Journal of Pharmaceutical Sciences 111 (2022) 417–431

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmaceutics, Drug Delivery and Pharmaceutical Technology

Resveratrol-Loaded Vesicular Elastic Nanocarriers Gel in Imiquimod-Induced Psoriasis Treatment: In Vitro and In Vivo Evaluation

Mahmoud A. Elgewelly[a], Soha M. Elmasry[a], Nesrine S El Sayed[b], Haidy Abbas[a,*]

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

ARTICLE INFO

Article history:
Received 1 June 2021
Revised 24 August 2021
Accepted 24 August 2021
Available online 28 August 2021

Keywords:
Resveratrol
Psoriasis
Spanlastics
Carbopol 934 gel
Sustained release

ABSTRACT

This work aimed to develop a new efficient approach for safe treatment of psoriasis. To achieve that, resveratrol-loaded spanlastics(F1-F12) were prepared and evaluated by complete in vitro characterization. The two optimal formulations (F10 and F11) had their particle size in the nano range with high entrapment efficiency and sustainable drug release. These two formulae were incorporated in carbopol 934 gel formulations (G1-G8) with different concentrations of drug and carbopol 934 polymer. G1 and G5 (1% w/w Carbopol 934 gel and 0.1% resveratrol) showed 40.13% ± 2.017% and 73.76% ± 2.46%,8 hours drug release, respectively. Their pH was accepted and non-irritant. At a shear stress of 500 s^{-1}, G1 and G5 showed a reasonable viscosity of 1048.5 ± 2.12 cps and 954 ± 2.15 cps, respectively. In the in vivo psoriasis study, mice treated by G5 gel showed significant improvement of erythema and scaling compared to positive control group and they maintained healthy skin as shown in histopathological observations. Moreover, this group showed the least changes in mRNA expression of inflammatory cytokines. Concisely, our results suggest that selected carbopol gel of resveratrol-loaded spanlastics could maximize resveratrol topical anti-psoriatic effect.

Introduction

Psoriasis is one of the most common immune-mediated chronic inflammatory diseases that affect approximately 2–3% of the world population.[1] It is mainly characterized by topical erythema, scaling, and increased epidermal thickness. It increases the number of T cells, CD + 4 and CD + 8. The pathogenesis of psoriasis is still unclear. However, it is already proved that psoriasis results from triggers such as trauma, stress, infections, and chemicals that stimulate and result in overexpression of cytokines, most probably interleukins (IL23/IL17) axis.[2] Overexpression of these cytokines in addition to others, such as IL-22 and TNFα, contributes to inflammation and keratinocyte proliferation.[3] Imiquimod-induced psoriasis represents an efficient psoriasis model. Imiquimod is an imidazoquinoline heterocyclic amine that is available in the market in the form of cream under the brand name Aldara® cream 5%. It induces synthesis of proinflammatory cytokines and results in overexpression of interleukins (IL23/IL17).[4]

Trans-resveratrol is a naturally occurring polyphenol found in nuts and berries. It has immune-mediated anti-inflammatory properties.[5] Resveratrol activates sirtuin enzyme (SirT1) in the skin and inhibits cell differentiation and regulates the cell cycle. SirT1 also controls the genetic transcription process, so it counteracts the overexpression of some cytokines that cause keratinocyte proliferation.[6] Resveratrol was also found to downregulate NF-κB, which is a family of transcription factors regulating immune response and inflammation.[7] The anti-inflammatory, anti-photoaging, and anti-wrinkling effects based on the concept of lowering inflammatory cytokine levels were reported in previous studies.[8,9] Resveratrol potential in the treatment of psoriasis has also been proposed in previous studies.[10]

Nanoparticles always represent an extremely good solution for the improvement and development of drug preparations.[11] Kakkar and Kaur developed novel surfactant-based nanovesicular drug carriers (spanlastics) composed of Span 60 as a non-ionic surfactant with an edge activator (EA) in nanosize ranges. These spanlastics showed elasticity, high penetration, and efficient drug loading. High elasticity is attributed to the use of EAs, which provide flexibility to the lipid bilayer membranes of these vesicles.[12] These surfactant

Abbreviations: IL, interleukin; NF-κ, nuclear factor kappa B; SirT1, sirtuin; SC, stratum corneum; TNFα, tumor necrosis factor alpha; ANOVA, analysis of variance; H&E, hematoxylin and eosin; MSE, mean standard error; PASI, psoriasis area and severity index; SD, standard deviation.

The following author have an ORCID Number: H. Abbas: https://orcid.org/0000-0003-4243-1442.

* Corresponding author at: Damanhour University, Department of Pharmaceutics, Egypt.

E-mail addresses: haidy_miu2002@yahoo.com, haidy.abass@pharm.dmu.edu.eg (H. Abbas).

https://doi.org/10.1016/j.xphs.2021.08.023
0022-3549/

molecules destabilize the lipid bilayers and, in turn, increase the deformability of the vesicles.[13] Incorporation of drug in suitable nano carriers is the best solution to maintain the active ingredient in its active form, enable it to pass through SC and reach more deep layers, hence maximize its effect and improve treatment.[14,15]

Cutaneous delivery of drugs is one of the most important routes used in the treatment of skin diseases. Topical application allows localizing higher concentration of the drug at the required site. This might lower systemic exposure, which can result in fewer or no adverse drug effects.[16] The complex structure of skin makes many barriers against drug penetration and localization at the deep skin layers. Stratum corneum (SC) is the major component of epidermal barriers which is physiological semi permeable membrane that acts as a biosensor which allows the essential elements to pass through it while prevents other foreign substances from passage.[17]

Gels are coherent systems composed of two phases: the interior one is composed of a polymer producing a coherent three-dimensional structure, which fixes the second phase (exterior phase). Intermolecular forces bind the molecules of these two phases so increase the viscosity by decreasing the mobility of these molecules.[18]

An important group of gels used in pharmaceutical purposes are hydrophilic gels, or hydrogels, usually made of hydrophilic polymers, which under certain situations, result in polymer concentration and jellify.[19] Carbopol is a polyacrylic acid polymer, which shows a sol-to-gel transition in aqueous solution as the pH is raised above its pka of about 5.5 and exhibits excellent bio-adhesive properties when compared with other polymers.[20] Carbopol 934 was selected to prepare the spanlastics gel owing to its hydrophilic nature and bio-adhesive properties. This may lead to increase in residence time of the drug at the site of absorption by interacting with the skin mucosa.[16,20]

This study aimed to design several spanlastics formulations using span 60 and edge activator(s) as tween 80, Birj 35 and cremophor EL. Then, the optimal formulae in term of in vitro characteristics were incorporated in carbopol 934 gel formulae with different concentrations of drug and polymer. Based on in vitro evaluation of the prepared gel formulae, the best two formulae were furtherly involved in the in vivo studies to assess their efficacy in the treatment of psoriasis in imiquimod-induced model in mice.

Materials and Methods

Materials

Resveratrol was purchased from Qingado Kaimosi Biochemical Technology Co., Ltd., China. Span 60 was purchased from El Nasr Pharmaceutical Chemicals Co., Egypt. Spectra Pore semipermeable membrane (20 KD cutoff) was obtained from Thermo Fisher Scientific (International Equipment Trading, USA). Tween 80, ethyl alcohol, and Birj35 were purchased from El-Gomhoria Chemical Co., Egypt. Cremophor EL was obtained as a gift from Pharco Pharmaceutical Co., Egypt. Vaseline lanette cream, Fagron, and imiquimod (Aldara® cream 5%) were purchased from MEDA Pharmaceuticals, Germany. Xylene, 10% neutral buffered formalin, polymerase chain reaction (PCR) buffer, and alcohol were of analytical grade, and hematoxylin and eosin were purchased from Sigma Aldrich, USA. Triethanolamine was obtained as a gift from Pharco pharmaceuticals Co. Egypt. Carbopol 934 (Goodrich Chemical Company, OH, USA). All other chemicals were of analytical grade.

Methods

Preparation of Resveratrol-Loaded Spanlastic Vesicles

Resveratrol spanlastic dispersions were prepared using the ethanol injection method as previously described by Kakkar and Kaur.[21] Resveratrol and Span 60 were dissolved in ethanol, and EAs were dissolved in a calculated volume of distilled water. The ratio of volume of the organic phase to aqueous phase was 1:5. The weight ratio of Span 60 to EA is shown in Table 1. Span alcoholic solution was wisely added drop by drop in the aqueous phase, while it was stirred by a magnetic stirrer (WiseStir, MSH-20A, PMI Lab Instrument, UK).

Continuous stirring was conducted to evaporate the ethanol until the final spanlastic dispersion was obtained containing the calculated dose of resveratrol.[22]

Water bath sonicator (POWERSONIC 405, USA) was used for 5 min to optimize the particle size of the produced dispersions.[23]

In Vitro Characterization of Spanlastics

Particle size, zeta potential, and polydispersity index (PDI). The average particle size, zeta potential, and PDI of the prepared spanlastic dispersions were determined using Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). Samples of each formulation were diluted using distilled water to obtain a final concentration of 0.01 w/v % to avoid aggregation of particles.[24] All measurements were performed in triplicate at 25°C, and the mean values ± standard deviation (SD) were reported.

Zeta potential (z) values of the systems were determined according to the electrophoretic light scattering technology using a laser Doppler anemometer coupled with the previously used Zetasizer (Malvern Instruments, Malvern, UK). The technique analyzes the electrophoretic mobility of vesicles under an electric field.[25] All measurements were performed in triplicate at 25°C, and the mean value ± SD was reported.

Determination of entrapment efficiency %. A portion from each formulation containing 1 mg of resveratrol was centrifuged using cooling centrifuge (PrO-Research K2015 R, Centurion Scientific Ltd., UK) at 15000 rpm for 1 h at 4°C, and the supernatant was separated. The sediment was rewashed by distilled water and vortex mixed (Vortex mixer, Bionex, USA), and the resulting dispersion was recentrifuged at the same conditions. The second supernatant was separated. Unentrapped resveratrol was calculated by determining the drug content in the two separated supernatants by spectrophotometric measurement at λ max =306 nm using UV-Vis spectrophotometer (Jenway 6305 single-beam spectrophotometer). The sum of drug content in both supernatants represents the entrapped drug. The entrapped drug percentage is calculated as follows:

$$\begin{aligned} EE\,\% = {} & \text{initial loaded amount of RES} \\ & - \text{un entrapped amount of RES} \\ & \times 100 \text{ Initial loaded amount of RES} \end{aligned}$$

Determination of drug release %. Provided samples containing 2-mg drug were added to a dialysis bag (20 KD cutoff, Spectra Pore, Thermo Fisher Scientific, IET, USA). The tested release buffer was phosphate buffer with pH 7.4.[26] The dialysis bag was sealed properly from both top and bottom and inserted into the 200-mL release buffer in the release cup. Paddle-type dissolution tester (RC-6, Nanbei, China) was used, rotating at 50 rpm with temperature adjusted to 32 ± 0.5°C.[27] At predetermined sampling points, 5 ml medium sample was withdrawn according to a pre-planned schedule and immediately replaced with another 5 ml of equally warmed buffer. Samples were measured at λ max using UV-Vis spectrophotometer (Jenway 6305 single-beam spectrophotometer). Release percent at each time point was determined and plotted against time.

Morphologic examination using transmission electron microscopy (TEM). Morphological examination was conducted for selected formulations according to the results of particle size, zeta potential, entrapment efficiency %, and in vitro drug release. One drop of spanlastic dispersion

Table 1
Composition of the Spanlastic Formulations.

Formula	Resveratrol	Edge Activators (EA)			Water	Span 60	Ethanol	Span: EA (s)	Solvent
		Tween 80	Birj35	Cremophor EL					
F1	10 mg	- - -			8.3 ml	200 mg	1.7 ml	100: 0	10 ml
F2	10 mg		20 mg - -		8.3 ml	180 mg	1.7 ml	90: 10	10 ml
F3	10 mg		40 mg - -		8.3 ml	160 mg	1.7 ml	80: 20	10 ml
F4	10 mg		60 mg - -		8.3 ml	140 mg	1.7 ml	70: 30	10 ml
F5	10 mg		80 mg - -		8.3 ml	120 mg	1.7 ml	60: 40	10 ml
F6	10 mg		100 mg - -		8.3 ml	100 mg	1.7 ml	50: 50	10 ml
F7	10 mg		- 20 mg -		8.3 ml	180 mg	1.7 ml	90: 10	10 ml
F8	10 mg		- - 20 mg		8.3 ml	180 mg	1.7 ml	90: 10	10 ml
F9	10 mg		- 100 mg -		8.3 ml	100 mg	1.7 ml	50: 50	10 ml
F10	10 mg		- - 100 mg		8.3 ml	100 mg	1.7 ml	50: 50	10 ml
F11	10 mg		- 50 mg 50 mg		8.3 ml	100 mg	1.7 ml	50:25: 25	10 ml
F12	10 mg		- 10 mg 10 mg		8.3 ml	180 mg	1.7 ml	90: 5: 5	10 ml

was applied, after appropriate dilution, to 300-mesh carbon-coated copper grid and allowed to settle for 5 min to adhere to the carbon substrate. After drying, the sample was investigated under TEM (JEM 1400 PLUS -JEOL, Japan) and TEM (JEM 2100 -JEOL, Japan) at 80 kV.

Differential scanning calorimetry (DSC) of selected formula. Thermal properties of selected modified spanlastic formulations were explored by differential scanning calorimetry using (SDT Q600 V20.9 Build 20 thermal gravimetric, New Castle, DE, USA). A sample of 3 −5 mg of lyophilized powder was heated in sealed aluminum pans at heating rate of 10°C/min in a temperature range from room temperature to 300°C under a nitrogen flow rate of 30 ml/min. Physical mixture of components and each component were scanned at the same conditions. The thermogram was recorded to check the degree of crystallinity and extent of interaction between components.

Formulation of Carbopol 934 gel of selected resveratrol Spanlastics

Weighed quantity of Carbopol 934 of different concentrations (0.1%, 2 % w/w %) was added gradually into 10 ml of the two spanlastics dispersions (F10-F11) and stirred with a mechanical stirrer at a high speed until a thin dispersion without lumps was obtained. The stirring speed was then reduced in order to break the foam. Few drops of triethanolamine were then added at once to form the base. Resveratrol concentration was maintained to be 0.05% w/w or 0.1% w/w according to the added weight of Carbopol 934.Composition of different Carbopol 934 gel formulae of the prepared spanlastics dispersion is shown in Table 2.

Evaluation of Carbopol 934 Gel Formulae

Visual inspection. The appearance and other physical properties, including color, precipitation, and homogenecity of freshly prepared gels were inspected by visual inspection under black and white background.[28]

pH measurement. The pH of various gel formulations was determined by using a digital pH meter(pH-009(III), Qingado TLEAD, Qingado, China. One gram of gel was dispersed in 10 ml of distilled water, warming over water bath if needed. Subsequently, the pH was measured after cooling to room temperature and storing for two hours. The measurement of the pH of each formula was done in triplicate and the average values were calculated.

Spreadability. Spreadability was determined by using two glass slides, the lower one is fixed and the upper one is movable. 1 g of the formulated gel was placed between the fixed slide and the upper movable slide. A fixed weight (100 g) was placed above the upper slide for 5 minutes. The distance diameter travelled by gel was determined. Measurement was made in triplicate for each formulation and the average was calculated.

In vitro release of resveratrol from Carbopol 934 gel formulae. Provided samples containing 2mg drug were added to a dialysis bag (20KD cut off, Spectra Pore, Thermo Scientific). The process and conditions of drug release study were the same of that was applied in spanlastics formulations as described previously in **2.2.2.3.**

Drug release kinetics analysis. Different kinetic models as zero order, first order, Higuchi, Weibull and Korsmeyer- Peppas kinetic equations were applied to determine the release mechanism of Resveratrol from different formulations using DDSolver, an add-in program for Microsoft Excel, for modeling and comparison of drug release profiles.[29]

Table 2
Composition of Carbopol 934 gel of Resveratrol spanlastics.

Gel formula	Spanlastics Formula (10 ml)	Carbopol 934 (%W/W)	Carbopol 934 weight	Resveratrol final concentration
G1	F10	1%	10 g	0.1% w/w
G2	F10	2%	10 g	0.1% w/w
G3	F10	1%	20 g	0.05% w/w
G4	F10	2%	20 g	0.05% w/w
G5	F11	1%	10 g	0.1% w/w
G6	F11	2%	10 g	0.1% w/w
G7	F11	1%	20 g	0.05% w/w
G8	F11	2%	20 g	0.05% w/w

Viscosity measurement and Rheology Studies. The viscosity of different bases prepared with different concentrations was determined using cone and plate viscometer with spindle 52 (Brookfield Engineering Laboratories, model HADV-II, Middleboro, MA). Freshly prepared gels were placed in the cup of the viscometer at 32°C ± 0.5. The sample was continuously sheared at shear rates ranged from 100 sec-1 to 500 sec-1. Measurements were made with 30 seconds interval between each two successive rates. measurements were done in duplicate and Viscosity in centipoises was calculated.

In Vivo Evaluation of Selected Modified Spanlastics

Animals. Adult male Swiss albino mice (18–22 g) were obtained from the animal facility of the National Research Center, Cairo, Egypt. Animals were allowed to acclimate for at least 1 week before the initiation of the experiment. The mice (5–6 per cage) were housed in a conditioned atmosphere at a temperature of 22 ± 3°C and humidity of 50–55% with a 12-h light/dark cycle and free access to food and water. The study complied with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 2011) and was approved by the Ethics Committee for Animal Experimentation of Faculty of Pharmacy, Damanhour University (ref. no. 1220PT20F, approved on 5 December 2020). All efforts were made to minimize animal discomfort and suffering.

Experimental design. Thirty-six mice were randomly allocated into six groups (n = 5-6). All mice had its back shaved. The study duration was full 7 days from the start of first application. In group1 (negative control/Vaseline), the vehicle cream (Vaseline lanette cream, Fagron) was applied on the shaved back and inner right ear of mice. In group 2 (negative control/empty gel): mice received empty 1% Carbopol 934 gel base. Group 3 (positive control): psoriasis was induced in mice by applying 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 4, Group 5 and Group 6 received the same dose of Imiquimod cream (ALDARA® cream 5%) as group 3, however the three groups were treated on daily basis by 1 g (containing 1mg of dug) of G1, G5 and free drug suspension in gel (Gel D) respectively. All groups were examined for erythema and scaling at day 0, 2, 4, 7. On day 7, animals were anesthetized using thiopental sodium (50 mg/kg i.p.), then sacrificed by cervical dislocation. Splenomegaly of mice was assessed. The shaved area of skin on their backs was immediately excised. A 4 mm punch biopsy of lesional skin was fixed in formalin and paraffin embedded for histological analysis. The remaining lesional skin was snap frozen in liquid nitrogen and stored at -80°C,[30] to be furtherly used in qRT-PCR tests for measuring gene expression of proinflammatory cytokines.

Evaluation of erythema and scaling. To score the severity of psoriasis, erythema and scaling were observed and scored. The scoring system is based on clinical psoriasis area and severity index.[10] Erythema and scaling scores were evaluated as follows: 0, none; 1, mild; 2, moderate; 3, severe; and 4, very severe. Scoring for each group was conducted on days 0, 2, 4, and 7. Day 0 measurement was performed before application and treatment. Day 2 is the third day of application and treatment; Day 4 is the fifth day, and day 7 is the end day of the study. The results were recorded as evidence on psoriasis progress or improvement.

Histopathological examination. Skin tissue samples were flushed and fixed in 10% neutral buffered formalin for 72 h. Samples were trimmed and processed in serial grades of alcohol, cleared in xylene, and were infiltrated and embedded into Paraplast tissue embedding media. Moreover, 4-μm thick tissue sections were cut by rotatory microtome for demonstration of skin layers in different samples.

Tissue sections were stained with hematoxylin and eosin and then examined using light microscope. All standard procedures for sample fixation and staining were according to **Culling, C.F.A. 2013**.[31]

Quantitative real-time PCR (qRT–PCR) IL17A, IL 22, and IL-23. Total RNA was extracted with TRIzol (Invitrogen), and cDNA was synthesized by reverse transcription using iScript cDNA synthesis Kit (Bio-Rad). The qRT–PCR was performed using iQ SYBR Green Supermix (Bio-RAd) according to the manufacturer's instructions. The following primers were used to analyze target mRNA expression: IL-17A, 5'-CCGACAGAAGCAGGAGATG-3' (forward) and 5'-CTCAGCCCAACCCAAGATAG -3' (reverse); IL-22, 5'-GCACCTCTGACACCGTCTAC–3'(forward) and 5'- GCGGTTTGATGGTAGTGTGC-3' (reverse); IL-23, 5'-GGTGTCACGGAGGAATCACA -3' (forward) and 5'- GCATGAGGTTCCGAAAAGCC -3' (reverse).

Statistical Analysis

Data were expressed as mean ± SD. Differences between groups were analyzed using one-way analysis of variance, followed by the least significant difference procedure using SPSS® (SPSS, Inc., Chicago, Illinois, USA). Statistical differences yielding p-value < 0.05 were considered significant.

Results and Discussion

Preparation of Resveratrol-Loaded Spanlastic Vesicles

The ethanol injection method is more efficient in the preparation of vesicular elastic nanoparticles.[32] The use of ethanol has an effect on decreasing the thickness of vesicles by interpenetrating the hydrocarbon chains, thus decreasing the particle size, contributing to the formula net negative charge (negative zeta potential), resulting in steric stabilization and improvement in the entrapment efficiency of vesicles. Span 60 is lipophilic non-ionic surfactant of HLB 4.7. Span is the main surfactant used in spanlastic preparation as it is responsible for its composition of well-designed mono- and/or multilamellar matrix vesicles.[33] Tween 80 is polyethylene glycol sorbitan monooleate and it is water soluble with HLB equals 15.Incorporation of Tween 80 as an EA in the six basic formulae (F1-F6), which was previously described by Kakkar and Kaur, increases the penetration

properties of vesicles and provides the formulation's squeezing properties through the pores of membranes. However, Tween 80 has been proven to result in some anaphylactoid reactions, which could cause undesirable effects on skin when used in topical preparation.[34] Hence, we prepared new six modified formulations (F7–F12) to reduce toxicity and simultaneously add more penetration and efficiency using other surfactants, such as Birj35 and Cremophor EL. They were modified from the two best basic formulations selected based on their particle size, zeta potential, entrapment efficiency, and drug release. Birj35, which is hydrophilic with HLB value of approximately 15, helped in modifying HLB of the formulation and increased its solubility in the biological media.[35] Cremophor EL is a hydrophilic non-ionic surfactant with HLB value ranging from 14 to 16. Incorporation of Cremophor EL in the formulation would increase its penetration and flexibility to pass through biological barriers, such as the stratum corneum (SC) of the skin.[36] Formulations containing the three surfactants Span 60, Birj35, and Cremophor EL are structurally more efficient because blending more surfactants optimizes HLB and enhances the formulation structure.[37] The HLB is calculated according to the following equation:

$$\text{HLB of resultant mixture} = [(\text{HLB1} \times \text{W1}\%) + (\text{HLB2} \times \text{W2}\%)]/100^{8}$$

Based on this equation, HLB of F10 was found to be 9.3 while HLB of F11 was found to be 10.1 which reflected that F11 is more hydrophilic and water soluble than F10.

In Vitro Characterization of Spanlastic Formulations

Particle Size, Zeta Potential, and PDI

The particle size, zeta potential, and PDI were measured, as shown in Table 3. For the six basic formulations (F1–F6), the mean vesicle sizes ranged from 503 ± 0.44 nm (F1) to 267.17 ± 10.64 nm (F6). This small vesicle size of all prepared formulae that falls in the nano range reflected the efficiency of the preparation method to produce such nano structures. The six modified formulations showed mean vesicle size ranging from 1880.46 ± 6.17 nm in F7 to 198.83 ± 8.98 nm in F10. The mean vesicle size was inversely proportional to the EA concentration in the formula. This can be explained by the fact that Tween 80, Birj35, and Cremophor EL are hydrophilic surfactants. This hydrophilicity increases the emulsification effect on the vesicles causing smaller particle size of the vesicles.[38]

Zeta potential is a key factor that reflects the steric stability of the formulation. All values of the six basic formulations were found to be negative ranging from -43.5 ± 0.64 mv in F1 to -23.4 ± 0.41 mv in F6. Zeta potential of the six modified formulations were in the range of -53.7 ± 2.01 mv in F12 to -21.8 ± 0.42 mv in F9, as shown in Table 3. The higher the concentration of EAs, the lower the negativity of zeta potential.

This may be attributed to the effect of EA, which, upon increasing its concentration, covers the surface of vesicles and thus has a shielding effect on the negative charge of the vesicles.[13] The negative zeta potential of the vesicles reflects the stability of formulation and low aggregation tendency.

PDI of all formulations was < 0.9. This confirms the uniformity of vesicles and low tendency of aggregation.[8]

Determination of Entrapment Efficiency %

Entrapment efficiency of the vesicles is extremely important to determine if the drug concentration will be sufficient to reach the treatment goal. In Table 3, entrapment efficiency % of the six modified formulations (F7:F12) were all found to be > 85%. It ranged from 85.8 ± 2.28% in F10 to 96.1 ± 1.35% in F9, which indicates that using other surfactants in the same ratio of hydrophilic to lipophilic surfactant concentration improved entrapment efficiency compared to the basic formulations, which showed entrapment efficiency % ranging from 87.11 ± 1.668% in F2 to 57.2 ± 1.92% in F5. The ratio of 90:10 of span and tween in F2 represented the most efficient formula in term of EE%. This is related to HLB value of the prepared formula. The combination of Span 60 and tween 80 in this ratio have developed the best HLB value suitable for more entrapment efficiency as the blending of surfactants help to form rigid vesicles due to incorporation of more alkyl chains in the structure of vesicle membrane.[39] When the edge activator concentration reaches a certain threshold, micelles and/or mixed micelles start to assemble and consequently higher drug percentages could escape into the external aqueous phase.[40] This fact can explain why there is no graduation of the value of EE% according to the concentration ratios of different surfactants in the formula. Modification of edge activators done in formulae (F7-F12) maintained the same concept and we could find different formulae to have comparable values of EE% with slight differences according to EAs concentration changes.

Determination of Drug Release %

Drug release from resveratrol-loaded basic spanlastic formulations (F1, F2, F3, F4, F5, F6) is shown in Figure 1. Formulation F2 showed the highest release after 8 h by 97.94 ± 0.86% of drug concentration. This is aligned with its high entrapment efficiency by 87.11 ± 1.668% and that confirms the high potential of this formula in not only drug entrapment but also its ability to release the drug in a modified manner in 8 h. The high saturation of alkyl chains in Span 60 beside the less saturated ones in Tween 80 provide a hybrid model that combines both high entrapment and efficient drug release.[41] F6 comes in second in drug release after 8 h by 67.24 ± 0.42% of the loaded drug released. However, the majority of drug release occurs during the first 4 h (approximately 60% of the drug released). This may be attributed to the results of particle size, which revealed that F6 has the smallest vesicles; hence, these small vesicles were able to

Table 3
Entrapment Efficiency (EE%), Vesicle Size, Zeta Potential, and Polydispersity Index (PDI) of the Spanlastic Formulations (Value = Mean ± SD).

Formula	EE%	Vesicle Size (nm)	Zeta Potential	PDI
F1	76.4 ± 1.45	503 ± 0.44	−43.5 ± 0.64	0.27 ± 0.07
F2	87.11 ± 1.668	425.76 ± 4.4	−30.6 ± 0.99	0.48 ± 0.04
F3	65.62 ± 3.47	347.04 ± 1.54	−26.36 ± 0.36	0.408 ± 0.006
F4	68.04 ± 2.02	314 ± 4.06	−25.9 ± 0.2	0.598 ± 0.017
F5	57.2 ± 1.92	287.7 ± 1.11	−24.67 ± 0.9	0.43 ± 0.05
F6	66.37 ± 2.003	267.166 ± 10.64	- 23.4 ± 0.41	0.62 ± 0.05
F7	92.5 ± 0.3	1880.46 ± 6.17	−33.27 ± 0.54	0.9 ± 0.059
F8	90.5 ± 3.76	781.48 ± 0.78	−35.5 ± 0.58	0.8 ± 0.14
F9	96.1 ± 1.35	438.55 ± 2.39	−21.8 ± 0.42	0.59 ± 0.037
F10	85.8 ± 2.28	198.83 ± 8.98	−28.66 ± 0.86	0.52 ± 0.03
F11	88.6 ± 0.84	207.2 ± 2.32	−28.4 ± 0.29	0.52 ± 0.045
F12	88.18 ± 1.02	490.27 ± 1.46	−53.74 ± 2.01	0.46 ± 0.059

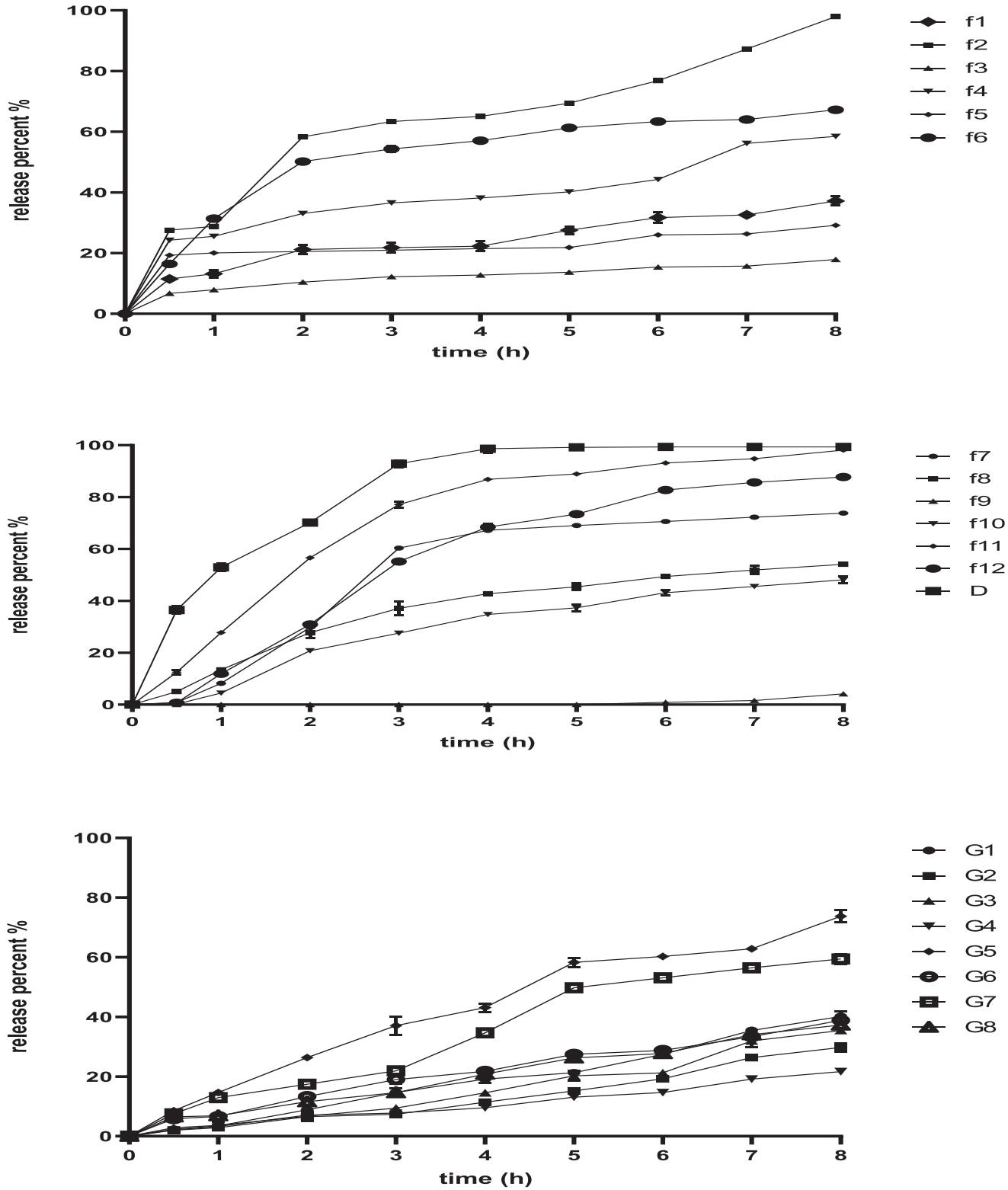

Fig. 1. In vitro drug release profiles of basic (F1:F6) spanlastics, modified (F7:F12) spanlastic, the free drug suspension D and different gel formulae (G1-G8). Values are calculated as mean ± SD.

rapidly release the drug in the external aqueous phase.[42] This is why this formulation is expected to have more potential in case we made modifications in its components, while maintaining the same ratio of aqueous and organic phases. The other formulations showed less efficient drug release, ranging from 58.48 ± 0.311% in F4 to 17.88 ± 0.078 % in F3. The HLB value of the different formulae has a direct impact on the ability of the vesicles to entrap and release drug.[43] Based on drug release results and previous findings of entrapment efficiency, particle size, and zeta potential, F2 and F6 were the two selected formulations that were further modified to new six formulations (F7–F12). The results of particle size, zeta potential, and entrapment efficiency of these newly modified formulations are

shown in Table 2, suggesting that F10 and F11 were more promising and this was confirmed in drug release, as shown in Fig. 1. It is clearly obvious that formulations containing the three types of surfactants (Span 60, Birj35, and Cremophor EL) represented the best drug release as these formulations have a balance between drug entrapment and release. This can be attributed to the fact that HLB value of the formulation is optimized by blending more surfactants.[37,40] F11 and F12 are the two formulations of this type, so they released the drug after 8 h 98.11 ± 0.3% and 87.74 ± 0.55%. F10 that consisted of Span 60: Cremophor in 50:50 ratio showed sustained release in 8 h despite its less drug release by 48.1 ± 1.575%. However, F10 may be potential in long-term treatment. Free drug suspension was also evaluated for drug release. It showed an extremely fast drug release of almost all drug contents after 4 h with no sustainability. Based on the previous findings alongside the preference to avoid formulations containing Tween 80, which results in skin anaphylactoid reactions, F10 and F11 were selected to undergo further in vivo investigations with the free drug suspension.

Morphologic Examination

Morphological examinations of the two selected modified span-lastics F10 and F11 that were selected to undergo further in vivo study are graphically illustrated in Fig. 2. The micrographs showed the development of nano spherical particles with uniform size and low tendency of aggregation, which matches the results of particle size and zeta potential measurements.

DSC

Thermo-analytical studies aims to discover the degree of crystallinity and presence of interactions between different components of the formula.[44] Since the heating of samples started from room temperature, thermotropic properties of Cremophor EL were not

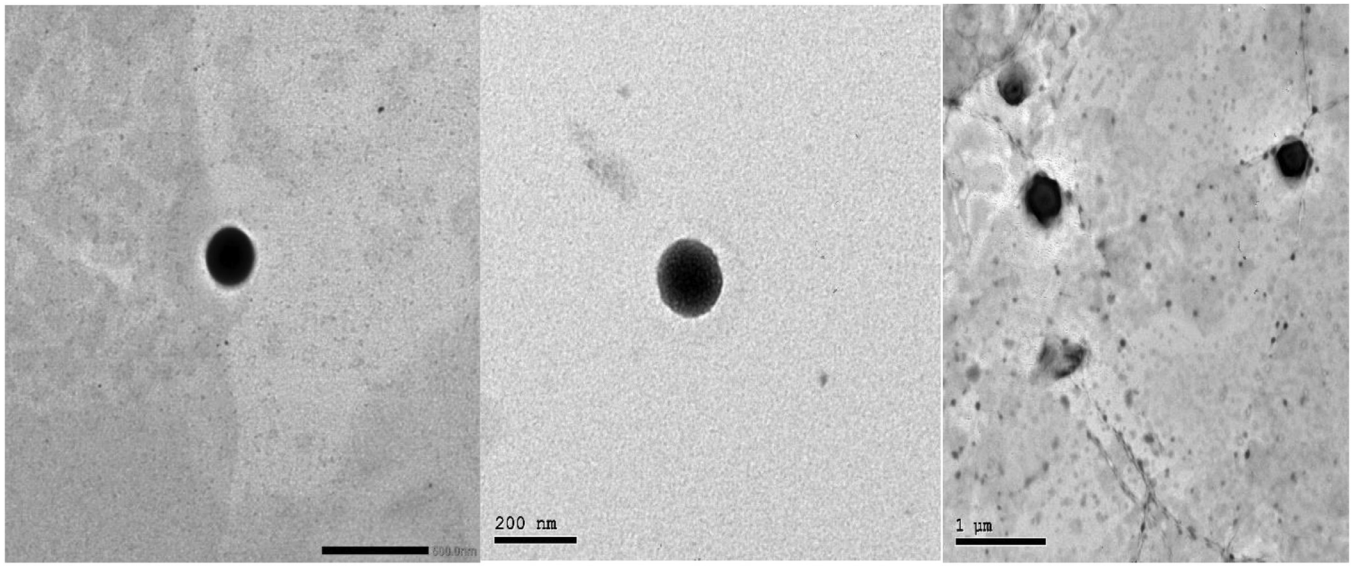

Fig. 2. TEM micrographs of F10 and F11.

evaluated because Cremophor EL exists in liquid form with melting point < 25°C.[45] Fig. 3 shows that Span 60, Birj35, and resveratrol showed endothermic peaks at 70 °C, 58 °C, and 262 °C, respectively, and this approximately matches with their melting points. Physical mixture of all components showed differences in peak positions but maintained the existence of different peaks. This is illustrated in the probable interaction between components. F11 Thermogram showed only two peaks at almost 238°C and 58°C with less melting enthalpies, which confirm the preparation of less ordered lattice arrangement and strongly prove the compatibility of different ingredients.[46] All previous results suggest the potentiality of resveratrol dispersion in the form of spanlastics.

Formulation of Different Resveratrol Spanlastics Carbopol 934 Gel Formulae

Carbopol 934 in different concentrations (1% w/w and 2% w/w) was used as gelling agent in preparation of different gel formulae. Carbopol was selected to prepare the spanlastics gel owing to its hydrophilic nature and bio-adhesive properties.[20] This may lead to increase in residence time of a drug at the site of absorption by interacting with the skin mucosa.[47] Resveratrol loaded Carbopol gel was prepared in different concentrations of polymer (1%, 2%) and different concentration of drug (0.05% and 0.1%) to formulate different 8 formulae of different characteristics regarding pH, In vitro release, spreadability and viscosity.

Evaluation of Resveratrol Carbopol 934 Gel Formulae

Visual Inspection

All formulae were evaluated for their appearance, color, homogeneity and precipitation. All gel formulae were homogenous and white with no precipitation.

pH Measurement

All formulae exhibited values of pH ranging from 5.4 and 7.44. These values were suitable and non- irritant to the skin and lying within the normal range of skin pH.[48]

Spreadability

Spreadability is an important characteristic for topical formulations which indicates that the prepared gel formulae are easy to be spreaded by a small application of shear and it shows the behavior of a gel when it comes out from its tube.[18,49] It was found that as the concentration of the polymer was increased, the viscosity of the gel was also increased and the spreadability was decreased. All the prepared formulations produced an acceptable spreadability in the range of (2.5 ± 0.7 to 5.6 ± 1.23cm).

Determination of Drug Release % from Gel Formulae

In vitro release of Resveratrol from different gel formulae was studied an is shown in Fig. 1. Regarding the gel formulae loaded by F10 spanlastics dispersion, G1 and G3 which contained the Carbopol 934 gel of concentration 1% were found to be the highest in term of drug release that reached 40.13% ± 2.017 and 35.43% ± 0.676 respectively after 8 hours. On the other hand, G2 and G4 which contained 2% Carbopol 934 showed less drug release by 29.77% ± 1.73 and 21.67% ± 1.16 after 8 hours. It was obvious that gel formulations of Carbopol 934 with polymer concentration 1% w/w showed higher release rate and percentage over those contained the higher polymer concentration (2%w/w). The same concept was achieved in gel formulations that contained F11 formula. G5 and G7 gels of F11 formula which contained the lower polymer concentration (1% w/w Carbopol 934) showed drug release 73.76% ± 2.46 and 59.42% ± 2.097 respectively after end of 8 hours, While G6 and G8 that contained Carbopol 934 of the higher polymer concentration showed lower release by 38.84% ± 0.909 and 37.30% ± 0.98 respectively. Dependence of drug release from gel on the polymer concentration used in gel base may be due to that at high polymer concentrations, the active substance was trapped in small polymers and was structured by its close proximity to that polymer molecule.[14] The density of chain structure which had been observed in gels microstructure increased at high polymer concentration and this limited the active substance movement area leading to decrease in the release of the active substance.[28] Viscosity also may play an important role in drug release rate as by increasing viscosity of gel,drug release would decrease.[20] Drug concentration of Resveratrol in different gel formulae also had an impact on drug release in formulae of the same polymer concentration.[20] G1 which contained Resveratrol 0.1% w/w was higher in drug release than G3 which had Resveratrol 0.05% w/w, G2 was also higher than G4. The same was observed in G5 which contained Resveratrol 0.1% that showed higher drug release than that of G7 which contained Resveratrol 0.05%w/w. Also, G6 was superior in drug release compared to G8 for the same reason that by increasing drug concentration in a gel formula of the same polymer concentration, drug release rate increases.[50] This point can be explained by the following equation that describes the performance of the formula in dissolution study:

$$Dr = (D/h)S(Cs - C),$$

Where Dr is dissolution rate, D: is diffusion coefficient, S: surface area, (Cs-C) is concentration gradient. According to previous equation, drug dissolution rate (Dr) is directly proportional to its concentration gradient (Cs-C) in the stagnant diffusion layer and its surface (S) available for dissolution. By increasing the drug concentration, the diffusion of drug through the stagnant layer will increase, and consequently the drug release in dissolution media will increase.

This indicates that the drug concentration in the prepared gel formula may be one of the main influences on the dissolution rate, hence the release profile of formulae.[50]

Drug Release Kinetics analysis

Release profile of the eight-resveratrol loaded Carbopol gel formulations was fitted into seven different kinetic models, including: zero order, first order, Higuchi, Hixson-Crowell, Korsmeyer – Peppas, Baker- Lonsdale and Weibull model. The best-fit model was selected on the basis of the highest correlation coefficients R2 and the lowest mean standard error (MSE) values. Data of kinetics models and kinetic parameters are shown in Table 4. As shown in Table 4, many of kinetic models could describe drug release behavior from different formulae, however by examining the Correlation coefficient and MSE of different spanlastics formulae, we found that most of gel formulations were best fitting to Weibull model. G2 (R2 = 0.9935, MSE = 0.8642), G3 (R2 = 0.9868, MSE=2.4940), G4(R2=0.9873, MSE = 0.7721) G5 (R2 = 0.9890, MSE = 7.6667), G7 (R2 = 0.9699, MSE=16.6425) and G8 (R2=0.9925, MSE=1.3149). This model can be explained by the following equation, $F = F\propto [1\text{-e-}(t - t_0/t_d)^{\beta}]$ where, F is the Fraction of the dose dissolved at time t, $F\alpha$ is the amount dissolved at infinite time, t is the time, t_0 is the lag time for dissolution after disintegration, td is the mean dissolution time, β is the shape factor (Weibull slope) and $\alpha = (td)^{\beta}$ is the time scale of the process. The Weibull model is used for comparing the release profiles of matrix type drug delivery systems. The dissolution rate is indicated by the mean dissolution time Td and the shape of the dissolution curve, indicated by parameter β.[51] The Weibull shape parameter, b, characterizes the curve as either exponential (b = 1) (case 1), sigmoid, S-shaped, with upward curvature followed by a turning point (b > 1) (case2) or parabolic, with higher initial slope and after that consistent with the exponential (b < 1)(case 3).[29] For all gel formulations, β was found to be more than 1 which indicated that they followed case 2,

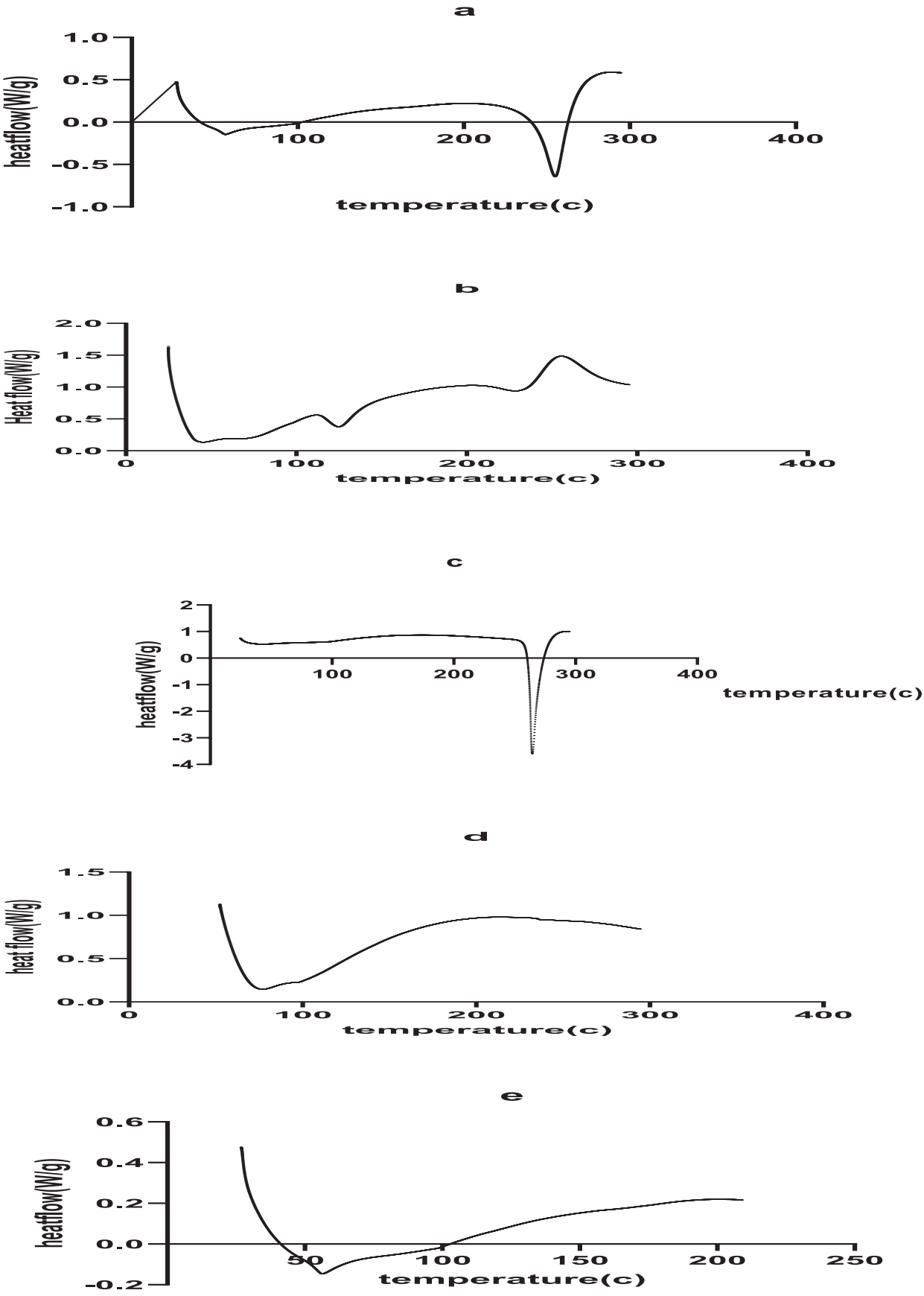

Fig. 3. DSC thermograms of (a) F11, (b) the physical mixture, (c) resveratrol (d) Span 60, and (e) Birj35.

with sigmoid, S-shaped curve, with upward curvature followed by a turning point except for G6 for which β was slightly smaller than 1 which means they related to Case 3 which indicated a parabolic curve, with higher initial slope and after that consistent with the exponential phase. Td is also a kinetic parameter used to determine the time needed for 63.2% of drug to be released from formulation. It was reasonable for all gel formulae except for G4 and G6 in which relatively long Td was found. On the other hand, G1 and G6 followed Korsmeyer- Peppas model. G1 (R2=0.9911, MSE=1.8220), G6 (R2=0.9910, MSE=1.3797). This model can be explained by the following equation, $Mt/M = a\ t^n$ where Mt/M is the released fraction of drug at time t, a is a constant incorporating structural and geometric characteristics of the drug dosage form and (n) is the release exponent, indicative of the drug release mechanism .Although Korsmeyer -Peppas model was not the best fitting model for most of formulations, but it was useful to determine the type of diffusion whether Fickian or non- Fickian diffusion.[52] The (n) value represents an indicative of the drug release mechanism. It was found that n value for all spanlastics formulae was more than 0.5 which indicated that diffusion of drug followed non Fickian diffusion mechanism. In case of G1, G2 and G3, n was found to be more than 1 which means the release mechanism in these gel formulations is probably non-Fickian super case II. Super Case II transport occurs when the concentration of penetrant in the highly stressed core is increased, promoting a more rapid relaxation-controlled transport.

Viscosity Measurement and Rheological Studies

Viscosity measurements and Rheological data of Resveratrol loaded gel formulae are represented in Table 5. It was found that all formulae exhibited non-Newtonian shear thinning (pseudoplastic) flow since the viscosity decreased with increasing the shear rate. As the shear rate is increased, the normally molecular structure of the gelling material is caused to align its long axes in the direction of a flow which in turns reduces the internal resistance of the material and hence decreases its viscosity.[18,53] By comparing the viscosity values of the gel formulae prepared by the same polymer in different concentration, it was found that by increasing the polymer concentration, there was an increase in the viscosity values in all the formulae.[28] That was obvious in G2 and G4 which consisted of 2% Carbopol 934 and showed higher viscosity compared to that of G1 and G3 which consisted of gel base of polymer concentration 1%. The same concept was achieved in G6 and G8 which showed higher viscosity than those of G5 and G7.

In Vivo Study of Selected Formula

Effect of Resveratrol-Loaded Spanlastics Gel Formulae on Erythema and Scaling

Erythema and scaling are the two major obvious clinical manifestations of psoriasis.[10] Application of imiquimod on the dorsal skin of mice is a well-established model of psoriasis.[3] Using resveratrol in an aqueous suspension or surfactant-based formulations may affect the progress of disease or inhibit the appearance of symptoms according to the formulation efficiency.[54] The two negative control groups (negative control/Vaseline only and negative control/empty gel did not show any changes in the erythema and scaling scores, while the positive control group in which animals were subjected only to imiquimod showed significant changes in terms of erythema and scaling compared to negative control groups. Skin inflammation symptoms in the model mice subjected to induction by imiquimod and treatment with different formulae started to be observed in day 2. It was moderate in group 3 (induced model) and mild in the three groups of treatment. In day 4, variations in symptoms started to be significantly noted. Group 5, which was treated with G5 gel, showed obvious improvement

Table 4
Release Kinetics of Carbopol 934 Gel of Resveratrol Spanlastics.

Release models	Kinetics parameters	Formulations							
		G1	G2	G3	G4	G5	G6	G7	G8
Zero order	R2	0.9864	0.9601	0.9612	0.9793	0.9298	0.9492	0.9472	0.9671
	MSE	2.4355	4.0008	5.4853	0.9433	36.6160	6.7941	21.9311	4.2978
First order	R2	0.9678	0.9391	0.9372	0.9765	0.9884	0.9777	0.9628	0.9795
	MSE	5.7800	6.1108	8.8787	1.0701	6.0286	2.9799	15.4603	2.6809
Higuchi	R2	0.7951	0.7366	0.7452	0.8375	0.9253	0.9205	0.8638	0.8902
	MSE	36.7264	26.4315	36.0273	7.3973	38.9701	10.6296	56.5444	14.3561
Hixon-Crowell	R2	0.9750	0.9464	0.9457	0.9778	0.9844	0.9708	0.9660	0.9775
	MSE	4.4785	5.3761	7.6732	1.0111	8.1281	3.9078	14.1070	2.9406
Baker-Lonsdale	R2	0.7728	0.7211	0.7262	0.8266	0.8801	0.9013	0.8251	0.8698
	MSE	40.7202	27.9911	38.7193	7.8968	62.5284	13.1910	72.6497	17.0224
Korsmeyer-peppas	R2	**0.9911**	0.9869	0.9812	0.9798	0.9860	**0.9910**	0.9631	0.9853
	MSE	**1.8220**	1.5049	3.0411	1.0531	8.3576	**1.3797**	17.5317	2.2019
	n	1.117	1.328	1.273	0.966	0.726	0.754	0.834	0.823
Weibull	R2	0.9902	**0.9935**	**0.9868**	**0.9873**	**0.9890**	0.9910	**0.9699**	**0.9925**
	MSE	2.3347	**0.8642**	**2.4940**	**0.7721**	**7.6667**	1.5974	**16.6425**	**1.3149**
	β	1.548	2.509	2.365	1.692	1.078	0.975	1.325	1.359
	$T_{d\ (h)}$	12.880	13.620	12.446	21.563	6.409	17.728	8.049	15.032

and scored mild erythema and scaling, while group 4, which was treated with G1 gel, showed mild scaling and severe erythema, and group 6, which was treated with the aqueous drug suspension in gel (Gel D), showed moderate scaling and erythema. Groups 4 and 6 did not show any significant change compared to the positive control group, which showed severe erythema and moderate scaling. In day 7 and by end of the study, group 3, which is the psoriasis model, showed severe erythema and extremely severe scaling due to the expected inflammatory effect of imiquimod due to induction of proinflammatory cytokines.[4] These observational symptoms were statistically significant compared to those in the negative control groups. By end of the study, group 5 mice were completely recovered from erythema and scaling induced by imiquimod with significant improvement compared to the positive control group. G5 formula was the most efficient treatment and showed the highest anti-inflammatory effect, which was reflected on observational symptom relief. These results were in agreement with the in vitro characterization regarding release %, entrapment efficiency, and particle size. This effect could be attributed to the potential of blending surfactants,[40] especially it contains Span 60, which is lipophilic-enhancing structure of vesicles, Cremophor EL, and Birj35, which have hydrophilic-enhancing drug solubility, sustained release, and penetration through the SC, leading to better deposition in different skin layers.[55] At the end of the study, group 4 still showed moderate scaling and erythema, while group 6 showed mild symptoms. Improvement in symptoms in these two groups was not significant compared to that in the positive control group.

Effect of Resveratrol-Loaded Spanlastics Gel Formulae on Histopathological Observations and Epiderma Thickness

Imiquimod-induced psoriasis results in skin thickness and infiltration of immune cells in the skin.[10] Histopathological observations are shown in Fig. 4. Group 1 demonstrated normal histological structures of skin layers with almost intact thin epidermal stratified squamous epithelium, intact dermal layer with abundant hair follicles, and normal organization of collagen fibers with minimal inflammatory cell infiltrates. Intact subcutaneous tissue was observed. Group 2 showed the same records as group 1 samples without records of abnormal alterations of epidermal/dermal structures (same annotation). Group 3 (positive control) revealed significant hyperplasia and increased thickness of epidermal layer accompanied with occasional intraepithelial lymphocytic infiltrates, and moderate subepidermal mononuclear inflammatory cell infiltrates with a higher density of dermal collagen bundles were observed. These changes in epidermal thickness and skin morphology were statistically significant compared to those in the negative control groups ($p < 0.05$), indicating the inflammatory effect of imiquimod on different skin layers. These results coincide with those reported in some literature.[10] Groups 4 and 6 showed only mild increase in epidermal thickening with persistence of inflammatory infiltrates and higher density of thick dermal collagen bundles records. Both groups showed mild to moderate degenerative changes and karyopyknosis in the epidermal basal cell layer. Existence of these inflammatory infiltrates confirms the inadequate anti-inflammatory action of these two formulae and that imiquimod inflammatory action still obviously exists. Group 5 showed significant improvement in morphological structures of most samples with almost well organized, thin epidermal epithelium with few scattered degenerated keratinocytes and normal dermal collagen fibers with mild few inflammatory cell infiltrates. The results of group 5 showed significant difference from that of the positive control group ($p < 0.05$). The advanced histological improvement and inflammation relief confirmed the effective anti-inflammatory action of resveratrol in G5 formula and its ability to counteract the action of imiquimod. Imiquimod is well known for its immune-mediated inflammatory effect that results in overexpression of inflammatory cytokines,

Table 5
Viscosity of Resveratrol Loaded Gel Formulae at Different Shear Rates. Value = Mean±SD.

Shear rate (1/S)	Viscosity (cps)							
	G1	G2	G3	G4	G5	G6	G7	G8
100	3340 ± 4.25	5200 ± 2.12	4700 ± 2.545	4900 ± 7.78	2589±3.6	4500 ± 4.45	3900 ± 4.48	4100 ± 7.95
200	2145 ± 7.07	3678 ± 9.89	3214 ± 3.1	3516 ± 0.85	1687 ± 2.16	2968 ± 3.9	2358 ± 5.25	2736 ± 3.53
300	1563 ± 2.82	2865 ± 2.26	2458 ± 3.54	2546 ± 0.92	1358 ± 1.58	2065 ± 1.77	1625 ± 1.15	1895 ± 1.75
400	1234 ± 4.24	2348 ± 1.55	1935 ± 3.1	2154 ± 2.26	1164 ± 1.9	1769 ± 3.32	1398 ± 1.07	1597 ± 3.55
500	1048.5 ± 2.12	1865 ± 1.84	1542 ± 3.25	1687 ± 3.75	954 ± 2.15	1398 ± 5.3	1158 ± 7.1	1269 ± 3.01

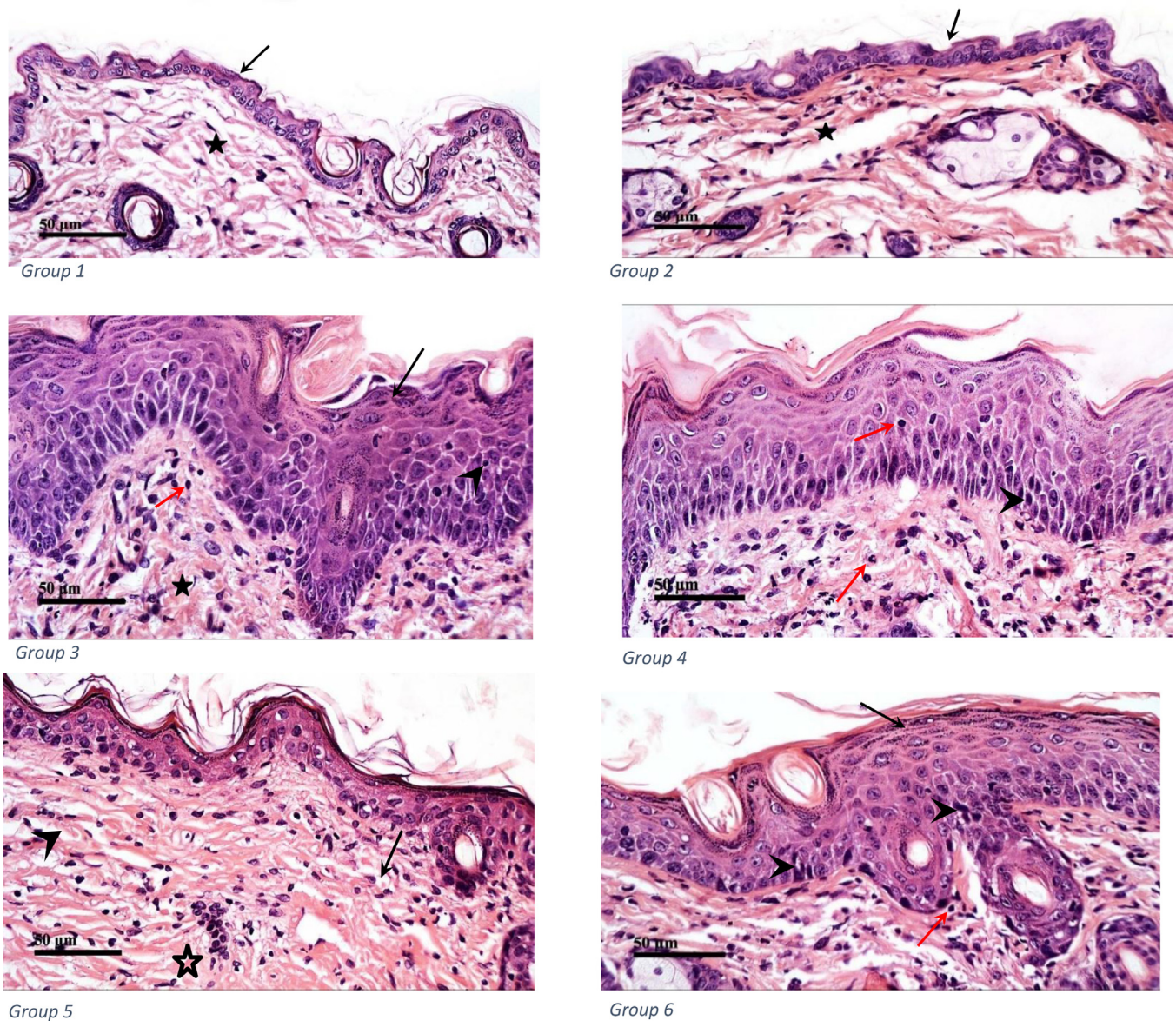

Fig. 4. Histopathological observations of the demonstrating epidermal thickness and morphological changes. Group (1) is the negative control treated with Vaseline only. Group (2) is the negative control treated with the empty gel only. Group (3) is the imiquimod-induced model without treatment. Groups 4, 5, and 6 are the psoriasis-induced models treated with G1, G5, and Gel D, respectively. Black arrows: epidermal layer, stars: dermal layer, arrow heads: lymphocytic infiltrates, red rrows: mononuclear inflammatory cell infiltrates.

which result in inflammation and keratinocyte proliferation.[4] Resveratrol as immune-mediated anti-inflammatory drug activates sirtuin enzyme (SirT1) in the skin and inhibits the cell differentiation and regulates the cell cycle. SirT1 also controls the genetic transcription process, so it counteracts the overexpression of some cytokines that cause keratinocyte proliferation.[6] The complex structure of the skin provides many barriers against drug penetration and localization at the deep skin layers. The SC is the major component of epidermal barriers that is a physiological semipermeable membrane that acts as a biosensor, which allows the essential elements to pass through it but prevents other foreign substances from passage[17]. Incorporation of drug in suitable nanocarriers is the best solution to maintain the active ingredient in its active form, enable it to pass through the SC, and reach more deep layers and hence maximize its effect and improve treatment.[15] Incorporation of resveratrol in spanlastic formulation with a ratio of Span 60, Birj35, and Cremophor EL, which was 50:25:25 as in F11 formula, in treatment group 5 provided an obvious improvement in skin histopathological and morphological characteristics, which proved the ability of this formulation to penetrate and localize efficiently throughout skin layers, including the deep layers. Selecting the proper blend of surfactants maximizes drug solubility and provides more penetration efficiency.[37,55]

Quantitative Real-Time PCR (qRT–PCR) IL17A, IL 22, and IL-23

Recent studies suggested a functional role of IL-23 /IL17A in pathogenesis of psoriasis.[56] IL-22 can synergize with interleukins (IL-23/IL17A) to induce many pathogenic phenotypes from keratinocytes and disease progression.[57] Imiquimod (Aldara® cream 5%) induces synthesis of proinflammatory cytokines and results in gene overexpression in interleukins (IL23/IL17A).[4] Fig. 5 shows that animals of the positive control group (psoriasis model) had significant increase in relative mRNA expression levels compared to the negative control groups 1 and 2. This could be attributed to the inflammatory effect of imiquimod that increases the levels of inflammatory cytokines, including IL-17A, IL-22, and IL-23. Animals treated by the G5 gel formula (group 5) showed comparable

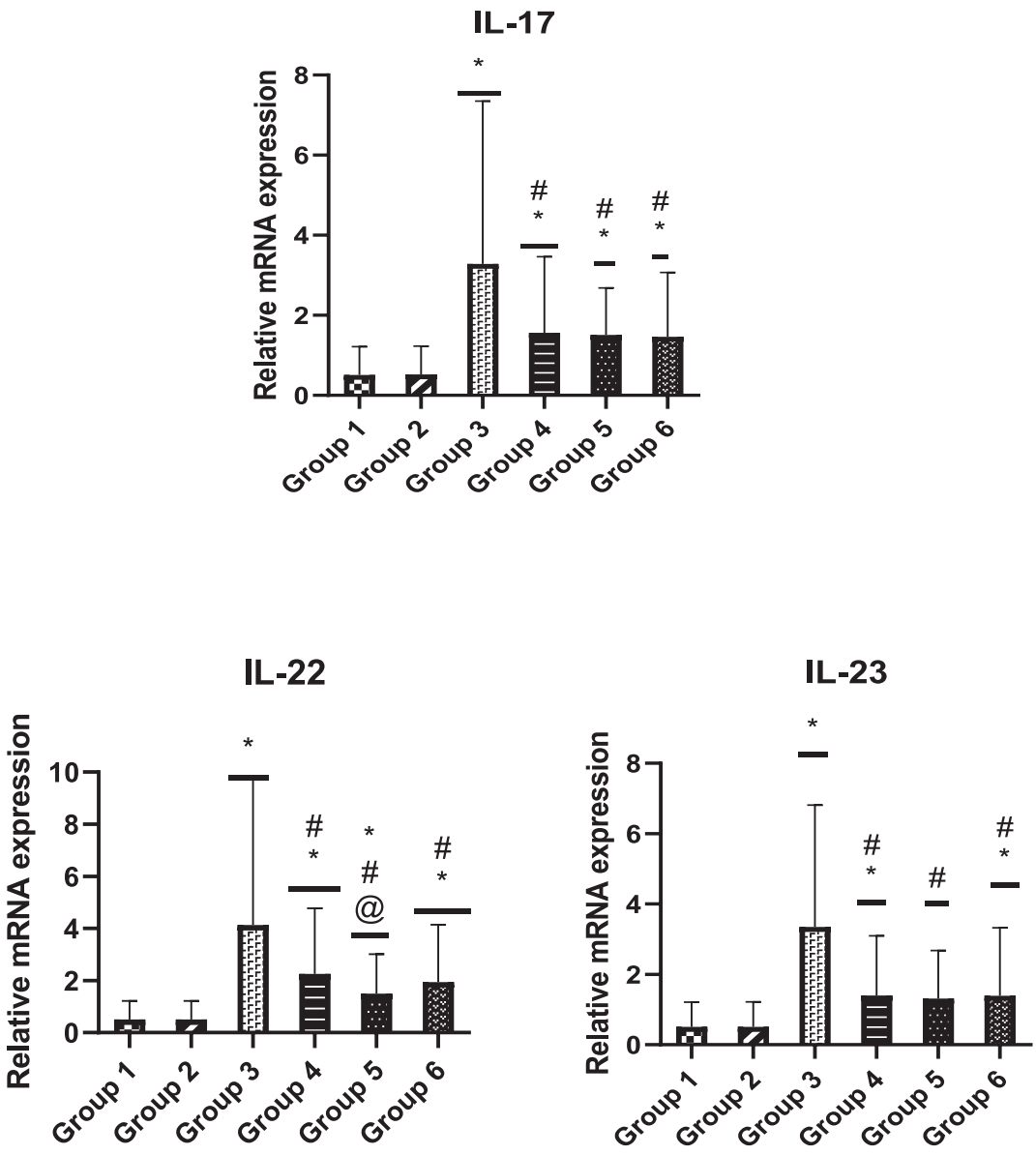

Fig. 5. Relative mRNA expression of inflammatory cytokines following treatment with resveratrol in different formulas: IL-17A, IL-22, and IL-23. Group (1) is the negative control treated with Vaseline only. Group (2) is the negative control treated with the empty gel only. Group (3) is the imiquimod-induced model without treatment. Groups 4, 5, and 6 are the psoriasis-induced models treated with G1, G5, and Gel D, respectively. Data are presented as mean ± SD. * statistically significant difference compared with the negative control groups (Group 1, Group 2) ($p < 0.05$). # statistically significant difference compared with the positive control group (Group 3) ($p < 0.05$). @ statistically significant difference compared with groups 4 and 6 ($p < 0.05$).

relative mRNA expression levels compared to the negative control groups. There was no significant difference between group 5 and negative control groups in terms of mRNA gene expression of IL-23. These results confirmed the efficient anti-inflammatory action of G5 formula and its high potential in psoriasis treatment. Relative mRNA gene expression of different interleukins was significantly decreased in animals treated by G1 gel formula (group 4) and free drug suspension in gel, Gel D (group 6) compared to positive control; however, animal group treated by G5 showed the most significant decrease in gene expression compared to the positive control group ($p < 0.05$). Animals treated by G5 showed significant decrease in mRNA gene expression of only interleukin IL22 compared to animals treated by G1 and animals treated by Gel D. The rest of differences between treatment groups were nonsignificant; however, the animal group treated by G5 had the best response in terms of mRNA gene expression levels of interleukins.

Gene expression results of this study made a clear explanation of the variations in erythema and scaling scores, splenomegaly, and histopathological and morphometric changes among different groups. Relative mRNA expression of different interleukins used to measure the efficiency of different drug formulae in the treatment of psoriasis. Induction of psoriasis by imiquimod-induced the synthesis of proinflammatory cytokines and resulted in overexpression of interleukin (IL23/IL17) axis and IL-22.[4] This was reflected in inflammatory cytokine levels and resulted in inflammatory signs (erythema and scaling).Induction of psoriasis by imiquimod also had a direct impact on keratinocyte proliferation and epidermal cell differentiation[10]; hence, significant histopathological and morphometric changes occurred in the positive control group compared to the negative control groups.

Resveratrol role in activating (SirT1) and downregulation of NF-κB resulted in inhibition of cell differentiation, counteracting the overexpression of some cytokines especially (IL-17–IL-23 axis) and regulating immune response and inflammation.[5] These effects occurred in different treatment groups but with some variations according to the composition of spanlastic formulae used in the treatment. G5 gel formula achieved the best results in terms of improvement of erythema and scaling, recovering normal epidermal thickness and skin morphology. This could be explained by the results of relative mRNA expression of proinflammatory cytokines in G5 formula, which showed the least differences from those of negative control groups. F11 composition of a certain ratio of surfactants (Span 60:Birj35:Cremophor EL equals 50:25:25) provided it with superior properties in terms of drug solubility, release, penetration, and localization in different skin layers.[15] This made F11 formula superior in the treatment of psoriasis when being incorporated in G5 gel formula.

Conclusion

Spanlastic formulations of resveratrol with concentrations of 50% Span 60, 25% Birj35, and 25% Cremophor (F11) showed superiority in drug entrapment, modified release, and penetration through skin barriers most probably due to blending three different surfactants that had an impact on formulation characteristics. F11 and F10 spanlastics were incorporated in Carbopol 934 gel formulations. G1 and G5 showed the best drug release of 40.13% ± 2.017% and 73.76% ± 2.46%, respectively with non-Newtonian shear-thinning (pseudoplastic) flow when tested for rheological properties; however, they maintained a reasonable viscosity by the end at a shear stress of 500 s−1, which reached 1048.5 ± 2.12 cps for G1 and 954 ± 2.15 cps for G5. In vivo study showed that Group 5, which was treated by G5, showed significant improvement of erythema and scaling symptoms and maintained healthy skin with the least changes in mRNA gene expression of inflammatory cytokines. Hence, G5, which contains F11 resveratrol-loaded spanlastics, was the best formulation in terms of imiquimod-induced psoriasis treatment.

Declaration of Competing Interest

The authors declare that they have no conflict of Interest.

Acknowledgement

The authors are grateful for Faculty of Pharmacy, Damanhour University.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Supplementary Materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.xphs.2021.08.023.

References

1. Liu Y, Krueger J, Bowcock A. Psoriasis: genetic associations and immune system changes. *Genes Immun*. 2007;8(1):1–12.
2. Kouris A, Pistiki A, Katoulis A, et al. Proinflammatory cytokine responses in patients with psoriasis. *Eur Cytokine Netw*. 2014;25(4):63–68.
3. Flutter B, Nestle FO. TLRs to cytokines: mechanistic insights from the imiquimod mouse model of psoriasis. *Eur J Immunol*. 2013;43(12):3138–3146.
4. Van der Fits L, Mourits S, Voerman JS, et al. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. *J Immunol*. 2009;182(9):5836–5845.
5. Oliveira ALdB, Monteiro VVS, Navegantes-Lima KC, et al. Resveratrol role in autoimmune disease—a mini-review. *Nutrients*. 2017;9(12):1306.
6. Kao C-L, Chen L-K, Chang Y-L, et al. Resveratrol protects human endothelium from H2O2-induced oxidative stress and senescence via SirT1 activation. *J Atheroscler Thromb*. 2010;17(9):970–979.
7. Panaro MA, Carofiglio V, Acquafredda A, Cavallo P, Cianciulli A. Anti-inflammatory effects of resveratrol occur via inhibition of lipopolysaccharide-induced NF-κB activation in Caco-2 and SW480 human colon cancer cells. *Br J Nutr*. 2012;108(9):1623–1632.
8. Abbas H, Kamel R. Potential role of resveratrol-loaded elastic sorbitan monostearate nanovesicles for the prevention of UV-induced skin damage. *J Liposome Res*. 2020;30(1):45–53.
9. Abbas H, Kamel R, El-Sayed N. Dermal anti-oxidant, anti-inflammatory and anti-aging effects of Compritol ATO-based Resveratrol colloidal carriers prepared using mixed surfactants. *Int J Pharm*. 2018;541(1-2):37–47.
10. Kjær TN, Thorsen K, Jessen N, Stenderup K, Pedersen SB. Resveratrol ameliorates imiquimod-induced psoriasis-like skin inflammation in mice. *PLoS One*. 2015;10:(5) e0126599.
11. Parveen S, Misra R, Sahoo SK. Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging. *Nanomed Nanotechnol Biol Med*. 2012;8(2):147–166.
12. Duangjit S, Opanasopit P, Rojanarata T, Ngawhirunpat T. Effect of edge activator on characteristic and in vitro skin permeation of meloxicam loaded in elastic liposomes. Trans Tech Publications Ltd. *Adv Mater Res*. 2011:537–540.
13. Tayel SA, El-Nabarawi MA, Tadros MI, Abd-Elsalam WH. Duodenum-triggered delivery of pravastatin sodium via enteric surface-coated nanovesicular spanlastic dispersions: development, characterization and pharmacokinetic assessments. *Int J Pharm*. 2015;483(1-2):77–88.
14. Hajjar B, Zier K-I, Khalid N, Azarmi S, Löbenberg R. Evaluation of a microemulsion-based gel formulation for topical drug delivery of diclofenac sodium. *J Pharma Investig*. 2018;48(3):351–362.
15. Küchler S, Radowski MR, Blaschke T, et al. Nanoparticles for skin penetration enhancement–a comparison of a dendritic core-multishell-nanotransporter and solid lipid nanoparticles. *Eur J Pharm Biopharm*. 2009;71(2):243–250.
16. Shrotriya S, Ranpise N, Vidhate B. Skin targeting of resveratrol utilizing solid lipid nanoparticle-engrossed gel for chemically induced irritant contact dermatitis. *Drug Deliv Transl Res*. 2017;7(1):37–52.
17. Levin J, Friedlander SF, Del Rosso JQ. Atopic dermatitis and the stratum corneum: part 1: the role of filaggrin in the stratum corneum barrier and atopic skin. *J Clin Aesth Dermatol*. 2013;6(10):16.
18. Al-Sakini SJ, Maraie NK. In vitro evaluation of the effect of using different gelling agents on the release of erythromycin from a nanocubosomal gel. *Al-Mustansiriyah J Pharma Sci*. 2019;19(1):34–43.
19. Negi P, Aggarwal M, Sharma G, Rathore C, Sharma G, Singh B, Katare O. Niosome-based hydrogel of resveratrol for topical applications: An effective therapy for pain related disorder (s). *Biomed Pharmacother*. 2017;88:480–487.
20. Amal El Sayeh F, El Khatib MM. Formulation and evaluation of new long acting metoprolol tartrate ophthalmic gels. *Saudi Pharma J*. 2014;22(6):555–563.
21. Kakkar S, Kaur IP. Spanlastics—a novel nanovesicular carrier system for ocular delivery. *Int J Pharm*. 2011;413(1-2):202–210.
22. Rajera R, Nagpal K, Singh SK, Mishra DN. Niosomes: a controlled and novel drug delivery system. *Biol Pharm Bull*. 2011;34(7):945–953.
23. ESSA Ebtessam. Effect of formulation and processing variables on the particle size of sorbitan monopalmitate niosomes. *Asian J Pharm*. 2014;4(4):227.
24. Tantra R, Schulze P, Quincey P. Effect of nanoparticle concentration on zeta-potential measurement results and reproducibility. *Particuology*. 2010;8(3):279–285.
25. Bhattacharjee S. DLS and zeta potential–what they are and what they are not? *J Control Release*. 2016;235:337–351.
26. Marques MR, Loebenberg R, Almukainzi M. Simulated biological fluids with possible application in dissolution testing. *Dissol Technol*. 2011;18(3):15–28.
27. Maddineni S, Chandu B, Ravilla S, Nama S, Pradesh A. Dissolution research-a predictive tool for conventional and novel dosage forms. *Asian J Pharm Life Sci*. 2012;2231:4423.
28. Abdelmonem R, El Nabarawi M, Attia A. Development of novel bioadhesive granisetron hydrochloride spanlastic gel and insert for brain targeting and study their effects on rats. *Drug Deliv*. 2018;25(1):70–77.
29. El-Kamel AH, Al-Shora DH, El-Sayed YM. Formulation and pharmacodynamic evaluation of captopril sustained release microparticles. *J Microencapsul*. 2006;23(4):389–404.
30. Kong HH, Andersson B, Clavel T, et al. Performing skin microbiome research: a method to the madness. *J Invest Dermatol*. 2017;137(3):561–568.
31. Culling CFA. *Handbook of histopathological and histochemical techniques: including museum techniques*. Butterworth-Heinemann. Trowbridge, Wiltshire, England; 2013.
32. Pando D, Matos M, Gutiérrez G, Pazos C. Formulation of resveratrol entrapped niosomes for topical use. *Colloids Surf B*. 2015;128:398–404.
33. Fahmy AM, El-Setouhy DA, Ibrahim AB, Habib BA, Tayel SA, Bayoumi NA. Penetration enhancer-containing spanlastics (PECSs) for transdermal delivery of haloperidol: in vitro characterization, ex vivo permeation and in vivo biodistribution studies. *Drug Deliv*. 2018;25(1):12–22.
34. Coors EA, Seybold H, Merk HF, Mahler V. Polysorbate 80 in medical products and nonimmunologic anaphylactoid reactions. *Ann Allergy Asthma Immunol*. 2005;95(6):593–599.

35. Popović-Nikolić MR, Popović GV, Agbaba DD. The effect of nonionic surfactant brij 35 on solubility and acid–base equilibria of verapamil. *J Chem Eng Data*. 2017;62(6):1776–1781.
36. Chen L, Annaji M, Kurapati S, Ravis WR, Babu RJ. Microemulsion and microporation effects on the genistein permeation across dermatomed human skin. *AAPS PharmSciTech*. 2018;19(8):3481–3489.
37. Weerapol Y, Limmatvapirat S, Nunthanid J, Sriamornsak P. Self-nanoemulsifying drug delivery system of nifedipine: impact of hydrophilic–lipophilic balance and molecular structure of mixed surfactants. *AAPS Pharmscitech*. 2014;15(2):456–464.
38. Otto A, Du Plessis J, Wiechers J. Formulation effects of topical emulsions on transdermal and dermal delivery. *Int J Cosmet Sci*. 2009;31(1):1–19.
39. Hao Y, Zhao F, Li N, Yang Y. Studies on a high encapsulation of colchicine by a niosome system. *Int J Pharm*. 2002;244(1-2):73–80.
40. van den Bergh BA, Wertz PW, Junginger HE, Bouwstra JA. Elasticity of vesicles assessed by electron spin resonance, electron microscopy and extrusion measurements. *Int J Pharm*. 2001;217(1-2):13–24.
41. Abdelbary G, El-gendy N. Niosome-encapsulated gentamicin for ophthalmic controlled delivery. *AAPS Pharmscitech*. 2008;9(3):740–747.
42. Klose D, Siepmann F, Elkharraz K, Krenzlin S, Siepmann J. How porosity and size affect the drug release mechanisms from PLGA-based microparticles. *Int J Pharm*. 2006;314(2):198–206.
43. Kamboj S, Saini V, Bala S. Formulation and characterization of drug loaded nonionic surfactant vesicles (niosomes) for oral bioavailability enhancement. *Sci World J*. 2014;2014:8. https://doi.org/10.1155/2014/959741.
44. Kiss D, Zelkó R, Novák C, Éhen Z. Application of DSC and NIRS to study the compatibility of metronidazole with different pharmaceutical excipients. *J Therm Anal Calorim*. 2006;84(2):447–451.
45. Elkordy AA, Bhangale U, Murle N, Zarara MF. Combination of lactose (as a carrier) with Cremophor® EL (as a liquid vehicle) to enhance dissolution of griseofulvin. *Powder Technol*. 2013;246:182–186.
46. Neves AR, Lúcio M, Martins S, Lima JLC, Reis S. Novel resveratrol nanodelivery systems based on lipid nanoparticles to enhance its oral bioavailability. *Int J Nanomed*. 2013;8:177.
47. Shin S-C, Kim J-Y, Oh I-J. Mucoadhesive and physicochemical characterization of carbopol-poloxamer gels containing triamcinolone acetonide. *Drug Dev Ind Pharm*. 2000;26(3):307–312.
48. Hashem FM, Shaker DS, Ghorab MK, Nasr M, Ismail A. Formulation, characterization, and clinical evaluation of microemulsion containing clotrimazole for topical delivery. *AAPS PharmSciTech*. 2011;12(3):879–886.
49. Bachhav Y, Patravale V. Formulation of meloxicam gel for topical application: In vitro and in vivo evaluation. *Acta Pharm*. 2010;60(2):153–163.
50. Nokhodchi A, Javadzadeh Y, Siahi-Shadbad MR, Barzegar-Jalali M. The effect of type and concentration of vehicles on the dissolution rate of a poorly soluble drug (indomethacin) from liquisolid compacts. *J Pharm Pharm Sci*. 2005;8(1):18–25.
51. Dash S, Murthy PN, Nath L, Chowdhury P. Kinetic modeling on drug release from controlled drug delivery systems. *Acta Pol Pharm*. 2010;67(3):217–223.
52. Costa P, Lobo JMS. Modeling and comparison of dissolution profiles. *Eur J Pharm Sci*. 2001;13(2):123–133.
53. Morris ER, Cutler A, Ross-Murphy S, Rees D, Price J. Concentration and shear rate dependence of viscosity in random coil polysaccharide solutions. *Carbohydr Polym*. 1981;1(1):5–21.
54. Švajger U, Jeras M. Anti-inflammatory effects of resveratrol and its potential use in therapy of immune-mediated diseases. *Int Rev Immunol*. 2012;31(3):202–222.
55. Alomrani AH, Al-Agamy MH, Badran MM. vitro skin penetration and antimycotic activity of itraconazole loaded niosomes: various non-ionic surfactants. *J Drug Delivery Sci Technol*. 2015;28:37–45.
56. Di Cesare A, Di Meglio P, Nestle FO. The IL-23/Th17 axis in the immunopathogenesis of psoriasis. *J Invest Dermatol*. 2009;129(6):1339–1350.
57. Ouyang W. Distinct roles of IL-22 in human psoriasis and inflammatory bowel disease. *Cytokine Growth Factor Rev*. 2010;21(6):435–441.