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Original Article

HAIR GROWTH PROMOTING ACTIVITY OF *CARTHAMUS TINCTORIUS* FLORETS EXTRACT-LOADED NANOSTRUCTURED LIPID CARRIERS

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ABSTRACT

Objectives: This work was aimed to formulate, characterize and determine hair growth promoting activity of the *Carthamus tinctorius* (safflower) florets extract-loaded nano structured lipid carriers (NLC) in C57BL/6 mice.

Methods: Safflower florets were extracted by maceration with ethanol, and then incorporated into NLC formula. Safflower extract-loaded NLCs were assessed for their physical properties, stabilities and hair growth promoting activity in C57BL/6 mice.

Results: Safflower-loaded NLCs had particle size around 100 nm, zeta potential in the range of -40 to -49 mV. The data from DSC and XRD suggested that this NLC occurred as an amorphous type NLC. Safflower extract-loaded NLC promoted hair growth in the mice better than minoxidil. Safflower yellow, the principle phytochemical in safflower extract, along with synergistic activity between other phytochemicals may account for hair growth promoting activity observed in mice. The further investigations in human volunteer should be conducted for the confirmation.

Conclusion: Safflower-loaded NLC that provided good physical properties and stabilities, exerted hair growth promoting activity in C57BL/6 mice.

Keywords: Alopecia, Hair growth promotion, Nanoparticles, NLC, Safflower extract, 5α-reductase.

INTRODUCTION

Although alopecia or hair loss is not a life-threatening event, it seriously affects mental health and quality of life in those sufferers. Within the various alopecia types, androgenic alopecia or AGA is the most common hair loss problem that affected a large number of peoples, both men and women. Moreover, AGA can occur in any stage of people's life [1, 2]. At present, there are two pharmacological classes to treat AGA, which are the 5α-reductase inhibitors and the topical minoxidil [2]. Unfortunately, medicines in the 5α-reductase inhibitors class come with unfavorable side effects that affected patients' quality of life, which are abnormality of sexual functions such as impotence (erectile dysfunction), abnormal ejaculation and decreased ejaculatory volume, gynecomastia (breast enlargement in men), testicular pain, impairment of muscle growth, and severe myopathy [3]. The more serious side effects such as higher grade of prostate cancer and central nervous system (CNS) disturbance, e. g. Depression, anxiety, suicidal thought and epilepsy have been reported [4]. The use of 2% topical minoxidil lotion also contains a risk for serious cardiovascular side effects. Moreover, when minoxidil was discontinued, newly grown hairs will loss again within three months [5]. From the drawbacks of the available medicines, natural products may be used as an alternative treatment for hair loss.

There are many plants reported with hair growth promoting activity. For examples, *Myrica rubra* (red bayberry) bark [6], *Thuja orientalis* (white cedar) seed [7], *Piper nigrum* (black pepper) leaf [8], *Rosmarinus officinalis* (Rosemary) leaf extract [9] and epigallocatechin-3-gallate (EGCG) found in green tea [10].

Carthamus tinctorius L. or safflower is the common medicinal plants found in the northern part of Thailand. This plant is known for its advantages in the treatment of many cardiovascular, cerebrovascular, and gynecological diseases [11]. In our previous work, we demonstrated that the ethanolic extract of safflower strongly inhibit the 5α-reductase enzyme with the finasteride equivalent 5α-reductase inhibitory activity of 24.30 mg equivalent per one gram of extract while the usual dosage regimen of finasteride to treat hair loss is only at 1 mg per day. In addition, the safflower extract promoted hair growth better than minoxidil [12]. The results were confirmed by Junlatat and Sripanidkulchai [13] whom reported a stimulation of hair follicle proliferation, synthesis of hair growth promoting markers; vascular endothelial growth factor (VEGF) and keratinocyte growth factor (KGF) of safflower extract. A reduction in the expression of transforming growth factor-β1 (TGFβ1) which is the signaling molecule that inhibits epithelial cell growth was also reported [13].

Delivery of substances into hair follicles depends on three factors, which are (i) follicular size, density and reservoir (ii) activity status of hair follicles and (iii) physicochemical properties of topically applied substances [14]. Factors (i) and (ii) cannot be controlled, but factor (iii) is an adjustable factor. It was reported that lipophilic vehicle made substances more penetrable to the hair follicle. For particle size, it was reported that the smaller of the size of particle, the deeper penetration was observed. Previous work indicated that particles that less than 1μm or 1000 nm in size can be absorbed in the hair follicle unit [14, 15]. From this reason, some special drug delivery system must be used in order to deliver the active substances in safflower to the hair follicle unit.

Over decades, lipid nanoparticles have been developed to be used as an alternative delivery system to those of traditional formulations [16]. These lipid nanoparticles provide more benefits in both pharmaceutic and cosmetic formulations, such as the protection of active substance from environmental degradation, release controlling of active substance and occlusion effects to protect skin from dryness [17, 18]. There are two generations of the lipid nanoparticles. The first generation is the Solid Lipid Nanoparticles or SLN that have some drawbacks such as lower entrapment efficacy, high water content, and the drug expulsion process. These drawbacks are the motivation for the researchers to develop the second generation of lipid nanoparticles, Nanostructured Lipid Carriers or NLCs to overcome those drawbacks seen in the SLN [19]. There are three types of NLCs; (i) the imperfect type, is the NLC that consists of lipids mixture, which cannot rearrange to the perfect crystalline; (ii) the amorphous type, is the NLC that have no crystalline structure; and (iii) oil in fat in water type, which its core contain the liquid lipid in the solid lipid seen like the multiple emulsion [20]. Emphases on the drug expulsion process, which is the process that occurred when the lipid material of SLN rearranged to

the crystalline structure where the drug can not be entrapped in this perfect crystal lipid structure, then the drugs are expulsed from the lipid matrix. Drug expulsion process can cause the nanoparticles to lose their control-release properties, and the drug in the solution may be degraded by the environment or the instability of the drug may occur. NLCs have been developed to overcome this problem by using the lipids combinations; thus the crystalline rearrangement and also the drug expulsion will not occur [20].

Normally, the NLCs are composed of the lipid matrix, water phase, and emulsifiers or surfactants combination, which added to improve the stability of the products. One contributing factor that affected the stability of all dispersion system is the zeta potential, which is the electrical phenomenon at the surface of the particle. The electrical charge at the surface of particles leads to the repulsion of the particle and prevent the aggregation or coalescence of the particle, which lead to the instability. According to the previous research, the preferable zeta potential that provides better stability for the dispersion system is greater than |30| mV [21, 22]. Within the various types of emulsifiers, the anionic surfactant is the preferable emulsifier due to their negative charge can increase the zeta potential of the system that may increase the stability of the colloidal products. In this experiment, sodium cocoyl isethionate (SCI) was used due to its mild, low irritation and the better safety profile. Monostearin was selected to be used as a wax matrix, combination with medium chain triglyceride, which is a liquid lipid, to improve the entrapment efficacy.

This work was aimed to the formulation and assessment the physical characteristics of developed NLC, to entrap and characterization of the safflower extract loaded-NLC and to determine the *in vivo* hair growth promoting efficacy of the safflower extracts loaded-NLC in C57BL/6 mice.

MATERIALS AND METHODS

Plant material and extraction

C. tinctorius fresh florets were purchased from a local market in Chiang Mai, Thailand. The florets were identified by comparison with the herbarium specimens at the Faculty of Pharmacy, Chiang Mai University, Thailand. After that, these florets were dried in a hot air oven at 45 °C until dried, then extracted with 95% ethanol by maceration for 5 days. The extract was then evaporated to dryness by using the rotary evaporator (Eyela, Tokyo, Japan).

Animals

Seven-week-old male C56BL/6Mlac mice were obtained from the National Laboratory Animal Center, Bangkok, Thailand, and housed under a 12 h light/dark cycles with free access to food and water. The protocol of this study was approved by the Animal Research Ethics Committee of the Faculty of Pharmacy, Ubon Ratchathani University, Ubon Ratchathani, Thailand.

Reagents and chemicals

Monostearin (TCI chemicals, Tokyo), capric/caprylic triglycerides (Miglyol® 812, Sasol, Germany), laureth-4 (Brij-L4, Croda, UK), sorbitan monostearate (span™ 60, Croda, UK), polysorbate 60 (Tween™ 60, Amresco, USA), Pluronic® F-68 (Sigma, St. Louis, MO) and DMDM hydantoin (Glydant™, Lonza, Switzerland) were purchased from their distributors in Thailand. DI water was produced from Milli-Q Synthesis (Millipore, USA). Methanol was purchased from Fisher Scientific (Fair Lawn, NJ). Absolute ethanol and 95% Ethanol was purchased from Liquor Distillery Organization Excise Department (Chachoengsao, Thailand). Safflower yellow was purchased from Tokyo Chemical Industry (Tokyo, Japan). Depilatory cream (Boots, UK) was purchased from the distributor in Thailand.

Preparation of NLC

NLC was prepared by using hot high pressure homogenization technique, by using high pressure homogenizer (Emulsiflex-C3, Avestin, Ottawa, Canada) under controlled temperature and pressure at 85 °C and 750 bars, respectively for 7 consecutive cycles. Briefly, the oil phase was melted in a water bath, the water phase was heated separately to 85 °C then the oil phase was slowly incorporated into the hot water phase using simple stirring technique. Then, the mixture was stirred for 5 min before forced through a high-pressure homogenizer. Resulting nanoemulsion was left cooled to room temperature to obtain the NLC. For *C. tinctorius* entrapped NLC (CT-NLC), various amounts of the extract (0.05, 0.1, 0.25, 0.5 and 1 % w/w) was added in the oil phase before melted in the water bath.

The compositions of investigated formulations are given in Table 1.

Table 1: Composition of investigated NLC formulations

	Chemicals	Concentration used (% by weight)					
		NLC base	CT1-NLC	CT2-NLC	CT3-NLC	CT4-NLC	CT5-NLC
Oil phase	Monostearin	7	7	7	7	7	7
	Medium chain triglycerides	3	3	3	3	3	3
	Span 60	9.5	9.5	9.5	9.5	9.5	9.5
	Laureth-4	1	1	1	1	1	1
	Safflower extract	-	**0.05**	**0.1**	**0.25**	**0.5**	**1**
Water phase	Pluronic F-68	2	2	2	2	2	2
	Tween 60	2	2	2	2	2	2
	Sodium cocoyl isethionate	0.7	0.7	0.7	0.7	0.7	0.7
	DMDM hydantoin	0.2	0.2	0.2	0.2	0.2	0.2
	DI water	to make 100 %					

Physicochemical properties of NLC and CT-NLC

Physical appearance

Prepared NLC base and CT-NLC were checked for their physical appearance by using organoleptic technique.

Photon correlation spectroscopy (PCS)

Mean particle size, polydispersity index, and zeta potential of each formulation was determined by using PCS (Zetasizer ZS, Malvern instruments, Malvern, UK).

The formulations were diluted in DI water to obtain the appropriate scattering intensity. The analysis was performed at 25 °C.

Accelerated stability, short term and long term stability study of NLC

In order to analyze the accelerated stability of prepared formulation, heating-cooling cycle was used. Briefly, the samples were kept at 45° C oven for 24 h followed by 4° C refrigerator for 24 h for six consecutive cycles.

Prepared formulations were kept in normal room temperature for 12 months to assess the short term and long term stability. For an assessment of short-term stability, particle size, size distribution and zeta potential were analyzed on day 1, 7, 14 and 28. For long-term stability study, the formulations were assessed for the same parameters after being kept for 3, 6, 9 and 12 months at room temperature.

Entrapment efficacy (EE)

Indirect method, which is the method that analyzed for un-entrapped safflower, was used for determining the EE. The NLCs were forced to precipitate by using the cold methanol precipitation technique as describe in our previous paper [23]. Briefly, formulations with entrapped safflower were accurately weighed to 0.5 g then mixed with methyl alcohol 4.5 g. After that, the mixtures were then centrifuged at 6000 rpm, 0 °C for 30 min. Clear supernatant was then filtered by 0.45μm nylon membrane filter before analyzed for safflower yellow by HPLC following the method reported by our group [23]. Briefly, samples of 10 μL were injected through the HPLC analytical column Hypersil®-ODS (Thermo Scientific, USA) 250 × 4.6 mm i. d. with 5μm internal particle size. The mobile phase used was 60% methanol in water with a flow rate of 1 ml/min. The temperature of the column was controlled at 40 °C. The detection wavelength was fixed at 401 nm. Total run time was 10 min per sample. EE was calculated by following equation:

EE = (Amount of CT added − Amount of CT found)/Amount of CT added × 100

Differential scanning calorimetry (DSC)

DSC technique was used to evaluate the lipid crystallinity and polymorphism of the selected formulation using Differential Scanning Calorimeter (Perkin Elmer, USA). Briefly, the samples and pure monostearin, which is the lipid matrix of NLC, were accurately weighed and sealed in the aluminium pans. An empty aluminium pan was used as the reference pan. The experiments were performed over the ranged of 35 to 85 °C, with constant heating rate of 5 K/min and flushed with nitrogen at the rate of 20 ml/min. The melting point, onset temperature and enthalpies (ΔH) were evaluated.

X-ray diffraction (XRD)

XRD was performed by using wide-angle X-ray scattering (WAXS) to investigate lipid crystallinity and polymorphism of the selected formulation and to confirm the results obtain from DSC. Analysis was performed by a D8 X-ray Diffractometer (Bruker AXS GmbH, Germany) with an X-ray tube equipped with copper anode (Cu-Kα radiation, 40 kV, 40 mA, λ = 1.54184 nm) using the Si(Li) semiconductor and scintillation counter as a detector. Analyses were done at 2 Theta from 2.0° to 40.0°; data collection was done with a step width of 0.04° and step time of 1 s. All WAXS experiments were conducted at 25 °C.

***In vivo* hair growth promoting efficacy of CT-NLC**

Hair growth promoting efficacy of the CT-NLC was assessed in the C57BL/6Mlac mice by the method reported by our group [12]. Briefly, twenty-five mice were randomly divided into five groups for the five treatments as followed: control, NLC blank, minoxidil, safflower extract, and CT-NLC. In the control group, DI water was used. In the NLC blank group, 5 % by weight of NLC base in DI water was used. In minoxidil group, 2% minoxidil in DI water was used. In safflower group, 0.1% by weight of safflower extract in DI water was used. In CT-NLC group, 5% by weight of CT-NLC in DI water was used. Prior to the experiment, the 3x4 cm (width (height) dorsal areas of seven-week-old mice were shaved by using a depilatory cream. On the next day, 100 μl of samples were applied gently on the mice back once daily, for 28 consecutive days. Hair growth promoting activities of tested components were assessed by observing the darkening of the skin and giving the hair growth score, as follows: score 0 = no growth observed; 1 = up to 20% growth; 2 = 20–40% growth; 3 = 40–60% growth; 4 = 60–80% growth; and 5 = 80% to full growth observed.

Digital images of total hair growth on day 28 were obtained using a Coscam® USB-225 (Seoul, South Korea) with a 40× magnification lenses.

Statistics

All of the data were reported in the term of mean ± SD. Experiment on particle size, size distribution, zeta potential, and entrapment efficacy were done in triplicate. DSC and XRD analysis was in three repeated measurements. Significance of differences was evaluated using Student's *t*-test and one-way ANOVA at the confidence level 95%.

RESULTS

Extraction of safflower florets gave a yield at 19.25% of its dry weight. Safflower yellow, a major constituent in safflower was found at 268.16 ± 7.28 mg/g extract.

In order to prepare CT-NLC, first the NLC base was formulated from different compositions and ratios of various ingredients (results not shown). Composition of the most favorable NLC base formula is the one that shown in table 1. From table 1, the investigated NLC consisted of five emulsifiers, which were Span 60, Laureth-4, Pluronic F-68, Tween 60 these four are nonionic emulsifiers and the anionic sodium cocoyl isethionate. Usually, safflower extract is sparingly soluble in oil but with the help of Laureth-4, safflower extract can completely soluble in the oil phase of NLC. DMDM hydantoin was selected as a preservative in the formulations. Prepared NLC occurred as a translucent milky viscous liquid and CT-NLC occurred as a translucent orange milky viscous liquid. The intensity of orange color of the CT-NLC depended on the concentration of safflower used. The viscosity of CT-NLC was increased from an increasing amount of safflower added. In formula with 1% by weight of safflower, the hard wax was obtained so that the NLC formula with 1% safflower was excluded from this study.

The size of the different NLCs formula were at around 103 – 109 nm and were not statistically different from each other. Polydispersity index of the NLC base was 0.225, and all CT-NLC formulas were at around 0.202 – 0.209. Zeta potential of all formulations was in the range of -40 – -49 mV. It was found that the entrapment of safflower into the wax matrix of NLC did not affect the particle size, size distribution or the zeta potential of the NLC formula, since there was no correlation between the concentration of the entrapped safflower extract and these parameters.

Accelerated heating-cooling cycle, short-term and long-term stability was used for investigating the stability of developed formula. Parameters of particle size, size distribution, and zeta potential were used to assess the stability of these formulas. The particle size of the NLC formulations after treated with heating cooling cycle was still in the range of 100 – 106 nm.

There was no any significant different on particle size, size distribution, and zeta potential between before and after treated with heating cooling cycles. The particle sizes of short-term and long-term stability study of the formulations was shown in fig. 1. There also were no any significant different on particle size and size distribution between day 1 and 12 months after prepared. This suggested that all NLCs were stable at room temperature for at least 1 year.

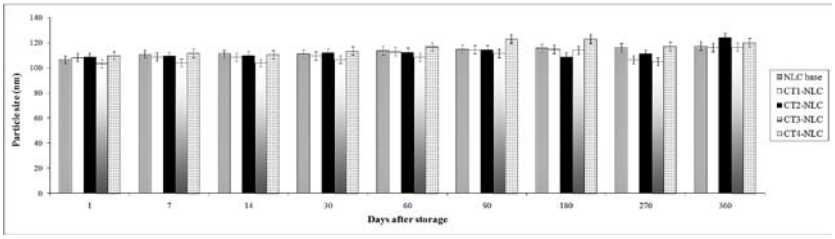

Fig. 1: Mean particle size of NLC base and safflower-loaded NLC (CT-NLC) after kept at room temperature for a certain period

Entrapment efficacy of safflower extract into the monostearin wax matrix was determined, and the results were shown in fig. 2. From fig. 2, it was observed that the more concentration of safflower used, the less entrapment efficacy was. The highest entrapment efficacy was achieved when using 0.05% safflower extract. From this result, the CT1-NLC, which was the NLC that entrapped with 0.05% by weight of safflower extract, was used in the next part of the study.

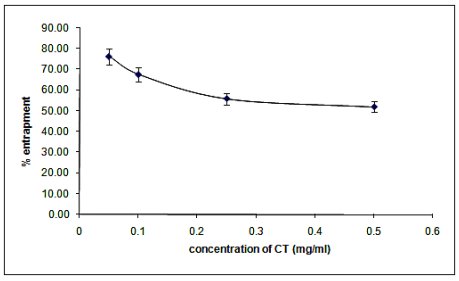

Fig. 2: Entrapment efficacy of safflower extract into NLC

In order to determine the lipid crystallinity and packing behavior of NLCs, both DSC and XRD were used. The DSC thermogram of monostearin, NLC base and CT1-NLC was shown in fig. 3. From the thermogram, monostearin gave melting onset at 54.346 °C and had a melting point at 59.593 °C. The enthalpy of melting was 121.608 J/g. It was observed that when monostearin was used as a wax matrix in NLC formulation, the melting peak was disappeared. This suggested that both NLC base and CT1-NLC occurred as an amorphous NLC.

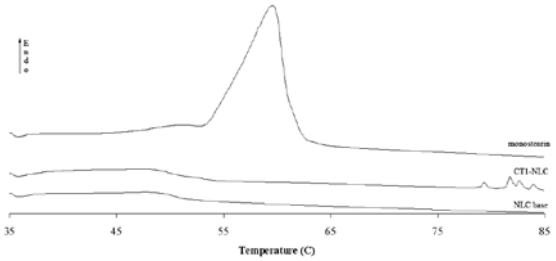

Fig. 3: DSC thermogram of monostearin, 0.05% w/w of safflower extract-loaded NLC (CT1-NLC) and NLC base

For confirmation, XRD was also used to identify the crystalline structure of monostearin, NLC base and CT1-NLC. The diffraction pattern was shown on fig. 4. From the diffractogram, pure monostearin gave two intense sharp peak at 2θ equal 5.48° and 19.56°, but in NLC and CT-NLC, these peaks were disappeared, suggesting that the NLC may occur as an amorphous NLC.

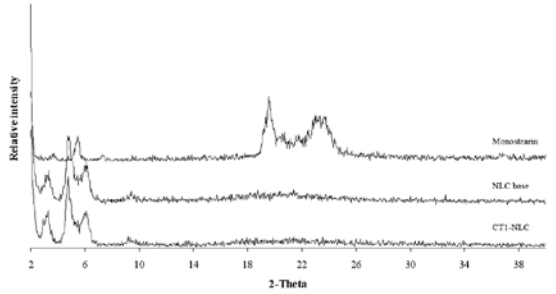

Fig. 4: X-ray diffraction pattern of monostearin, 0.05% w/w of safflower extract-loaded NLC (CT1-NLC) and NLC base

The results on hair growth promoting activity of CT-NLC compared with the control, NLC base, minoxidil and crude safflower extract were shown in fig. 5. From fig. 5, CT-NLC and 1% safflower extract exert the strongest hair growth promoting activity, followed by 2% minoxidil and NLC blank. Water, which was used as control showed the normal hair growth rate of mice.

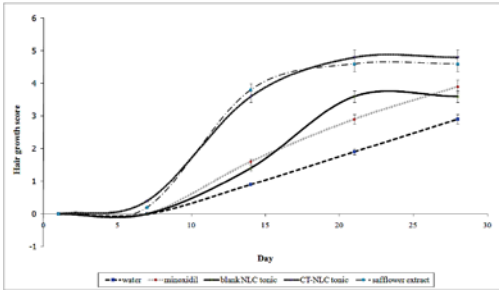

Fig. 5: Hair growth promoting efficacy of safflower-loaded NLC (CT1-NLC) in comparing with NLC base, minoxidil, and safflower extract

Total hair growth at day 28 was shown in fig. 6, which was obtained from Coscam, a special camera that used for viewing the skin. The pictures from Coscam suggested that the mice received CT-NLC, and 1% safflower extract had the highest hair growth. None of any irritation occurred in mice when treated with water, NLC base, CT-NLC or safflower extract.

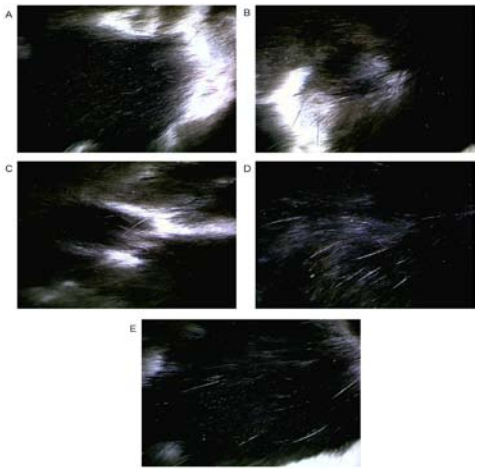

Fig. 6: Total hair growth in C57BL/6 mice at day 28 where: (A) control (B) minoxidil treated (C) NLC base treated (D) safflower-loaded NLC (CT-NLC) treated and (E) safflower extract treated. Digital images obtained from Coscam® USB-225 with 40x magnification lens

DISCUSSION

Safflower florets contain various phytochemicals, including flavonoids that may account for its activity. Safflower yellow is a major constituent in safflower florets. In this study, yield of extraction and safflower yellow contents were differ from the experiment done by Junlatat and Sripanidkulchai [13], whom reported the yield of extraction at 28.30% and safflower yellow, in a term of hydroxysafflor yellow A, content at 212.00 ± 17.56 mg/g extract. Compared to our results, the extraction yield was lower but safflower yellow content was higher.

In order to deliver the active ingredients in safflower into the hair follicle unit, we selected NLC as the carrier system due to previous characteristics as described in the introduction. In this investigation, we used monostearin as a wax matrix for entrapping the safflower extract. Medium chain triglycerides or synonyms caprylic/capric triglycerides was used as a liquid lipid. The addition of liquid lipid into the wax matrix of NLC helps increase the entrapment efficacy due to the more solubility of the entrapping drug in the liquid lipid than the solid lipid [24]. Five emulsifiers were used in combination

to obtain the most stable NLC. Sodium cocoyl isethionate is an anionic surfactant intended to use in this experiment for increasing the zeta potential value of the dispersion. DMDM hydantoin was selected as a preservative. Hot high-pressure homogenization technique was used to prepare the NLC, this technique is an effective method for both laboratory- and factory-scale production of NLC. The particle size of all formulations was in the range of 100 to 110 nm, which was conformed to the criteria of transfollicular penetration described by Schaefer and Lademann [15]. Zeta potential is the electric potential at the surface of a particle and is an important factor for predicting physical stability of colloidal preparation. The charge at the surface plays a role in electric repulsion between the particles, thus preventing aggregation and coalescence of the particles, which lead to the system stability. The higher zeta potential, the more stability of the nanoparticle observed. The zeta potential in these formulations was at around -40 to -49 mV. According to the previous reports [21, 22], this formulation should possess a considerable stability. For confirmation of this hypothesis, both of the accelerated stability study, short term and long term stability were investigated. All of the formulation possessed the stability at least one year at room temperature. This suggests that the zeta potential can be a marker to predict the stability of this NLC formulation.

For the determination of entrapment efficacy, safflower yellow, which is the most abundant phytochemical found in the safflower florets, was used as a phytochemical marker. In this experiment, we used an indirect method to determine the entrapment efficacy of safflower, from unincorporated safflower, which was solubilized in the medium. Cold methanol precipitation technique was used to extract the unincorporated safflower since methanol caused the wax to be dehydrated and precipitated [23]. The amount of safflower added minus by the amount of safflower found in the matrix is the entrapped safflower so the entrapment efficacy can be calculated after an analysis by HPLC. The lower entrapment efficacy observed in the formula with higher safflower may be a result from a saturated solubility of the safflower in the oil matrix.

In order to determine the lipid crystallinity of NLC, two methods; DSC and XRD were used. Fig. 3 and 4 suggested that both NLC base and CT1-NLC occurred as an amorphous lipid core. The benefit of amorphous lipid core is the prevention of drug expulsion from the wax core. Since the first generation of lipid nanoparticles or SLN, which use one type of the lipid, after being kept the wax matrix will recrystallize into the more stable and preferable form and the entrapped drugs are expulsed from the core. Drug expulsion process may lead to the physical instability of drugs and the loss of controlled release properties of the nanoparticles [20]. From previous research, Hu et al. [25] also used the monostearin and various contents of medium-chain triglycerides as a wax matrix for NLC, but they used only poloxamer 188 as an emulsifier. Monostearin-based NLC from that experiment still had the crystalline structure, seen from crystallinity index, which were ranged from 60 to 90%. This suggested that SCI and the four emulsifiers combination in this experiment may play a role in the occurrence of amorphous NLC. This may lead to the application of this SCI stabilized monostearin-based NLC in an entrapment of various substances.

The C57BL/6 mice are usually used for assessment of hair growth promoting activity due to their special characteristics; which were their melanin are found only in the hair and the melanogenesis processes is occurred only in the anagen phase of the hair growth cycle [26]. At seven-week-old, all of the hair follicles of these mice are in the telogen stage, when shaved; the blackening of the skin is observed only when the anagen phase is started. There was an interesting finding on the hair growth promoting activity of the NLC base which was equal to the minoxidil, which may be a result from the lipid material in the wax matrix since there was a reported on 5α-reductase inhibitory activity of the fatty acids [27] and there was a strong correlation between 5α-reductase inhibitory activity and hair growth promoting activity [12]. In order to confirm this hypothesis, the further investigation would be considered. Another point is the equal hair growth promoting activity of the CT-NLC and the crude safflower extract. However, the amount of safflower extract in the CT-NLC was 40-fold diluted than 1% solution of safflower extract, this suggested that the entrapment of safflower ion the NLC increased the hair growth promoting activity of safflower, which may be a result from the succession of the delivery process of the substances in the NLC into the hair follicle of the mice.

CONCLUSION

Safflower extract-loaded NLC in this study provided long term stability. NLC prepared from monostearin and medium chain triglycerides that stabilized by a combination of sodium cocoyl isethionate and other emulsifiers, occurred as an amorphous form with particle size of around 100 nm. The study revealed that NLC with entrapped safflower of 0.05% by weight shown hair growth promoting activity in C57Bl/6 mice than minoxidil, which is the drug that used to treat alopecia. Further investigation on the development of CT-NLC into several hair products and efficacy evaluation of these products in healthy human volunteer would be further analyzed.

ACKNOWLEDGEMENT

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ABBREVIATIONS

AGA=Androgenic alopecia

CT=*Carthamus tinctorius* L. (safflower)

CT-NLC=*Carthamus tinctorius* L.-loaded NLC

EE=Entrapment efficacy

SCI=Sodium cocoyl isethionate

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Trüeb RM. Molecular mechanisms of androgenetic alopecia. Exp Gerontol 2002;37:981-90.
2. Sinclair RD. Male androgenetic alopecia. J Men Health Gender 2004;1(4):319-27.
3. Lacy CF, Armstrong LL, Goldman MP, Lance LL. Drug information handbook with international trade names index, 17th ed. Ohio: Lexi Comp Inc; 2008. p. 652-3.
4. Traish AM, Mulgaonkar A, Giordano N. The dark side of 5α-reductase inhibitors' therapy: sexual dysfunction, high gleason grade prostate cancer and depression. Korean J Urol 2014;55:367-79.
5. Abramowicz M. Propecia and rogain extra strength for alopecia. Med Lett Drugs Ther 1998;40:25-7.
6. Matsuda H, Yamazaki M, Matsuo K, Asanuma Y, Kubo M. Anti-androgenic activity of Myricae cortex-isolation of active constituents from bark of Myrica rubra. Biol Pharm Bull 2001;24:259–63.
7. Park W-S, Lee C-H, Lee B-G, Chang I-S. The extract of Thujae occidentalis semen inhibited 5α-reductase and adrochronogenetic alopecia of B6CBAF1/j hybrid mouse. J Dermatol Sci 2003;31:91–8.
8. Hirata N, Tokunaga M, Naruto S, Iinuma M, Matsuda H. Testosterone 5α-reductase inhibitory active constituents of Piper nigrum leaf. Biol Pharm Bull 2007;30:2402–5.
9. Murata K, Noguchi K, Kondo M, Onishi M, Watanabe N, Okamura K, *et al*. Promotion of hair growth by rosmarinus officinalis leaf extract. Phytother Res 2013;27:212-7.
10. Kwon OS, Han JH, Yoo HJ, Chung KH, Cho KH, Eun HC, *et al*. Human hair growth enhancement in vitro by green tea epigallocatechin-3-gallate. Phytomed 2007;14:551-5.
11. Tian Y, Yang Z-F, Li Y, Qiao Y, Yang J, Jia Y-Y, *et al*. Pharmacokinetic comparisons of hydroxysafflower yellow a in normal and blood stasis syndrome rats. J Ethnopharmacol 2010;129:1-4.
12. Kumar N, Rungseevijitprapa W, Narkkhong NA, Suttajit M, Chaiyasut C. 5α-reductase inhibition and hair growth

promotion of some Thai plants traditionally used for hair treatment. J Ethnopharmacol 2012;139:765-71.
13. Junlatat J, Sripanidkulchai B. Hair growth-promoting effect of Carthamus tinctorius floret extract. Phytother Res 2014;28:1030-6.
14. Patzelt A, Knorr F, Blume-Peytavi U, Sterry W, Lademann J. Hair follicles, their disorders and their opportunities. Drug Discov Today Dis Mech 2008;5(2):e173-81.
15. Schaefer H, Lademann J. The role of follicular penetration. A differential view. Skin Pharmacol Appl 2001;14(S1):23-7.
16. Müller RH, Lucks JS. Medication vehicles made of solid lipid particle (Solid lipid nanospheres SLN); 1996.
17. Wissing SA, Müller RH. Cosmetic applications for solid lipid nanoparticles (SLN). Int J Pharm 2003;254:65-8.
18. Pardeike J, Hommoss A, Müller RH. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. Int J Pharm 2009;366:170-84.
19. Müller RH, Petersen RD, Hommoss A, Pardeike J. Nanostructured lipid carriers (NLC) in cosmetic dermal products. Adv Drug Deliv Rev 2007;59:522-30.
20. Müller RH, Radtke M, Wissing SA. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Adv Drug Deliv Rev 2002;54(S1):S131-5.
21. Mehnert W, Mäder K. Solid lipid nanoparticles: production, characterization and applications. Adv Drug Deliv Rev 2001;47:165-96.
22. Mishra PR, Al Shaal L, Müller RH, Keck CM. Production and characterization of hesperidin nanosuspensions for dermal delivery. Int J Pharm 2009;371:182-9.
23. Kumar N, Tharatha S, Chaiyasut C. Development and validation of simple isocratic high performance liquid chromatography-ultraviolet (HPLC-UV) method for determination of safflower yellow in Carthamus tinctorius L.-loaded nanostructured lipid carriers (NLC). Afr J Pharm Pharmacol 2011;5(20):2335-41.
24. Jenning V, Thünemann AF, Gohla SH. Characterization of a novel solid lipid nanoparticle carrier system based on binary mixtures of liquid and solid lipids. Int J Pharm 2000;199:167-77.
25. Hu F-Q, Jiang S-P, Du Y-Z, Yuan H, Ye Y-Q, Zeng S. Preparation and characteristics of monostearin nanostructured lipid carriers. Int J Pharm 2006;314:83-9.
26. Slominski A, Paus R, Plonka P, Chakraborty A, Maurer M, Pruski D, *et al*. Melanogenesis during the anagen–catagen–telogen transformation of the murine hair cycle. J Invest Dermatol 1994;102:862–9.
27. Liang T, Liao S. Inhibition of steroid 5-alpha-reductase by specific aliphatic unsaturated fatty acids. Biochem J 1992;285:557-62.