The activity of candlenut oil in the nanostructured lipid carrier system on hair growth in rats

Tristiana Munandar Erawati, Noorma Rosita, Intan Rachmania

Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

Abstract

Background: Candlenut oil (*Aleurites moluccana* L. Willd), which is also called Aleurites moluccana Seed (AMS) oil, is empirically effective as a hair growth agent, it is an unstable substance. Nanostructured Lipid Carrier (NLC) is a delivery system that is proven to increase the effectiveness and stability of the material, and the usage of solid lipid combination: beeswax-oleum cacao can produce good NLC characteristics.

Objective: To determine the effectiveness of NLC_AMS oil with different combination of beeswax-oleum cacao (100:0; 50:50; 25:75; and 0:100) as a hair growth agent, using rats as subjects.

Methods: NLC-AMS oil was made using 20% of total lipid with 5% AMS oil as liquid lipid and 15% solid lipid; combinations of beeswax-oleum cacao were of different ratios (100:0; 50:50; 25:75; and 0:100). NLC was made by High Shear Homogenization (HPH) method. Hair growth activity test carried out on male white rats using the research methods of Yoon (2010).

Results: The addition of oleum cacao as a solid lipid did not affect the pH, but increased the consistency and decreased the particle size of NLC- AMS oil. There was a relationship between the characteristic of NLC and the hair growth activity test: the small particle size and low viscosity had greater hair growth activity.

Conclusion: The usage of solid lipid combination: beeswax-oleum cacao can produce better NLC characteristics and had higher hair growth activity than the formulas that used single lipid.

Correspondence: Noorma Rosita, Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, 60115, Indonesia.
Tel.: +62.315933150 - Fax: +62.315935249
E-mail: noorma-r@ff.unair.ac.id

Key words: Candlenut oil, AMS oil, Nanostructured lipid carrier (NLC), Hair growth.

Acknowledgments: The Authors would like to thank Universitas Airlangga and Directorate General of Higher Education for grant (Grant No. 398/UN3.15/PT/2021) and Faculty of Pharmacy Universitas Airlangga for research support and facilities.

Contributions: TEM, contributions to the design of the work, validation, formal analysis, original drafting, data analysis, data interpretation; NR, validation, data collection, data analysis, data interpretation, drafting manuscript; IR: data collection, formal analysis. All the authors approved the final version to be published.

Conflict of interest: The authors declare no potential conflict of interest.

Funding: None.

Ethical approval and consent to participate: The procedure for treating experimental animals in this study had received approval from the Research Ethics Commission of the Faculty of Veterinary Medicine, Universitas Airlangga (Certificate number 2.KE.052.01.2018). All methods were performed in accordance with ARRIVE guidelines and general animal guidelines respectively.

Availability of data and material: Data and materials are available by the authors.

Informed consent: The manuscript does not contain any individual person's data in any form.

Received for publication: 24 November 2022.
Revision received: 22 December 2022.
Accepted for publication: 31 December 2022.

Journal of Public Health in Africa 2023; 14(s1):2519
doi:10.4081/jphia.2023.2519

Introduction

Hair is a part of the body that serves to protect the scalp from external disturbances that support one's appearance. Factors that affect hair growth were stress, hormones, metabolism, nutrition, and drugs.[1] According to Satriyo *et al*. the amount of hair loss ranges from 50-100 strands per day, but it can also exceed this range that can cause baldness.[2] In order to prevent hair loss, promote hair growth and baldness, treatments are necessary.

The use of synthetic materials for cosmetic products is considered less safe because it can cause various side effects, especially in long-term use.[3] Therefore, innovation is needed to reduce these side effects. Several studies have described alternatives that use natural ingredients, including candlenut oil which is also called AMS oil.

Aleurites moluccana Seed (AMS) oil is derived from the seeds of the candlenut plant: Aleurites moluccana L. Willd used as hair loss preventive empirically.[4] Candlenut seeds containing palmitic acid, arachidic acid, oleic acid, linoleic acid and linoleic acid showed hair growth activity on male white rabbit's hair.[5] Observation of rabbit hair growth for 18 days using AMS oil in the form of cream preparations was able to increase hair growth by 11.26 mm.[6] In Prasojo's research (2012), the concentration of AMS oil based on previous research in stimulating hair growth in male rabbits was 5%.[7] The largest content contained in AMS oil is linoleic acid and linoleic acid of 66.2% where Omega-6 contained in linoleic acid and Omega-3 contained in linoleic acid are able to improve blood circulation in the head area and stimulate hair growth.[8,9] AMS oil has volatile and oxidized properties because it has a high Iodine number of 136-167.[10,11] To prevent evaporation and oxidation of AMS oil, a system is needed, namely using a nano lipid carrier system.[12]

NLC is one of the nano lipid carriers that has a particle size of

50-1000 nm, consisting of a mixture of solid lipid and liquid lipid which makes the matrix have an amorphous crystalline structure that allows the active ingredients entrapped firmly in the system.[13] The advantage of this system is lipophilic and has a particle size of <700 nm, therefore it can penetrate through the follicular pathway.[14] In addition, NLC has adhesive properties which is able to make occlusive effect that will hydrates the skin, controlled release and increase absorption or penetration as well as increase the chemical stability of the active.[15] In its application, viscosity of the NLC is low due to the presence of liquid lipids. This causes the contact time with the skin is short so that the absorption of the active ingredients also decreases and the acceptability is less.[16] The addition of the base will increase the viscosity and contact time on the skin. It will increase skin hydration so that the active ingredients penetrate more easily into the skin.

The NLC system consists of solid lipid, liquid lipid, aqueous phase, surfactant and co-surfactant. The solid lipids commonly used are glyceryl monostearate, stearic acid, cetyl alcohol or triglycerides such as tristearate. The liquid lipids commonly used are natural or synthetic oils such as oleic acid, castor oil and mustard oil.[17] According to Krasodomska *et al*. (2016), the use of seed oil with beeswax in the NLC system in ratio 1:3 had good stability and particle size.[18] Solid lipids such as beeswax have stable physical properties but their ability to entrap active ingredients is low, so it is necessary to combine it with other lipid to make it more amorphous.[19] Based on the of Attama research (2006), the value of the recrystallization index (RI) of the solid lipid combination of beeswax-oleum cacao with a ratio of 50:50 and 25:75 is smaller than the RI value the single lipid. The RI value affects the crystal structure so that it will increase drug entrapment in lipids.[19]

Materials and Methods

Materials

All ingredients, if the grade is not stated, then what was used is pharmaceutical grade: *Aleurites moluccana* seed oil (Natures), beeswax (PT Kurniajaya Multisentosa, Indonesia), oleum cacao (Research Center for Coffee and Cocoa, JemberIndonesia), Span 80 (Sigma Aldrich), Tween 80 (Sigma Aldrich), Propylenglycol (PT Kurniajaya Multisentosa), Sodium benzoate (PT Brataco), Sodium acetate and Glacial Acetic acid pro analysis (E.Merck), and CO2-free d,emineralized water.

Experimental animals

This study used 28 male white rats (*Rattus nivergicus*) Sprague Dawley strain, approximately 2 months old and weighing around 150-200 grams.[20] The procedure for treating experimental animals in this study had received approval from the Research Ethics Commission of the Faculty of Veterinary Medicine, Universitas Airlangga (Certificate number 2.KE.052.01.2018). All methods were performed in accordance with ARRIVE guidelines and general animal guidelines respectively.

Table 1. Formula of NLC_AMS oil.

Formula (F)	AMS oil (%)	Solid Lipid (15%) with ratio: Beeswax	Oleum cacao	Aqueous Phase (%)
FI	5	100	0	ad 100
FII	5	50	50	ad 100
FIII	5	25	75	ad 100
FIV	5	0	100	Ad 100

Table 2. Average particle size (nm) and PI NLC- AMS oil.

Formula	Mean Particle Size ± SD (nm)	Mean PI ± SD
Placebo	473.17±129.45	0.476±0.07
FI	591.23±88.65	0.399±0.04
FII	230.60±16.41	0.335±0.05
FIII	272.60±9.27	0.290±0.01
FIV	400.27±39.72	0.452±0.008

Table 3. Average hair length results of each treatment.

Test Groups	Treatment	Average Hair Length (mm) ± SD Day 7	Day 14	Day 21
I	Negative control	3.56±0.38	6.31±0.34	8.69±0.40
II	Placebo	2.79±1.87	6.16±1.42	8.63±1.28
III	FI	0.00±0.00	5.84±0.05	8.81±0.26
IV	FII	2.72±3.14	8.01±2.28	9.79±1.51
V	FIII	1.97±2.33	8.55±2.19	10.22±0.93
VI	FIV	1.05±2.11	5.51±2.12	8.92±0.84
VII	Positive Control (Minoxidil 2%)	5.94±3.97	10.83±0.82	13.02±0.4

Methods

NLC preparation

In this study, NLC-AMS oil was made using 20% of total lipid with 5% AMS oil as liquid lipid and 15% solid lipid, combination of beeswax- oleum cacao with different ratio (100:0; 50:50; 25:75; and 0:100). The formula could be seen in Table 1. The aqueous phase was acetate buffer pH 5.0±0.5 with sodium benzoate as preservative, and a combination of surfactants Span 80 and Twee 80 and propylene glycol as co-surfactant. NLC was made by HPH method.

Evaluation of NLC characteristics includes organoleptic of NLC preparation, pH value using pH-meter Eutech Instrument pH 700, viscosity using Cone and Plate viscometer. Sample was measured for the particle size and poly dispersity index using Particle Analyser Delsa® Nano Submicron Particle Size, after validating a 1:50 dilution using distilled water and vortexing for one minute.

Test on the effectiveness of hair growth

Before conducting activity tests on experimental animals, rats were acclimatized for 1 week to be able to adapt to new environmental conditions. Then the rats were divided into 7 groups, where each group consisted of 4 rats. The hair on the back was shaved with an area of 4x4 cm. The area that was used as a test area was only 2x2 cm in size. Before testing, the rat was rested for 24 hours. The sample test was applied to the area once a day as much as 0.2 mL for 3 weeks.[21] The procedure for treating experimental animals in this study had received approval from the Research Ethics Commission of the Faculty of Veterinary Medicine, Universitas Airlangga.

Group 1 was not smeared with the preparation or as a negative control, group 2 was smeared with the preparation that did not contain the active ingredient (NLC base), group 3 was smeared with the formula I (FI), group 4 was smeared with the formula II (FII), group 5 was smeared with the formula III (FIII), group 6 was smeared with the formula IV (FIV) and group 7 was smeared with the product "X" which was contain minoxidil 2% as a positive control. The first day of smearing sample was considered to be day 0. This test had 2 test parameters [(24)], namely: i). The average length of rat hair was obtained by randomly measuring the 10 longest strands hair using a shovel and was performed every week that were: on day 7, 14 and 21 after smearing. The results were expressed as the mean hair length ± SD of 10 hairs. ii) The weight of rat hair was obtained by weighing all the hairs that grew on the polishing area on the 21st day, the results were expressed as hair weights ± SD of 10 hairs of 4 rats.

Results

Preparation characterization

Organoleptic

The addition of oleum cacao as a solid lipid increased the consistency, but the texture was smooth, the color was more yellowish and it had specific smell.

Value of pH

pH value of placebo, FI, FII, FIII and FIV were 5.07±0.01; 5.27±0.02; 4.98±0.02; 5.17±0.02 and 5.37±0.01, respectively. All formulas had pH range 4.96-5.38, in between of the stability range of AMS oil and pH balance with skin.

Viscosity

The viscosity of placebo, FI, FII, FIII and FIV were 16527.33±67.26; 82.00±6.56; 2696.87±12.00; 14277.00±981.91 and 19999.00±884.37 cps, respectively. The addition of oleum cacao increased the viscosity.

Particle size and polydispersity index (PI)

It can be seen in Table 2. From the results of one-way ANOVA statistical analysis, p value <0.05, showed that the particle size of placebo, FI, and FIV were significantly different with FII and FIII. By Tukey HSD test results, it is known that the particle size of placebo = FI > FII, FIII, FIV. An increase of oleum cacao decreased the particle size of NLC- AMS oil.

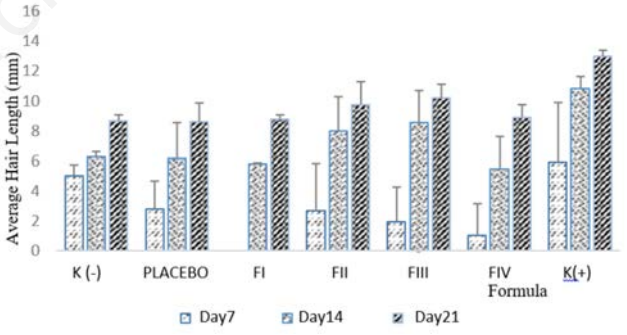

Figure 1. Histogram of average hair length of each treatment.

Table 4. Scoring results of acceptability test.

Criteria	Score	Indicators	Total respondent		Total score	
			F2	F3	F2	F3
Odour	1	Very strong smell	3	0	3	0
	2	Slightly strong smell	7	0	14	0
	3	Slightly smelly*	0	10	0	30
	4	Odourless*	0	0	0	0
Ease smeared	1	With strong emphasis the preparation cannot be evenly distributed	0	0	0	0
	2	Strong emphasis is needed so that the preparations are evenly distributed	0	0	0	0
	3	A little emphasis is needed for the preparations to be evenly distributed*	7	4	21	12
	4	Without emphasis the preparations are easily and evenly distributed*	3	6	12	24
Ease of washing	1	It does not go away even if it is given water and rubbed repeatedly	0	0	0	0
	2	Disappeared after being given water and rubbed for many times	0	0	0	0
	3	Lost after watering and rubbing*	6	5	18	15
	4	Immediate preparations missing after being given water*	4	5	16	20

*Preparations considered acceptable.

Activity test results

Hair length measurement

The results of average hair length of each treatment on the 7th, 14th, and 21st day can be seen in Table 3 and Figure 1. Positive control showed the longest followed by Formula II and Formula III. Based on Kruskal Wallis and Mann-Whitney statistical test results, there were a significant difference between FII and FIII with the negative control, placebo, and positive control, while there were no significant difference between FI and FIV with negative control and placebo.

Hair weight measurement

Observations were also made on hair weight on 21st day. The hair in each test area of each treatment was shaved and weighed. The results of hair weight measurements showed that positive control was the heaviest, followed by Formula III.

Based on statistical calculations using Kruskal Wallis and Mann-Whitney, there were significant difference between FII and FIII with negative control, placebo, and positive control, while there were no significant difference between FI and FIV with the negative control and placebo.

Relationship between NLC characteristics and the activity

Figure 2 showed the relationship between the NLC characteristics and the hair growth activity test on 21st day in each of the NLC formulas. It showed that particle size and viscosity affected the activity of NLC as delivery system. The small particle size and the low viscosity had greater hair growth activity.

Acceptability test results

Table 4 showed the result of acceptability test of 4 formulas carried out on 10 respondents. If each criterion in the acceptability aspect is summed based on the desired acceptability criteria (scores 3 and 4) and ignores unacceptable criteria (scores 1 and 2), it could be concluded that Formula III (beeswax-oleum cacao 25:75) had better acceptability than Formula II (beeswax-oleum cacao 50:50)

Discussion

In the NLC_AMS oil formula that used a single lipid, either beeswax or oleum cacao did not show good activity, it was very clear on the first seven days. This could be due to the use of single beeswax which was too crystalline caused the NLC to be too rigid, while the use of single cacao oleum had a very loose NLC structure.[19] It caused the entrapment of the active ingredients to not be optimal, thus affected the release of active ingredients and their effectiveness.

From Figure 2, which correlates particle size with activity, it was known that the small particle sizes of FII and FIII provided good activity. There was no significant difference between FII and FIII on particle size. The small size of NLC allows NLC-AMS oil to enter the deeper layers of the skin through all penetration pathways.[16] In addition, the smaller particle size caused higher active ingredient to be loaded.

Theoretically, the effect of viscosity on activity is related to the ease of active ingredients released from the base. Low viscosity indicates low flow resistance, making it easier for the active ingredient to release from the base.[22] However, this study showed the opposite result. It could be caused by the increasing viscosity that enlarged the occlusive properties of the preparation. This caused the skin hydration due to the retention of skin water on the surface of the stratum corneum. This hydration provided a spongy effect to the stratum corneum cells and facilitated penetration of the active ingredients.

FIII had better acceptability than FII (beeswax-oleum cacao 50:50), especially in ease of smearing. It was because FIII had more oleum cacao that had smoother consistency than beeswax.

The results of this study can be used to develop AMS oil formulas into cosmetic preparations that are acceptable and effective for hair growth.

Conclusions

Based on the results of the study, it can be concluded that all

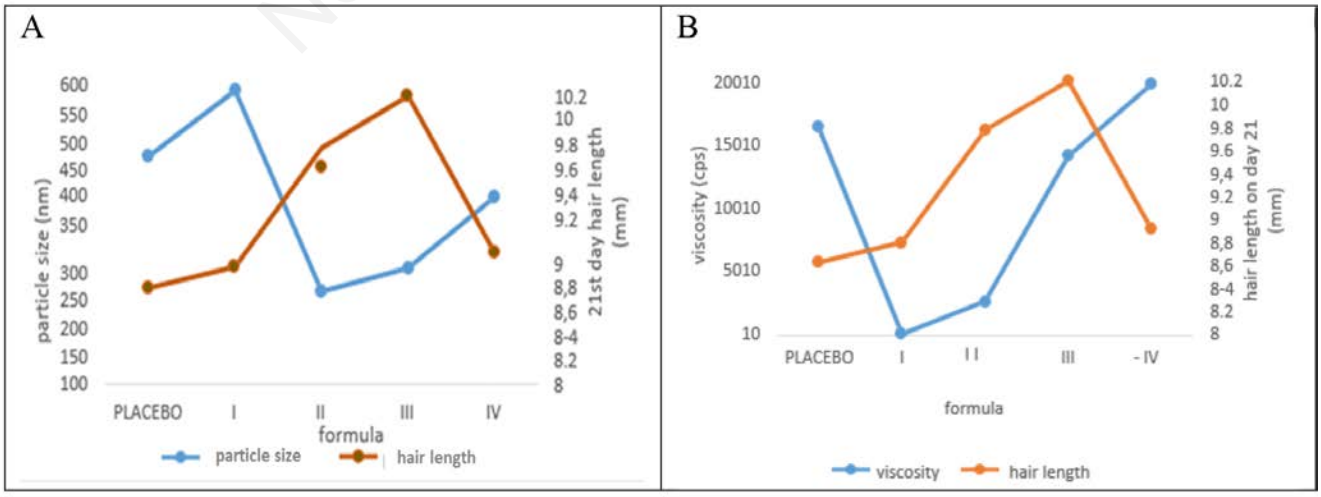

Figure 2. Graph of particle size A) and viscosity B) relationship with activation test on day 21st of each NLC formula.

NLC-AMS formulas were effective as hair growth agents, but the use of a combination of solid lipid: beeswax-oleum cacao produced better characteristics and higher hair growth activity compared to formulas using a single fat. NLC-AMS oil with a combination of solid lipids beeswax:oleum cacao 25:75 had the same effectiveness as NLC-AMS oil with a combination of lipids beeswax:oleum cacao 50:50, but had better acceptability.

References

1. Soepardiman L, Legiawati L. Hair Abnormalities. Seventh edition of Science of Skin Diseases and Gender. FKUI Issuing Agency, Jakarta 2015.
2. Satriyo A, Legiawati L, Agustin T, Sugito TL. Anagen effluvium. MDVI. 2011;38:41-48.
3. Li Y, Han M, Lin P, et al. Hair growth promotion activity and its mechanism of Polygonum multiflorum. Evid Based Complement Alternat Med 2015;2015:1-11.
4. Krisnawati H, Kallio M, Kanninen M. Aleurites moluccana (L.) Willd. Ecology, silviculture and productivity. CIFOR, Bogor 2011.
5. Erawati TM, Asha Denara P, Maharani SA, et al. Widji. Characteristics and Stability of Nanostructured Lipid Carrier (NLC) Aleurites Moluccana Seed Oil (AMS oil) Using Various Combinations of Beeswax and Oleum Cacao. Int J Drug Deliv Technol 2019; 9; 94-97.
6. Wulansari EDE, Masruriati DD. Rabbit Hair Growth Test with Celery Extract Cream, AMS Oil Cream, Celery Extract Cream - AMS Oil and Physical Testing of Preparations. MPI 2013;2013:186-192.
7. Prasojo, Mulyani, Mufrod. Effect Of Storage Length On Physical And Chemical Stability Of Hair Growth Lotion Containing Candlenut Extract (Aleurites Moluccana L. Willd.). Majalah Obat Tradisional 2012; 17:1–7.
8. Martín C, Andres M, Giraldo M, Eugenio C. Fractional characterisation of jatropha, neem, moringa, trisperm, castor and candlenut seeds as potential feedstocks for biodiesel production in Cuba. Biomass Bioenergy 2010;34:533-538.
9. Jaffar.The effectiveness of coconut oil mixed with herbs to promote hair growth. Iran J Electr Electron Eng 2014;1:1-4.
10. Arlene A. Extraction of AMS Oil by Soxhlet Method and Characterization of AMS Oil. Chem Eng J 2013; 2:6–10.
11. Pasaribu VD, Rohanah, A. Ichwan N. Designing Threaded Oil Forging Tools. myJAFE 2014;2:145-150.
12. Montenegro L. Lipid-Based Nanoparticles as Carriers for Dermal Delivery of Antioxidants. Curr Drug Metab 2017;18:469-480
13. Puri A, Loomis K, Smith B, et al. Lipid-based nanoparticles as pharmaceutical drug carriers: from concepts to clinics. Crit Rev Ther Drug Carr Syst 2009;26:523-580.
14. Rosen J, Landriscina A, Friedman A. Nanotechnology-Based Cosmetics for Hair Care. Cosmetics 2015; 2:211-224.
15. Müller RH, Petersen RD, Hommoss A, Pardeike J. Nanostructured lipid carriers (NLC) in cosmetic and dermal products. Adv. Drug Deliv. Rev 2007;59:522-30.
16. Tichota, Silva. Design, characterization, and clinical evaluation of argan oil nanostructured lipid carriers to improve skin hydration. Int J Nanomedicine 2014;9:3855-64.
17. Kaur S, Nautiyal U, Singh R, Singh S. Nanostructure Lipid Carrier (NLC): the new generation of lipid nanoparticles. Asian PAC. J. Health Sci 2015; 2:76-93.
18. Krasodomska O, Paolicelli P, Cesa S, et al. Protection and viability of fruit seeds oils by nanostructured lipid carriers (NLC) nanosuspensions. J. Colloid Interface Sci 2016;479:25-33.
19. Rosita N, Setyawan D, Soeratri W, Mrtodihardjo S. Physical Characterization Of Beeswax And Glyceryl Monostearate Binary System To Predict Characteristics Of Solid Lipid Nanoparticle (SLN) Loaded. Int J Pharm Pharm Sci 2014;6:939-45.
20. Indiwinani. White Rats Hair Growth Activity, Physical Stability and Safety Test of Hibiscus Leaves Extract Gel (Hibiscus tiliaceus Linn). Universitas Gadjah Mada, Yogyakarta; 2007.
21. Yoon JI, Al-Reza SM, Kang SC. Hair growth promoting effect of Zizyphus jujuba essential oil. Food Chem Toxicol 2010;48:1350-4.
22. Avramov I. Relationship between Diffusion, Self-Diffusion and Viscosity. J Non-Cryst Solids 2009;355:745-7.