/mL) while the mean diam- eter of these vesicles remained unchanged. This increase can be related to an improvement of interactions between vesicles, which decreased the kinetic energy.

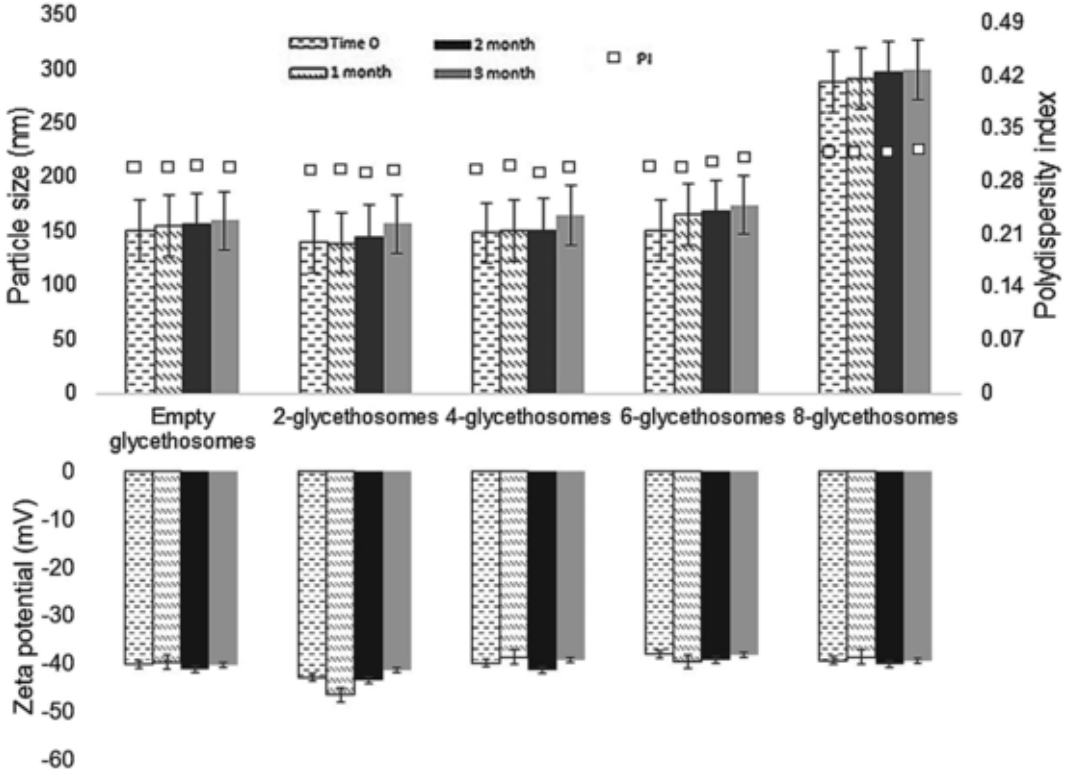
3.3. In vitro skin penetration and permeation studies

The ability of the new vesicles to facilitate the skin delivery of mangiferin was evaluate by using franz diffusion cells (Manca et al., 2016). Additionally, mangiferin (8 mg/mL) was dispersed in a blend of water, ethanol, and glycerol and the obtained dispersion was used as ref- erence to compare the vesicle performances (Fig. 4).

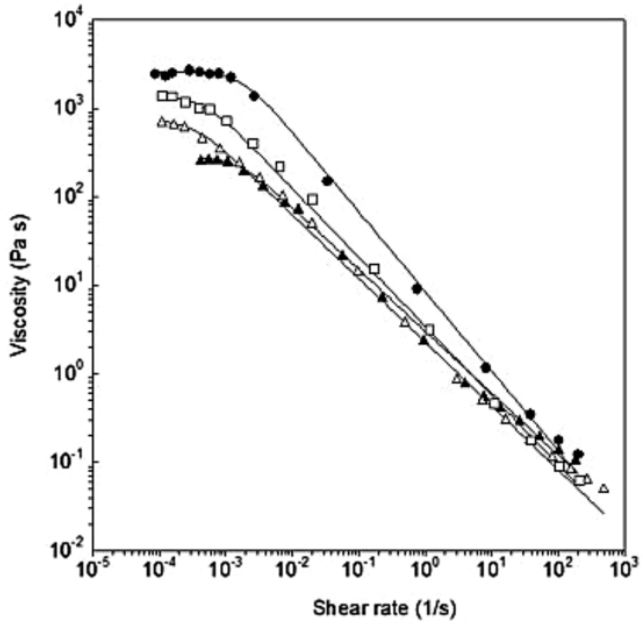
Using the mangiferin (8 mg/mL) in dispersion the deposition in the skin was ~15 μg like to that provided by the vesicles (~15 μg, $p > 0.05$) loading the lowest concentration of mangiferin (2 mg/mL). Using the mangiferin loaded in vesicles the amount of phytodrug ac- cumulated in the epidermis was closely related to the administered dose. Indeed, the amount deposited in the skin after the application of 8-glycethosomes which loaded 8 mg/mL of mangiferin was 9-fold
higher than that accumulated after the application of mangiferin dis- persion which contained the same amount of phytodrug. Results dis- closed the optimal performance of glycethosomes in the skin delivery of mangiferin.



/ig. 1. Representative cryo-TEM images of 2-glycethosomes (A), 4-glycethosomes (B), 6-glycethosomes (C) and 8-glycethosomes (D).



/ig. 2. Mean diameter (MD), polydispersity index (PI) and zeta potential (ZP) of mangiferin loaded into 2-glycethosomes, 4-glycethosomes, 6-glycethosomes and 8-glycethosomes stored for 3 months at 4 °C. Data are reported as mean values ± standard deviations (error bars).



/ig. 3. Representative flow curves fitted to Carreau model for 2-glycethosomes (▲), 4-glycethosomes (Δ), 6-glycethosomes (□) and 8-glycethosomes (●).

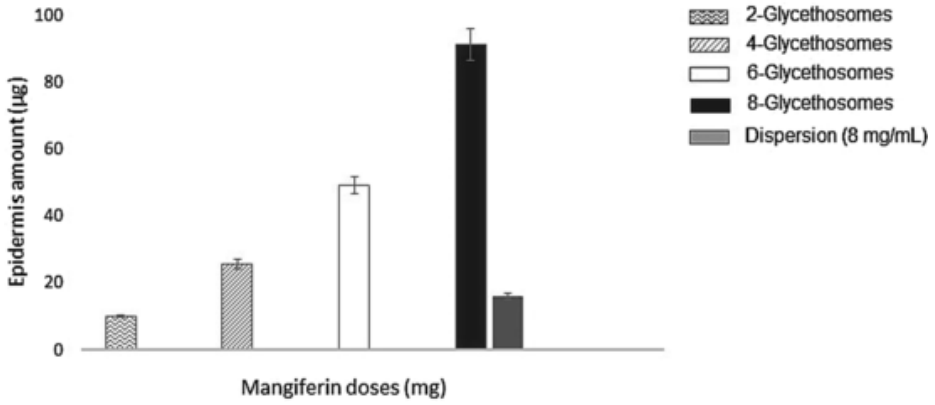
The amount of phytodrug which permeated in the receptor fluid was the lowest using the dispersion and increased as a function of the loaded mangiferin in the vesicles, as follows: mangiferin disper- sion < 2-glycethosomes (0.81%) < 4-glycethosomes (0.86%) < 6-glycethosomes (1.52%) < 8-glycethosomes (1.70%) ($p < 0.05$). The lower permeation of mangiferin in dispersion may be related to its high molecular weight (~422.34 g/mol) and hydrophilicity (log P oct = −0.65), which make difficulty its permeation through the stra- tum corneum. Moreover, it was observed that the permeation of the drug through the skin was independent of the vesicle size as the best results were obtained with 8-glycethosomes, which were significantly larger than the other vesicles. Given that, the accumulated amount in the RC was ~1.70% or lower, thus it can be considered negligible.

3.4. In vitro cell viability and protection against oxidative stress

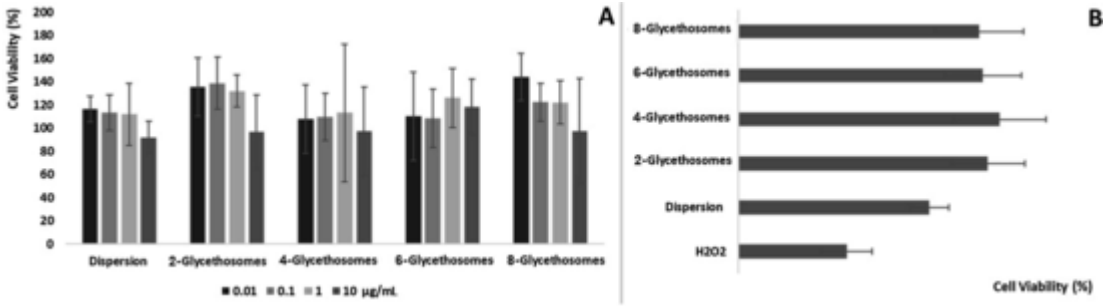
The biocompatibility of mangiferin in dispersion or loaded into glycethosomes was evaluated by using fibroblasts (3T3 cells, Fig. 5A). The vesicle viability was slight lower using the lower dilution corre- sponding to 10 μg/ml of mangiferin irrespective to the used formulation. Using the other dilutions any mortality was detected and the viability of cells was ≥100%. Results confirmed a great bioavailability of mangiferin dispersion which was not affected by the loading in vesicles.

The ability of mangiferin in dispersion or loaded in glycethosomes to protect the cells against oxidative stress was evaluated (Fig. 5B).

The cells were stressed with hydrogen peroxide and simultaneously treated with formulations. The viability of stressed cells was very low (~40%). The addition of mangiferin in dispersion was able to slightly counteract the oxidative stress as the viability increased up to (~70%). However, the best results were obtained when the mangiferin was loaded in glycethosomes as the viability reached ~100% irrespective of the used amount of phytodrug ($p < 0.01$ versus result provided by mangiferin dispersion). These results underlined the ability of the vesi- cles to promote the protection of cells against oxidative stress by re- ducing the damages caused by the oxygen reactive species. Consider- ing that this process is involved in the pathogenesis of psoriasis, these new mangiferin loaded glycethosomes represent a promising tool for the treatment of this pathology (Lai et al., 2018).



/ig. 4. Mangiferin accumulated in epidermis (EP) after 24 h of experiment. Bars represent the mean ± standard deviation of at least six independent experimental determinations.



/ig. 5. A Viability of 3T3 cells incubated for 48 h with different concentrations of mangiferin in dispersion or loaded into glycethosomes. **B** Protective effect of mangiferin in dispersion (8 mg/mL) or loaded in glycethosomes against hydrogen peroxide-induced oxidative stress in 3T3 cells. Data are reported as mean values ± standard deviations (error bars) of cell viability expressed as the percentage of control (100% viability).

3.5. Inflammatory TPA mice models

Psoriasis is an inflammatory skin disorder characterized by the hy- perproliferation of basal epidermal cells. It has been postulated that po- tent anti-inflammatory and antioxidant agents can play an important role in restoring physiological conditions (Pivetta et al., 2018; Zengin et al., 2019).

In the present study, TPA was applied topically on mice skin to in- duce ulceration and inflammation effects. The therapeutic efficacy of mangiferin in dispersion or loaded in glycethosomes was evaluated in vivo by measuring the inhibition of oedema and inflammatory cell acti- vation, typical events associated with skin lesion. To evaluate the effect of the vesicles alone, empty vesicles were also tested.

TPA-induced oedema was significantly reduced ($p < 0.05$) by treat- ing the wounded skin with mangiferin loaded vesicles, (~51% inhi- bition), irrespective to the mangiferin concentration used ($p > 0.05$), while the inhibition was only ~6 and 9% ($p > 0.05$) when empty vesi- cles and mangiferin loaded vesicles were used (Table 3).

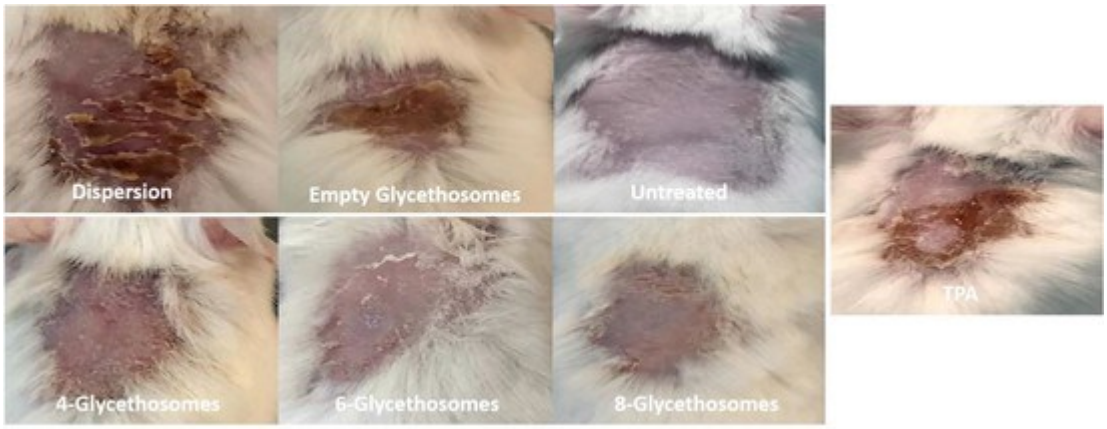
MPO activity was inhibited as follows: mangiferin loaded vesicles (~83%) > mangiferin dispersion (~30%) > empty vesicles (~25%). These findings demonstrated that the delivery of mangiferin at the wound site using phospholipid vesicles, improves the effectiveness of phytodrug against skin inflammation and this effect should be facilitated by the penetration effect of ethanol and emollient and hydrating proper- ties of glycerol. In addition, different studies suggested that the combi- nation of anti-inflammatory and antioxidant compounds as mangiferin (Biswas et al., 2015; Bairy et al., 2002) with emollients should be used as second and third-line psoriasis therapies (Khosravi et al., 2017). However, all consensus conferences and guidelines supported the use of emollients as a first-line therapy for the treatment of atopic dermatitis (Ring et al., 2012; Eichenfield et al., 2014).

Table 3
Inhibition of oedema and MPO activity in mice exposed to TPA and treated with mangiferin in dispersion or loaded into glycethosomes. Mean values ± standard deviation is reported. * indicate values significantly different from glycethosomes, # indicate values significantly different from 8-glycethosomes ($p < 0.05$).

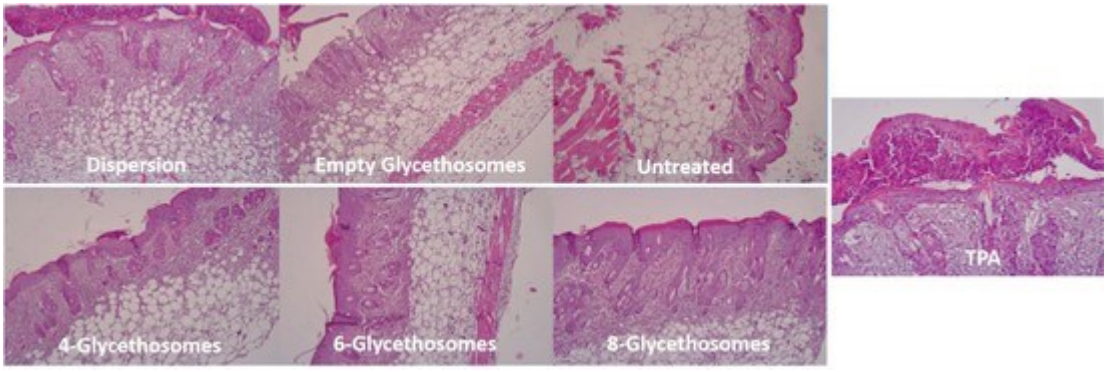
Formulations	Oedema inhibition (%)	MPO inhibition (%)
TPA	0	0
Mangiferin dispersion	9 ± 0.1*	30 ± 0.6*
Empty glycethosomes	6 ± 0.1*	26 ± 2.4*
4-glycethosomes	47 ± 3.2	80 ± 5.1
6-glycethosomes	51 ± 2.9	81 ± 4.4
8-glycethosomes	58 ± 2.6	87 ± 3.0 #

Macroscopic evaluation of the treated skin underlined the superior ability of glycethosomes to heal the wound in comparison with the dis- persion used as reference, irrespective of the concentration tested (Fig. 6), even if better results were obtained using 6- and 8-glycethosomes (loading 6 and 8 mg/mL of mangiferin). Histological analysis (Fig. 7) showed that topical application of mangiferin loaded vesicles, irrespec- tive to the used mangiferin concentration, were able to reduce the pathological effect induced by TPA in comparison with mangiferin dis- persion and empty glycethosomes. In agreement with the macroscopic observation, 4-glycethosomes showed a slight accumulation of inflam- matory cells in the epidermis with parakeratosis evidences and a moder- ate inflammatory infiltration in dermis.

However, as the mangiferin concentration increased (6- and 8-glycethosomes), the protection against damaging effects of TPA also increased. Otherwise, skin treated with TPA only, displayed inflamma- tory infiltrates of mononuclear cells, eosinophils and neutrophils, along with severe dermal and subcutaneous alteration. These alterations are slightly reduced by treating the skin with mangiferin dispersion.



/ig. 6. Mice skin lesions caused by TPA application and treated with empty glycethosomes, mangiferin in dispersion or loaded into 4-glycethosomes, 6-glycethosomes and 8-glycethosomes, and untreated skin.



/ig. 7. Histological determination of the mice skin exposed to TPA, treated with empty glycethosomes and with mangiferin in dispersion or loaded into 4-glycethosomes, 6-glycethosomes and 8-glycethosomes, and untreated skin. Original magnification 10x.

4. Conclusions

The delivery of mangiferin in glycethosomes, along with the high vis- cosity of these vesicles, favoured the retention of the drug in the epi- dermis and provided a depot of mangiferin on the skin that could be released slowly. All vesicles tested were highly biocompatible and pro- moted the effectiveness of mangiferin against the oxidative stress in- duced in fibroblasts using hydrogen peroxide. In vivo results underlined one time more the superior ability of vesicles to promote the heal of the wound induced by TPA. Overall results seemed to indicate a remarkable therapeutic potential of mangiferin loaded glycethosomes for the treat- ment of psoriasis or other skin disorders.

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Declaration of Competing Interest

The authors declare that they have no known competing financial in- terests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abrahams, S., Haylett, W.L., Johnson, G., Carr, J.A., Bardien, S., 2019. Antioxidant effects of curcumin in models of neurodegeneration, aging, oxidative and nitrosative stress: A review. Neuroscience 406, 1–21.

Alves, M.P., Scarrone, A.L., Santos, M., Pohlmann, A.R., Guterres, S.S., 2007. Human skin penetration and distribution of nimesulide from hydrophilic gels containing nanocar- riers. Int. J. Pharm. 341 (1–2), 215–220.

Angelova-Fischer, I., Rippke, F., Richter, D., Filbry, A., Arrowitz, C., Weber, T., Fischer, T.W., Zillikens, D., 2018. Stand-alone emollient treatment reduces flares after discon- tinuation of topical steroid treatment in atopic dermatitis: A double-blind, random- ized, vehicle-controlled, left-right comparison study. Acta Dermato-Venereologica 98 (5), 517–523.

Bairy, I., Reeja, S., null Siddharth, Suganghi Rao, P., Bhat, M., Shivananda, P.G., 2002. Evaluation of antibacterial activity of mangifera indica on anaerobic dental mi- croglora based on in vivo studies. Indian J. Pathol. Microbiol. 45 (3), 307–310.

Biswas, T., Sen, A., Roy, R., Maji, S., Maji, H.S., 2015. Isolation of mangiferin from flow- ering buds of *Mangifera Indica* L and its evaluation of in vitro antibacterial activity. J. Pharm. Anal. 4 (3), 8.

Body-Malapel, M., Djouina, M., Waxin, C., Langlois, A., Gower-Rousseau, C., Zerbib, P., Schmidt, A.-M., Desreumaux, P., Boulanger, E., Vignal, C., 2018. The RAGE signaling pathway is involved in intestinal inflammation and represents a promising therapeutic target for inflammatory bowel diseases. Mucosal Immunol. 25–35.

Doppalapudi, S., Mahira, S., Khan, W., 2017. Development and in vitro assessment of pso- ralen and resveratrol co-loaded ultradeformable liposomes for the treatment of vi- tiligo. J. Photochem. Photobiol. B,Biol. 174, 44–57.

Du, S., Liu, H., Lei, T., Xie, X., Wang, H., He, X., Tong, R., Wang, Y., 2018. Mangiferin: An effective therapeutic agent against several disorders (Review). Mol. Med. Rep. 18 (6), 4775–4786.

Eichenfield, L.F., Tom, W.L., Berger, T.G., Krol, A., Paller, A.S., Schwarzenberger, K., Bergman, J.N., et al., 2014. Guidelines of care for the management of atopic dermati- tis: Section 2. Management and treatment of atopic dermatitis with topical therapies. J. Am. Acad. Dermatol. 71 (1), 116–132.

Fang, C.-L., Wang, Y., Tsai, K.H.-Y., Chang, H.-I., 2018. Liposome-encapsulated baicalein suppressed lipogenesis and extracellular matrix formation in Hs68 human dermal fi- broblasts. Front. Pharmacol. 256, 45–50.

Furue, M., Uchi, H., Mitoma, C., Hashimoto-Hachiya, A., Chiba, T., Ito, T., Nakahara, T., Tsuji, G., 2017. Antioxidants for healthy skin: the emerging role of aryl hydrocarbon receptors and nuclear factor-erythroid 2-related factor-2. Nutrients 9 (3).

Gu, P.-C., Wang, L., Han, M.-N., Peng, J., Shang, J.-C., Pan, Y.-Q., Han, W.-L., 2018. Com- parative pharmacokinetic study of mangiferin in normal and alloxan-induced diabetic rats after oral and intravenous administration by UPLC-MS/MS. Pharmacology 103 (1–2), 30–37.

Guo, H.-W., Yun, C.-X., Hou, G.-H., Jun, D., Huang, X., Yi, L., Keller, E.T., Zhang, J., Deng, J.-G., 2014. Mangiferin attenuates Th1/Th2 cytokine imbalance in an ovalbumin-in- duced asthmatic mouse model. PLoS ONE 9 (6).

Huang, C., Quinn, D., Sadovsky, Y., Subra Suresh, K., Hsia, J., 2017. Formation and size distribution of self-assembled vesicles. Proc. Natl. Acad. Sci. 114 (11), 2910–2915.

ussain, A., Singh, S., Sharma, D., Webster, T.J., Shafaat, K., Faruk, A., 2017. Elastic li- posomes as novel carriers: recent advances in drug delivery. Int. J. Nanomed. 12, 5087–5108.

Imran, M., Arshad, M.S., Butt, M.S., Kwon, J.-H., Arshad, M.U., Sultan, M.T., 2017. Mangiferin: A natural miracle bioactive compound against lifestyle related disorders. Lipids Health Dis..

Janeczek, M., Moy, L., Lake, E.P., Swan, J., 2018. Review of the efficacy and safety of top- ical mahonia aquifolium for the treatment of psoriasis and atopic dermatitis. J. Clin. Aesthetic Dermatol. 11 (12), 42–47.

Khosravi, H., Siegel, M.P., Van Voorhees, A.S., Merola, J.F., 2017. Treatment of inverse/ intertriginous psoriasis: updated guidelines from the medical board of the national psoriasis foundation. J. Drugs Dermatol.: JDD 16 (8), 760–766.

Lai, R., Xian, D., Xiong, X., Yang, L., Song, J., Zhong, J., 2018. Proanthocyanidins: novel treatment for psoriasis that reduces oxidative stress and modulates Th17 and Treg cells. Redox Rep.: Commun. Free Rad. Res. 23 (1), 130–135.

Manca, M.L., Matricardi, P., Cencetti, C., Peris, J.E., Melis, V., Carbone, C., Escribano, E., Zaru, M., Fadda, A.M., Manconi, M., 2016. Combination of argan oil and phospho- lipids for the development of an effective liposome-like formulation able to improve skin hydration and allantoin dermal delivery. Int. J. Pharm. 505 (1–2), 204–211.

Manca, M.L., Mir-Palomo, S., Caddeo, C., Nacher, A., Díez-Sales, O., Peris, J.E., Pedraz, J.L., Fadda, A.M., Manconi, M., 2019. Sorbitol-penetration enhancer containing vesi- cles loaded with baicalin for the protection and regeneration of skin injured by oxida- tive stress and UV radiation. Int. J. Pharm. 555, 175–183.

Manconi, M., Manca, M.L., Caddeo, C., Valenti, D., Cencetti, C., Diez-Sales, O., Nacher, A., et al., 2018. Nanodesign of new self-assembling core-shell gellan-transfersomes load- ing baicalin and in vivo evaluation of repair response in skin. Nanomed.: Nanotech- nol., Biol., Med. 14 (2), 569–579.

Martinez, R.M., Pinho-Ribeiro, F.A., Steffen, V.S., Silva, T.C.C., Caviglione, C.V., Bottura, C., Fonseca, M.J.V., et al., 2016. Topical formulation containing naringenin: efficacy against ultraviolet B irradiation-induced skin inflammation and oxidative stress in mice. PLoS ONE 11 (1).

Mir-Palomo, S., Amparo Nácher, M.A., Busó, O.V., Caddeo, C., Manca, M.L., Manconi, M., Díez-Sales, O., 2019. Baicalin and berberine ultradeformable vesicles as potential ad- juvant in vitiligo therapy. Colloids Surf. B, Biointerfaces 175, 654–662.

Owczarek, K., Jaworski, M., 2016. Quality of life and severity of skin changes in the dy- namics of psoriasis. Postepy Dermatologii I Alergologii 33 (2), 102–108.

Perez, A.P., Altube, M.J., Schilrreff, P., Apezteguia, G., Celes, F.S., Zacchino, S., Indiani, C., de Oliveira, E., Romero, L., Morilla, M.J., 2016. Topical amphotericin B in ultrade- formable liposomes: formulation, skin penetration study, antifungal and antileishma- nial activity in vitro. Colloids Surf. B, Biointerfaces 139, 190–198.

Pimentel-Moral, S., Teixeira, M.C., Fernandes, A.R., Arráez-Román, D., Martínez-Férez, A., Segura-Carretero, A., Souto, E.B., 2018. Lipid nanocarriers for the loading of polyphe- nols – A comprehensive review. Adv. Colloid Interface Sci. 260, 85–94.

Pivetta, T.P., Simões, S., Araújo, M.M., Carvalho, T., Arruda, C., Marcato, P.D., 2018. Development of nanoparticles from natural lipids for topical delivery of thymol: in- vestigation of its anti-inflammatory properties. Colloids Surf. B, Biointerfaces 164, 281–290.

Pleguezuelos-Villa, M., Amparo Nácher, M.J., Hernández, M.A., Buso, O.V., Sauri, A.R., Díez-Sales, O., 2019. Mangiferin nanoemulsions in treatment of inflammatory disor- ders and skin regeneration. Int. J. Pharm. 564, 299–307.

Rauf, A., Imran, M., Khan, I.A., Ur-Rehman, M.-, Gilani, S.A., Mehmood, Z., Mubarak, M.S., 2018. Anticancer potential of quercetin: A comprehensive review. Phytotherapy Res.: PTR 32 (11), 2109–2130.

Ring, J., Alomar, A., Bieber, T., Deleuran, M., Fink-Wagner, A., Gelmetti, C., Gieler, U., et al., 2012. Guidelines for treatment of atopic eczema (atopic dermatitis) Part I. J. Eur. Acad.

Dermatol. Venereol.: JEADV 26 (8), 1045–1060.
Rodríguez-Luna, A., Talero, E., Del Carmen, M., Terencio, M.L., González-Rodríguez, A.M., de Los Rabasco, C., Motilva, R.V., Ávila-Román, J., 2017. Topical application of glycolipids from isochrysis galbana prevents epidermal hyperplasia in mice. Marine Drugs 16 (1).
Saha, S., Sadhukhan, P., Sinha, K., Agarwal, N., Sil, P.C., 2016. Mangiferin attenuates ox- idative stress induced renal cell damage through activation of PI3K induced Akt and Nrf-2 mediated signaling pathways. Biochem. Biophys. Rep. 5, 313–327.
Szandruk, M., Merwid-Ląd, A., Szeląg, A., 2018. The impact of mangiferin from be- lamcanda chinensis on experimental colitis in rats. Inflammopharmacology 26 (2), 571–581.
Tanaka, M., Kishimoto, Y., Sasaki, M., Sato, A., Kamiya, T., Kondo, K., Iida, K., 2018. Ter- minalia Bellirica (Gaertn.) Roxb. extract and gallic acid attenuate LPS-induced inflam- mation and oxidative stress via MAPK/NF-KB and Akt/AMPK/Nrf2 pathways. Oxid. Med. Cell. Longevity 2018, 9364364.
Zengin, G., Stefanucci, A., Rodrigues, M.J., Mollica, A., Custodio, L., Aumeeruddy, M.Z., Mahomoodally, M.F., 2019. *Scrophularia Lucida* L. as a valuable source of bioactive compounds for pharmaceutical applications: in vitro antioxidant, anti-inflammatory, enzyme inhibitory properties, in silico studies, and HPLC profiles. J. Pharm. Biomed. Anal. 162, 225–233.
Zou, B., Wang, H., Liu, Y., Qi, P., Lei, T., Sun, M., Wang, Y., 2017. Mangiferin induces apoptosis in human ovarian adenocarcinoma OVCAR3 cells via the regulation of Notch3. Oncol. Rep. 38 (3), 1431–1441.