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Promising role of topical caffeine mesoporous gel in collagen resynthesis and UV protection through proline assessment

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Abstract

Background: Caffeine, an alkaloid agent, has been globally used regularly in drinks, for the reduction in skin cancers and wrinkle formation. As a result of the previous, attempts have been carried out to use caffeine in cosmetology due to its antioxidant and UV ray protection effects. Our aim was to evaluate the effect of caffeine on collagen resynthesis via its effect on proline and prolidase biosynthesis on mice, orally and topically as mesoporous silica at three levels, and the influence on UV protection. In skin biopsies of orally and topically treated mice, the following was assessed using ELISA and Western blot techniques, the activity of prolidase, together with the concentrations of proline, beta integrin, insulin growth factor, protein kinases beta, and mitogen-activated protein kinase. Moreover, we loaded the caffeine on mesoporous silica and assessed the aforementioned parameters together with checkpoint kinase 1 and Rad3-related protein.

Results: Caffeine promoted collagen resynthesis in a dose-dependent manner. The mechanism of this process was found at the level of prolidase activity as caffeine significantly increased the enzyme activity. Caffeine also had a protective effect against UV exhibited by the over-expression of beta integrin, insulin growth factor together with the under-expression of protein kinases beta, mitogen-activated protein kinase, checkpoint kinase 1, and Rad3-related protein.

Conclusions: Our study revealed the superiority of SYL-C12 (mesoporous silica-loaded caffeine gel), compromising the high level of the three independent factors, in terms of the measured responses in mesoporous silica with caffeine. Moreover, caffeine promoted collagen resynthesis with significant protective effect against UV apoptotic damage.

Keywords: Prolidase, Caffeine, B integrin, Insulin growth factor, Protein kinases beta, Checkpoint kinase 1, Rad3-related protein

Background

The skin, the outer body cover, comprised of outer epidermis and inner dermis, shields the body in averse to several extrinsic dangerous stimulants as pathogens, harmful agents, and mechanical stress [1]. Nevertheless, several phenomena can promote the process of skin aging by reducing healing of wounds, increased wrinkles formation, decreased skin elasticity, and compromising oxygen and nutrient supply in addition to pale discoloration of the skin [2].

Numerous elements can promote skin senescence; they can be either internal or external [3]. Internal processes involve irreversible natural aging by time, under the effect of hereditary factors, while external ones can enhance intrinsic factor, through the continuous exposure to

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harmful ultraviolet (UV) sunrays, stress, and unhealthy lifestyle [4].

During stress, prolidase (PEPD), an enzyme that promotes collagen resynthesis through proline amino acid, is decreased [5]. Decreased enzymatic activity associated with PEPD deficiency can be seen in massive imidodipeptiduria, aging, hard-to-heal wounds, mental retardation, and impaired immune system [6]. At cellular bases, PEPD regulates epidermal growth factor receptor and epidermal growth factor receptor 2 (HER2)-dependent signaling pathways [7], p53 activity [8], and expression of the interferon α/β receptor [8], all of which take part in the resynthesis of collagen through phospho-extracellular signal-regulated kinase.

UV rays are one of the non-ionizing radiations, with wavelengths shorter than that of visible light [5]. Sunlight and UV exposure in moderate amounts are essential for vitamin D synthesis [9]. However, increased exposure to UV will result in increased synthesis of reactive oxygen species (ROS) which will in turn promote photoaging [4]. This is accompanied by a set of processes that lead to the breakdown of extracellular matrix (ECM) molecules as collagen, which is needed for skin preservation [10].

Another damaging effect of UV radiations is DNA damage; elements associated with this UV-induced DNA damage signaling response are the protein kinases beta (AKT), checkpoint kinase 1 (CHK1), and Rad3-related protein (ATR), which are interconnected with the phospho-extracellular signal-regulated kinase, eventually leading to the prevention of collagen resynthesis [11, 12].

Caffeine, an alkaloid agent, has been globally used regularly in drinks [13]. In addition, it had been suggested that daily consumption of caffeine aids in reducing both skin cancer and wrinkle formation [14]. Despite caffeine gaining popularity in a number of food and beverages, trials have been carried out to use caffeine in cosmetology because of its antioxidant, thermogenic, lipolytic, and UV ray protection effect [15]. In cosmetics, caffeine-based creams and lotions have already been shown to delay the photoaging process of skin and hinder skin cancer caused by harmful UV radiations, hence preventing the development of tumors after skin exposure to sunlight by functioning as a sunscreen [16]. However, to the best of our knowledge, the protective role of caffeine against skin aging and UV rays has not been fully studied.

Adding to the previous, drug loading onto nonporous as well as porous silica moieties has been long investigated [17] due to its proven biocompatibility and safety [18]. Not only does the mesoporous silica augment the dissolution profiles of poorly water-soluble drugs [19] but also its huge surface area in addition to the presence of the surface silanol groups expedites its surface modification [19] and stabilizes drugs in their amorphous forms [20] due to size confinement effects with a decrease in the Gibbs free energy of the drug/silica system. Syloid® 244 FP is a newly introduced non-ordered mesoporous silica which offers a promising carrier for many drugs [21]. Thus, in order to assess the collagen-enhancing ability together with the protective effect of caffeine against UV, we measured the activity of PEPD, together with the concentrations of proline, beta integrin (β integrin), insulin-like growth factor 1 (IGF-1), AKT, and mitogen-activated protein kinase (MAPK), orally and topically. Moreover, we loaded the caffeine on mesoporous silica and assessed the aforementioned parameters together with CHK1 and AKT.

Methods

This study was approved by the Research and Ethics committee for Experimental and Clinical studies at Faculty of Pharmaceutical sciences and Pharmaceutical Industries, Future University in Egypt, Cairo, Egypt, with approval number FUE (1954). The study was conducted in accordance with the U.K. Animals (Scientific Procedures) Act 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments. Our study was carried out on mice BALB/c albino. A total of 12 mice were used and categorized over four groups as follows: two groups, each comprised of 3 mice, one control and the other was treated with caffeine in drinking water 300 mg/kg, once per day, over a period of 28 days after which the 6 mice were exposed to UV light over a period of 6 days, exposure was carried every other day, then the mice were slaughtered, and our parameters were assessed within the obtained skin biopsies using ELISA and Western blot techniques.

The remaining 2 groups were composed of 3 mice each, control and treated with caffeine loaded on mesoporous silica cream. Application was done at a fixed time in the morning throughout 28 days for 4 weeks after which the 6 mice were exposed to UV light over a period of 6 days, exposure was carried every other day, then the mice were slaughtered, and our parameters were assessed within the obtained skin biopsies using ELISA and Western blot techniques.

On the 6 mice that were treated orally the following was carried out

Assessment of proline concentration ELISA was done using *P4HA1,* Polyclonal Antibody catalog number MBS7612021.

PEPD activity was assessed by ELISA using PLD ELISA Kit: Rat PEPD (PLD) ELISA Kit, catalog number MBS3808214, and Western blot by Mouse anti-Human PEPD Monoclonal Antibody, catalog number MBS6008175. The concentrations of β integrin were

determined using both ELISA and Western blot using anti-ITGAV antibody: beta 6 Monoclonal Antibody, catalog number MBS4382448.

Regarding IGF-1, it was assessed by ELISA using anti-IGF1R antibody: anti-IGF1R antibody: Rabbit anti-Mouse IGF1R (Insulin-Like Growth Factor Receptor 1) Polyclonal Antibody, Catalog number MBS556390, Western blot using: anti-IGFBP-3 antibody: Rabbit anti-Human, Mouse IGFBP-3 Polyclonal Antibody. As for AKT, it was measured by ELISA using anti-RPS6KB2 antibody: anti-Pan-AKT antibody: Pan-AKT Polyclonal Antibody, catalog number: MBS7611954, and Western blot using RPS6KB2 Polyclonal Antibody, catalog number MBS7611791.

Measurement of MAPK was done using ELISA by rat mitogen-activated protein kinase 8 ELISA Kit, catalog number MBS7269597, and Western blot using anti-RPS6KA5 antibody: Mouse RPS6KA5/MSK1 Monoclonal Antibody, catalog number MBS4382372.

On the other 6 mice that were treated topically, the following was carried out

Moreover, we loaded the caffeine on mesoporous silica and assessed the aforementioned parameters together with CHK1 that was assessed by ELISA using anti-Chk1 antibody: Mouse Chk1 Monoclonal Antibody, catalog number MBS767885, and Western blot using anti-CHK1 antibody: Mouse anti-Human CHK1 Monoclonal Antibody catalog number MBS418378, and ATR was measured by ELISA using ATR ELISA Kit: Mouse Serine/threonine-protein kinase ATR ELISA Kit, catalog number MBS2887956, and by Western blot using anti-ATR antibody: Mouse anti-Human ATR Monoclonal Antibody, catalog number MBS6146055.

Mesoporous cream preparation

Sodium chloride, sodium dihydrogen phosphate monohydrate, and disodium hydrogen phosphate heptahydrate were purchased from El Nasr Pharmaceutical Chemicals (Egypt). Raspberry seed oil, carrot oil, wheat germ oil, peppermint oil, sandalwood oil, and tea tree oil were purchased from Harraz Co. (Egypt). Propylene glycol, methyl paraben, propyl paraben, alpha-tocopherol, butylated hydroxytoluene, and Lanette-N (cetearyl alcohol (and) sodium cetearyl sulfate) were purchased from CISME (Italy).

Preparation and evaluation of the prepared SYL-C-loaded formulations

Loading of caffeine on Syloid® 244 FP (SYL-C)

Exactly weighed amounts of caffeine were dissolved in 3.5% w/v nicotinamide (hydrotropic agent) solution [22] in distilled water followed by the dispersion of the calculated amount of Syloid® 244 FP (2:1 drug/Syloid). The dispersion was frozen at − 80 °C for 24 h followed by lyophilization in the freeze-dryer (Christ ALPHA 2–4 LD plus, Germany) running at a temperature of − 80 °C and vacuum of 7×10^{-2} mbar for 48 h. The obtained powders from the lyophilized samples were stored in desiccators until use.

Formulation of the SYL-C-loaded Creams

Full-factorial $3^1.2^2$ experimental design was utilized for the preparation and optimization of SYL-C-loaded creams using micro-fluidization [23]. Water phase comprised propylene glycol (5% w/w) and glycerine (5% w/w) for their humectant effect. Methyl paraben (0.15% w/w) and propyl paraben (0.3% w/w) were added to protect against effects of microbial contamination. The water phase was kept at 75 °C. Lanette-N (10%) was melted followed by the addition of BHT (0.1% w/w), wheat germ oil (5% w/w), and/or carrot and raspberry oils with their different levels and heated to 75 °C to form the oil phase. The oil phase was kept at 15% w/w even at the lower levels of carrot and raspberry oils using wheat germ oil. The oil phase was mixed with the water-phase solution, which was then homogenized (Heidolph, Germany) for 3 min at 10000 rpm to form a coarse emulsion. The calculated amount of SYL-C lyophilized powder was then incorporated with homogenization for an extra 2 minutes. The essential oil blend (2.5% w/w consisting of tea tree oil (1.25% w/w), peppermint oil (0.75 w/w), and sandalwood oil (0.5% w/w)) as well as alpha-tocopherol (0.5% w/w) was added to the cooling phase. All prepared SYL-C-loaded creams were kept at room temperature for further investigations.

Ex vivo Permeation study

In vitro passive permeation studies were conducted using Hanson–Franz-type diffusion cells (model 60–301–106, CA, Los Angeles, USA) with an effective permeation area of 1.76 cm^2area using dorsal mice skin cut into squares as the diffusion medium [24]

The skin was prepared by immersing PBS (pH 7.4) for an hour before starting the experiment. The donor compartment was filled with 0.4 g of the formulations. The receptor compartment was filled with 7.5 mL phosphate buffer solution (pH 7.4) and kept at 37 °C ± 2 °C under constant stirring (100 rpm). The experiment was conducted for 24 h. Aliquots of 2 ml sample were withdrawn from the receptor medium at the predetermined times: 0.25, 0.5, 0.75, 1, 2, 4, 6, 10, 12, 18, and 24 h. Withdrawn samples were compensated by an equal volume of PBS (pH 7.4). Samples were collected and stored in − 80 °C to be analyzed using a validated, selective, and sensitive isocratic UPLC method which

was developed and validated prior to the beginning of the study. Filtration of samples was done using a 0.45-µm Millipore® filter followed by injection into the UPLC column (Agilent UHPLC, 1290 Infinity LC, binary pump, autosampler, multicolumn thermostat, USA). Acetonitrile and water (80:20 v/v) represented the isocratic mobile phase which was filtered, degassed, and set to a flow rate of 0.1 mL/min. The column effluent was continuously checked through UV detection at 275 nm. The column used was C18 kept at a set temperature of 30 °C ± 0.2. The injection volume was set to 5 µL.

The cumulative amount of drug permeated (Qs) through the membrane in the receptor compartment at the nth sampling (Qs) was estimated by the following equation:

$$\mathrm{Qs} = \frac{V}{S}.Cs + \sum_{n-i}^{n-1} \frac{Vi}{S}.Ci$$

where *Cn* represents the concentration of the drug in the receptor solution at the nth sampling time, *Ci* is the drug concentration, and *V* and *Vi* represent the volumes of the receptor solution and the withdrawn sample, respectively. *S* is the effective diffusion area.

The cumulative amount of the permeated caffeine was plotted against time. The slope and intercept of the linear part of the plot were contributed by regression. The caffeine permeation rate at steady state (J_{SS}, µg/cm^2/h) was computed from the calculated slope of linear portion. The following equation was used to obtain the permeability coefficient Kp (cm/h):

$$\mathrm{Kp} = J_S/C_0$$

where Kp represents the permeability coefficient, J_S is the flux at steady state, and C_0 represents the initial concentration of the drug in the donor compartment [25].

Determination and characterization of the optimized formulation for further investigations

The desirability function of the Design Expert® software was utilized to select the formulations for further investigations. The targeted criteria were to maximize Qs, J_{ss}, and Kp.

pH determination of the optimized formulation

The pH of optimized formulation was tested at room temperature using a Jenway pH meter (model 3510, UK) that was calibrated before each use with standard pH 4, 7, and 9.2 buffer solutions [26].

In vitro determination of sun protection factor of the optimized formulation

The Transpore® tape methodology was adopted to determine the UV blocking of the optimized formulation. The ultraviolet radiation absorbance through a Transpore® tape sample was determined. Exactly 2 mg/cm^2 formulation was evenly applied onto a Transpore® tape of 4.5 cm^2 area and positioned on a quartz cuvette. The sample was left for 15 min to dry followed by scanning at a range from 400 to 290 nm utilizing a double-beam spectrophotometer (Ultraviolet Spectrophotometer, Shimadzu (401 PC) Double Beam Spectrophotometer, Tokyo, Japan) [27]. The method described by Diffey and Robson was adopted for the SPF determination [28]. The obtained in vitro SPF is an indicator of both the UV-A and UV-B protective property of a sunscreen product which is computed from the mean value of the monochromatic protection factor, the solar irradiance, and the erythemal constants according to the following equation [29]:

$$\mathrm{SPF} = \sum_{290}^{400} E_\lambda B_\lambda / \sum_{290}^{400} (E_\lambda B_\lambda \sqrt{MPF_\lambda})$$

where E_λ represents the spectral irradiance of terrestrial sunlight under defined conditions and denotes the midday midsummer sunlight; MPF_λ is the mean monochromatic protection factor; and B_λ is the erythemal effectiveness. The erythema action spectrum utilized in the calculation was adopted by the International Commission on Illumination (CIE) as a "reference action spectrum" [28].

Statistical analysis of data

Data among the study groups of mice were assessed as average ± standard deviation (SD), with the significant difference among the groups assessed using *T* test, at $P < 0.05$.

Regarding the cream preparations models and stats, the generated models were computed utilizing Design Expert® v. 11.0 (Stat Ease, Minneapolis, MN). Other results were investigated exploiting *t* test or ANOVA to determine the significance of the obtained models at $P < 0.05$.

Results

The study mice groups

Regarding the two groups of mice that were treated orally with caffeine, as shown in Fig. 1, Table 1, there was a significant increase in proline concentration in the treated group when compared to the control one. In addition, all of the following were significantly increased in the orally

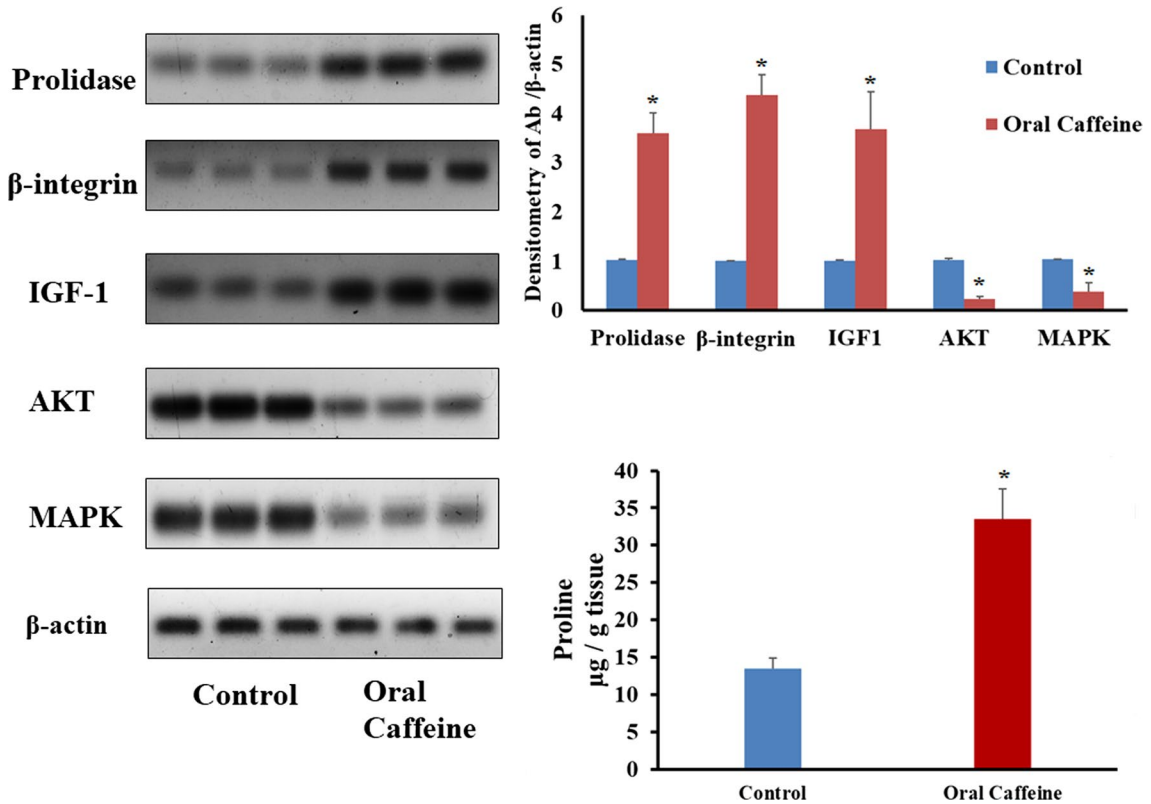

Fig. 1 Parameters assessed in mice treated with oral caffeine. B integrin, beta integrin; IGF-1, insulin-like growth factor 1; AKT, protein kinases beta; MAPK, mitogen-activated protein kinase; CHK1, checkpoint kinase 1; ATR, Rad3-related protein

Table 1 Parameters assessed in caffeine-treated mice orally

	Control	Oral caffeine
Proline µg/g tissue	13.5 ± 1.412	33.533 ± 3.972*
Prolidase	1.0233 ± 0.025	3.603 ± 0.405*
B-integrin	1.003 ± 0.006	4.366 ± 0.416*
IGF-1	1.01 ± 0.01	3.68 ± 0.766*
AKT	1.03 ± 0.026	0.236 ± 0.041*
MAPK	1.033 ± 0.015	0.372 ± 0.181*

B integrin, beta integrin; IGF-1, insulin-like growth factor 1; AKT, protein kinases beta; MAPK, mitogen-activated protein kinase

* significant at $p < 0.01$

treated group when compared to the control, PEPD activity, β integrin, IGF-1, while MAPK and AKT were significantly decreased, all at $p < 0.01$.

Regarding the two groups of mice that were treated topically with caffeine, as shown in Fig. 2, Table 2, there was a significant increase in all of the following in the topically treated group when compared to the control, PEPD activity, β integrin, and IGF-1, while MAPK, AKT, CHK1, and ATR were significantly decreased, all at $p < 0.01$.

Preparation, statistical analysis of the experimental design, and evaluation of the prepared SYL-C-loaded formulations

Formulation of the SYL-C-loaded creams

Micro-fluidization was utilized for the preparation of SYL-C-loaded creams at all levels of the investigated factors. All formulations achieved emulsification and exhibited acceptable texture as well as spreadability upon application on the skin (Fig. 3).

Ex vivo Permeation study

The cumulative amount of drug permeated after 24 h (Q_{24}), the permeation rate of caffeine at steady state (Jss), and the permeability coefficient (Kp) are presented in Table 3, while the generated models and the ANOVA results of the prepared formulations are displayed in Table 4 and Fig. 4. The permeated amount versus time relationship is displayed in Fig. 3.

ANOVA results revealed the significance ($p < 0.05$) of the suggested 2FI models. The obtained values for r-squared, adjusted *r*-squared, and predicted r-squared were more than 0.7 for all models signifying their good accordance as well as the high fitting of the model equations to the results [30].

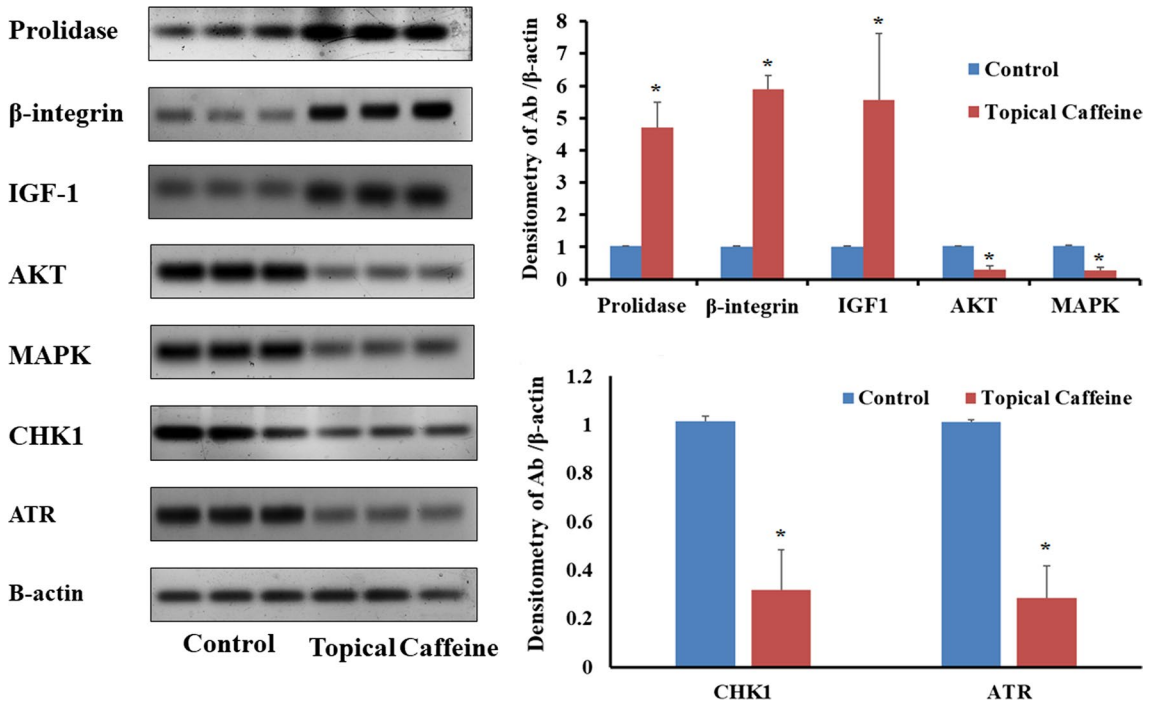

Fig. 2 Parameters assessed in mice treated with topical caffeine. B integrin, beta integrin; IGF-1, insulin-like growth factor 1; AKT, protein kinases beta; MAPK, mitogen-activated protein kinase; CHK1, checkpoint kinase 1; ATR, Rad3-related protein

Table 2 Parameters assessed in caffeine-treated mice topically

	Control	Topical caffeine
Prolidase	1.03 ± 0.015	4.7 ± 0.781*
B-integrin	1.02 ± 0.017	5.896 ± 0.433*
IGF-1	1.02 ± 0.02	5.566 ± 2.05*
AKT	1.026 ± 0.005	0.29 ± 0.127*
MAPK	1.043 ± 0.015	0.28 ± 0.087*
CHK1	1.013 ± 0.023	0.32 ± 0.165*
ATR	1.01 ± 0.01	0.286 ± 0.132*

B integrin, beta integrin; IGF-1, insulin-like growth factor 1; AKT, protein kinases beta; MAPK, mitogen-activated protein kinase; CHK1, checkpoint kinase 1; ATR, Rad3-related protein

*significant at $p < 0.01$

Furthermore, the adequate precision for the three models exhibited a value more than 4 which indicates the high signal-to-noise ratio and the adequacy of the model to navigate the experimental space of the three models [31].

Validation of the models was studied using the Box-Cox transformation test which determines the appropriate power (lambda) that is required to augment the normality of both the positively and negatively skewed factors to work at the best lambda set within the confidence intervals of the test [32].

Results revealed the significance of the three independent factors, as well as the factor interaction AC as validated by the coded equation displayed in Table 4. The increase in caffeine concentration accompanied with the increase in carrot oil concentration led to a significant ($p = 0.0331$) increase in Q24.

Furthermore, the results also revealed the significance of all factors as well as the factor combinations AB and AC on the Kp model. The presence of both the caffeine concentration and the carrot and/or raspberry oil concentrations in their higher levels led to a significant ($p < 0.05$) increase in Kp values. Finally, the ANOVA results proved the significance of the significance of the caffeine–raspberry oil (AB) interaction ($p < 0.05$) on J_{ss}.

Determination and characterization of the optimized formulation for further investigations

The desirability function of the Design Expert program suggested the superiority of SYL-C12 formulation compromising the high levels of the three independent factors based on the maximization criteria of the responses adopted. Consequently, SYL-C 12 was chosen for further investigations.

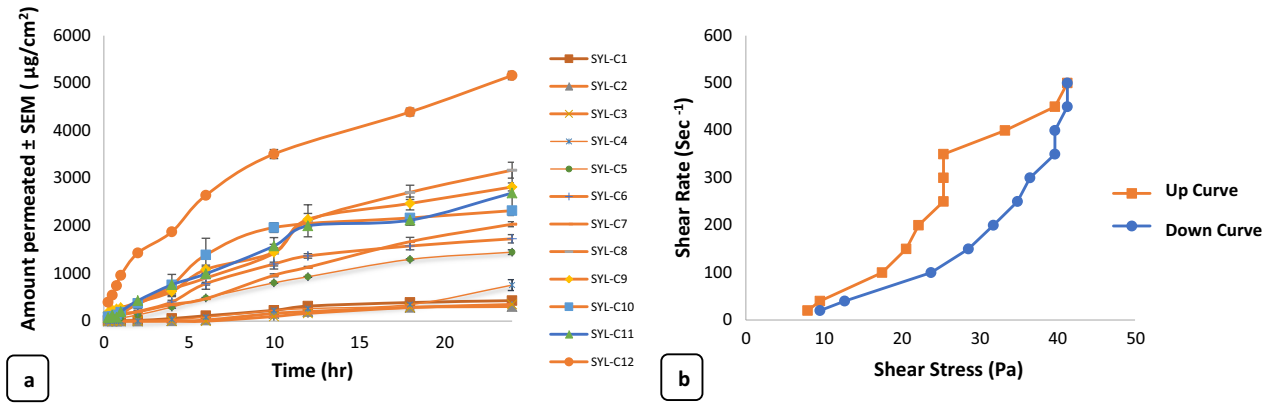

Fig. 3 **a** Permeation profiles of the prepared SYL-C-loaded formulations and **b** rheogram of SYL-C 12. *SYL-C: Caffeine on Syloid® 244 FP*

Table 3 Composition and responses of the full-factorial design

Formula	Composition*			Responses		
	Caffeine concentration (CAFF. %)	Carrot oil concentration (CARR. %)	Raspberry oil concentration (RASP. %)	Q_{24} (µg/cm^2) (A)	Kp (cm/hr) (B)	Jss (µg/cm^2/hr.) (C)
SYL-C1	1	0	0	369.29 ± 61.94	7.50 ± 0.98	30 ± 2.93
SYL-C2	1	5	0	307.10 ± 50.78	5.38 ± 0.85	21.5 ± 1.34
SYL-C3	1	0	5	350.95 ± 45.91	5.00 ± 0.56	20.00 ± 3.01
SYL-C4	1	5	5	757.76 ± 55.21	5.85 ± 0.69	23.40 ± 0.99
SYL-C5	3	0	0	1216.52 ± 120.67	6.19 ± 1.04	74.25 ± 10.30
SYL-C6	3	5	0	1731.13 ± 90.39	9.67 ± 1.99	116 ± 12.94
SYL-C7	3	0	5	2038.15 ± 120.34	7.28 ± 1.09	87.4 ± 7.63
SYL-C8	3	5	5	3174.08 ± 201.33	11.50 ± 0.95	138 ± 10.01
SYL-C9	5	0	0	2820.52 ± 190.10	7.95 ± 1.00	159 ± 15.55
SYL-C10	5	5	0	2322.66 ± 301.14	10.27 ± 2.03	205.4 ± 8.99
SYL-C11	5	0	5	2693.32 ± 120.33	7.80 ± 0.87	156 ± 25.08
SYL-C12	5	5	5	5163.844 ± 208.11	15.78 ± 2.34	315.6 ± 16.67

* All formulations contain 10% Lanette N, 5% glycerin, 5% propylene glycol, 5% wheat germ oil, 2.5% total essential oil blend (1.25% tea tree oil, 0.75 peppermint oil, and 0.5% sandalwood oil), 0.5% alpha-tocopherol, 0.15% methyl paraben, 0.3% propyl paraben, and 0.1% butylated hydroxytoluene

Table 4 Generated models and the ANOVA results of the prepared formulations

ANOVA results	Response		
	Q_{24}	Kp	Jss
Model	2FI	2FI	2FI
Coded equation	Q24 = +1791.22 + 1205.14 * A + 451.55 * B + 571.80 * C + 400.27 * A * B + 481.98 * A * C + 217.33 * B * C	Kp = +8.10 + 1.90 * A + 1.64 * B + 0.76 * C + 1.81 * A * B + 1.29 * A * C + 0.54 * B * C	Sqrt(Jss) = +9.63 + 4.53 * A + 1.05 * B + 0.48 * C + 1.12 * A * B + 0.70 * A * C + 0.29 * B * C
P value	0.0031	0.0052	0.0006
R^2	0.9767	0.9700	0.9903
Adjusted R^2	0.9418	0.9250	0.9757
Predicted R^2	0.7671	0.7778	0.9223
Adequate precision	16.803	15.229	23.943

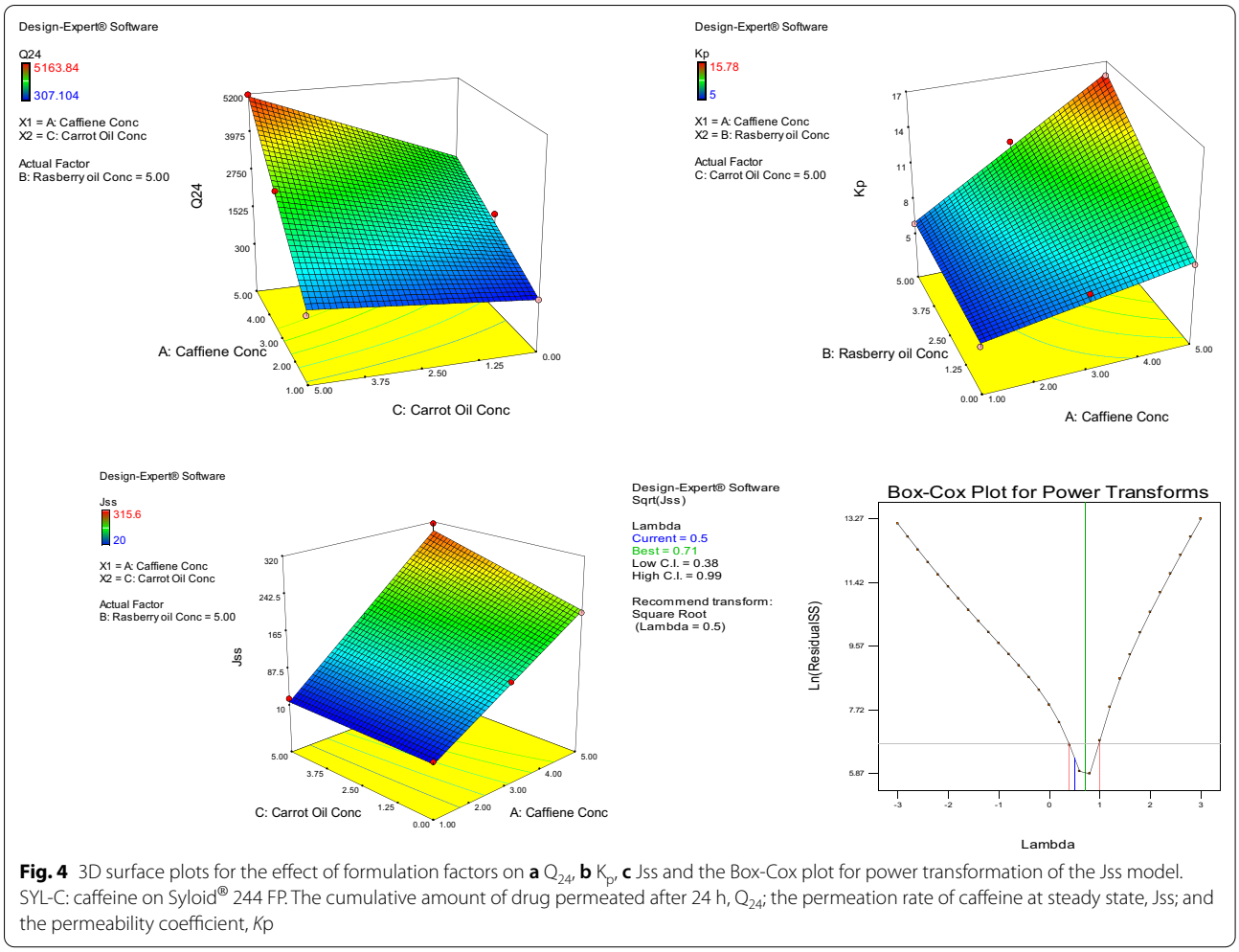

Fig. 4 3D surface plots for the effect of formulation factors on **a** Q_{24}, **b** K_p, **c** Jss and the Box-Cox plot for power transformation of the Jss model. SYL-C: caffeine on Syloid® 244 FP. The cumulative amount of drug permeated after 24 h, Q_{24}; the permeation rate of caffeine at steady state, Jss; and the permeability coefficient, *Kp*

pH determination of the optimized formulation

The pH significance of cutaneous epithelium represents a major factor that determines its physical, chemical, and microbiological barrier function [33]. SYL-C12 showed a pH of 6.01 ± 0.2 which is in accordance with the normal physiological skin pH [34]; consequently, no further pH adjustment was performed.

In vitro determination of sun protection factor of the optimized formulation

In order to investigate whether SYL-C12 exhibits a UV-blocking effect or not, the prepared formulation as well as its caffeine-free analogue was examined. SYL-C12 showed an SPF value of 3.19 ± 0.27, while its caffeine-free analogue measured an SPF value of 2.00 ± 0.21 which was significantly different ($p < 0.05$) from SYL-C12. This confirms the UV-protecting effect of caffeine that positions it as a valuable candidate for further investigation as a UV protective agent.

Rheological studies of the optimized formulation

The rheogram of SYL-C12 is presented in Fig. 3b. By applying Farrow's equation, N computed a value of 1.84 ± 0.19 which indicates a pseudoplastic flow. This shear thinning behavior with the hysteresis loop ensures thixotropic behavior that is desirable for topical formulations [35].

Discussion

3,7-trimethylxanthine, caffeine, is a biologically active compound that exists naturally in many drinks and other food products. Caffeine produces different effects that is dose dependent, including relaxation of muscles, release of ions, anti-oxidative stress, and metabolism of lipids [10]. The main target of caffeine is the G-protein-coupled adenosine receptors located in both the central and peripheral nervous systems, in addition to other organs as the heart and lung [36]. Caffeine shows the highest affinity (~ 10 μM) toward the A2AR subtype of adenosine receptors [37]. At high dose (mM range),

caffeine stops the mutated ataxia–telangiectasia and ATR kinase and subsequently the DNA damage response, thus suggesting a protective role of caffeine against oxidative stress and senescence [38].

In our study, both orally and topically caffeine-treated mice showed significant elevation in both proline and PEPD activity, when compared to their control groups, thus promoting collagen biosynthesis. This could be explained, as PEPD activity was found to resynthesize proline required for the resynthesis of collagen and therefore the enzyme may play a step-limiting role in the regulation of collagen biosynthesis [17]. Moreover, the role of PEPD in regulation of collagen biosynthesis was well documented in fibroblast treated with anti-inflammatory drugs [7]. The enzyme aids in the conservation of imino acids from various protein sources, mainly collagen; moreover, PEPD plays an essential role in the resynthesis of proline for collagen synthesis and cell growth and aiding as an interface between protein nutrition and matrix breakdown [39].

There are controversial resources correlating caffeine-treated mice with proline and PEPD [14]. Most of the work in the literature showed negative or slow enhancement of collagen biosynthesis [14], where caffeine in coffee has been shown to decrease the synthesis of collagen in the dermis layer of the skin, affecting also the biosynthesis of DNA [40]. Moreover, despite the fact that caffeic acid may help decrease inflammation having both antioxidant and antimicrobial effects and promote collagen synthesis in the skin, this effect will not surpass the damaging effect of caffeine [41]. Also, it has been shown that drinking a maximum of two coffee cups is healthy, but exceeding this limit can be harmful causing aging and loss of firmness [41].

The protective role of caffeine skin care topical treatment' includes decreasing fat cells by dehydrating such cells, decreasing dark circles round the eyes by vasoconstricting and draining excess lymphatic fluid or blood, and decreasing cellulite appearance [41]. All of the previously mentioned studies are concomitant with our finding where caffeine showed its protective role through promoting the synthesis of proline and consequently increasing collagen resynthesis, in both topically and orally treated groups. However, it is important to note that despite caffeine being an effective treatment topically, it is better to ingest drinks that contain caffeine in moderate quantities because of the reasons mentioned above, as caffeine can be damaging to the skin.

We further tried to explain this caffeine enhancing/protective effect, through studying the unique function of PEPD with mostly parameters involved in the regulation of PEPD activity, where b1-integrin receptor signaling was found to increase PEPD activity [11] in addition to collagen biosynthesis [42]. The exact mechanism of b1-integrin receptor signaling-dependent functions involved several processes with concomitant inhibition of ATR proteins and MAPK, which in turn will increase collagen resynthesis [8]. From the previous, several lines of evidence suggest that PEPD activity and collagen resynthesis are regulated together through b1-integrin signaling pathway, where IGF-1 synthesis was enhanced and both AKT and MAPK production was decreased in caffeine-treated mice [8], thus concomitant with our findings, where PEPD activity increased together with over-production of proline in caffeine-treated mice, whether orally or topically. However, this is the first study correlating these parameters in orally and topically treated mice; thus, further molecular insight is needed to aid our findings of caffeine as a procollagen stimulus via the b1-integrin pathway.

Moreover, the results regarding the effect of caffeine on beta cyclin are controversial [43], whereas in one study caffeine was found to enhance beta cyclin; on the other hand, when caffeine was administered in drinking water prior to UVB exposure, it inhibited UVB-induced phosphorylation of Chk1 and interfered with UVB-enhanced decrease in mitotic cells with cyclin B1 as well as the decrease in total mitotic cells; all of the previous findings agree with our findings regarding the protective role of oral caffeine against UVB [44].

Adding to the previous caffeine protective effect, and due to the finding that caffeine has a UV absorption capacity between 260 and 300 nm (with a peak at $\sim$ 273 nm) [45], we studied the effect of topical application of caffeine loaded on mesoporous silica prior to and after UVB irradiation. It has been found that caffeine-treated primary human keratinocytes inhibited UVB-induced increase in ATR-mediated formation of p-Chk1 (Ser 345), and tumorigenesis was decreased [43]. Moreover, it was found that the enhancement of ATR that resulted from DNA breakdown stopped chromatin condensation through Chk1 protein kinase modification, and caffeine stopped this process, that in return caused chromatin condensation [46]. In addition, it has been shown that caffeine interrupts the ATR/Chk1 pathway [47], where ATR protein has binds to the DNA of cells damaged by UVB rather than undamaged DNA [48]. Thus, from the previous findings, caffeine has a protective effect against the damaging effect of UV-B, aiding our results.

Further assessment regarding the mesoporous caffeine cream was done as follows:

Preparation, statistical analysis of the experimental design, and evaluation of the prepared SYL-C-loaded formulations

Ex vivo permeation study Results revealed the significance of the three independent factors, as well as the factor interaction AC as validated by the coded equation dis-

played in Table 4. The increase in caffeine concentration accompanied with the increase in carrot oil concentration led to a significant ($p = 0.0331$) increase in Q24. According to Fick's first law for diffusion, concentration gradient is the driving force in the diffusion process; mathematically, the rate of transfer of diffusing substance per unit area is proportional to the concentration gradient; thus, the higher caffeine concentration leads to a higher concentration gradient across the diffused membrane which consequently contributes to the highest permeation parameters. Moreover, it is reported that carrot oil contains a much higher concentration of terpenes as compared to raspberry oil. Terpenes were proved to mainly act on the intercellular lipid structure between corneocytes to increase the fluidity of SC lipids and thus has been proved as effective penetration enhancers [49]. Carrot oil is rich in monoterpene hydrocarbons that constituted about 81–90% of the total oil composition, compared to only 24% and 10%–15%, for wild and cultivated raspberry, respectively [50]. On the other hand, wheat germ oil which was used as a base oil to compensate the amount of the two other investigated oils has a very low monoterpene content represented as only 6.8% of limonene [51].

Furthermore, results also revealed the significance of all factors as well as the factor combinations AB and AC on the Kp model. The presence of both the caffeine concentration and the carrot and/or raspberry oil concentrations in their higher levels led to a significant ($p < 0.05$) increase in Kp values. Generally, the permeation effect of the used oil may be either as a result of its effect on the lipid on the stratum corneum by its incorporation into the lipid lamellae leading to loosen their packing and fluidize the lipid chains or through loosening the packing of stratum corneum protein by modifying the conformation of keratin to cause swelling and increased hydration, or act on the corneodesmosomes to alter cohesion between corneocytes [52].

Finally, the ANOVA results proved the significance of the caffeine–raspberry oil (AB) interaction ($p < 0.05$) on J_{ss}. The increase in both factors significantly increased J_{ss} which is a logical consequence of the increase in the other two measured responses.

Conclusions

Our study revealed the superiority of SYL-C12, compromising the high level of the three independent factors, in terms of the measured responses in mesoporous silica with caffeine. Moreover, caffeine promoted collagen resynthesis with significant protective effect against UV apoptotic damage.

Abbreviations

UV: Ultraviolet rays; HER2: Epidermal growth factor receptor 2; PEPD: Prolidase; ROS: Reactive oxygen species; ECM: Extracellular matrix; AKT: Protein kinase beta; IGF: Insulin-like growth factor; CHK1: Checkpoint kinase 1; ATR: Rad3-related protein; MAPK: Mitogen-activated protein; SYL-C: Caffeine on Syloid® 244 FP; SD: Standard deviation; Q_{24}: The cumulative amount of drug permeated after 24 h; Jss: The permeation rate of caffeine at steady state; Kp: The permeability coefficient.

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Author contributions

Animal medication and slaughter was handled by Dr. Yasmine Abulfadl; ELISA and Western blot and statistical analysis were carried out by Dr. Mae Seleem and Dr. Heba A. Ewida; mesoporous cream and analysis were done by Dr. Nada El Hoffy and Dr. Nancy Lotfy. Manuscript writing and revision were done by all participants in this study. All authors read and approved the final manuscript.

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Availability of the data and materials

All data and materials have been provided with the manuscript as tables and figures.

Declarations

Ethical approval and consent to participate

This study was approved by the Research and Ethics committee for Experimental and Clinical studies at Faculty of Pharmaceutical sciences and Pharmaceutical Industries, Future University in Egypt, Cairo, Egypt (FUE), with approval number (REC-FPSPI-19/54). The study was conducted in accordance with the U.K. Animals (Scientific Procedures) Act 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments.

Consent for publication

All authors have approved the publication of this study.

Competing interests

All authors hereby declare that there is no conflict of interest.

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