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Original Research Article

Enhancing Hair Follicle Stimulation: A Nanoliposome-Based Delivery of Lavender Essential Oil (*Lavandula angustifolia* Mill.)

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ABSTRACT

Lavender (*Lavandula angustifolia* Mill.), a popular garden herb widely cultivated for its essential oil (EO), has long been used traditionally for its therapeutic benefits. The use of Lavender EO (LEO) has already been proven to have hair growth stimulating activity. However, traditional use of LEO often has low bioavailability, making them less effective. This study aims to develop a novel nanoliposome-based delivery system of LEO, enchancing its delivery to accelerate hair growth. Nanoliposomes were prepared by a combination of LEO, Lipoid Phospholipon 80 H, and Lipoid PhytoSolve 4021 using thin layer hydration method. The resulting LEO-Nanoliposomes (LEON) had a slightly fatty thick consistency, distinctive lavender aroma, homogenous milky yellow color, as well as morphology of single layer spherical shape without aggregation as observed through Transmission Electron Microscope (TEM). Particle Size Analyzer (PSA) results was 83.0 nm ± 3.2 in size with Polydispersity Index (PDI) value of 0.4 PDI ± 0.0 and Zeta Potential (ZP) value of -14.4 mV ± 0.3. The LEON gel preparation met the acceptability requirements for pH, viscosity, spread ability, adhesiveness, and microbial contamination tests. Irritation and hair growth effectiveness tests were carried out on male white rats of the Sprague Dawley strain for 1 month where it showed no irritating effects in topical use. Statistical analysis using One-Way Anova and post-Hoc test resulted in LEON gel exhibiting significant hair growth length higher than that in the other groups ($p<0.0001$). These findings indicated that LEON has improved effectiveness compared to traditional use of EO.

***Keywords*:** Lavender, Nanoliposome, Hair growth, Effectiveness.

Introduction

Hair is a crown of pride for women and men, providing warmth, protection, and support for appearance.[1] The growth cycle of hair consists of three phases: the anagen phase (growth), the catagen phase (rest), and the telogen phase (hair loss).[2] In a day, hair can lose 50-100 strands,[3] due to various factors, such as genetics, age, hormones, nutrition, illness, stress, and inappropriate hair care.[4,5]
Meanwhile, EO are oils derived from plants, and their physical properties include being liquid and volatile at room temperatures.[6] One of the most effective EO for accelerating hair growth is lavender. Lavender (*Lavandula angustifolia* Mill.) is an ingredient that can be used to increase hair growth and reduce hair loss, with the chemical content in the form of linalool and linalyl acetate.[7] These compounds can help improve blood circulation in the scalp, thus bringing more oxygen and nutrients to the hair follicles which can support better hair growth.[8] LEO has aromatherapy properties and is good potential as an antifungal, antimicrobial, antibacterial, and antioxidant compound beneficial for hair growth.[9,10] However, the potential of LEO as a therapeutic agent when applied directly as a topical preparation cannot be optimally utilized because EO are lipophilic, which means that they are difficult to dissolve in water, thus leading to low bioavailability

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On the other hand, EO has a disadvantage of being volatile and easily decomposed. This happens because the barrier system on the skin membrane is difficult to penetrate,[11] which therefore requires an effective transport system to increase the stability and bioavailability of LEO. Nanoliposomes include a transport system that can improve the stability, biodistribution, and bioavailability of LEO.[12] Liposomes in cosmetic preparations are helpful to facilitate the penetration of the active substance in the preparations into the skin. Therefore, this study modified the development of topical preparation using a nanoliposome delivery system to increase penetration into the skin. Nanoliposomes have previously been studied and developed as a hair treatment,[13] while LEO has been proven to be effective in promoting hair growth in C57BL/6 mice.[14] However, combining the two to strengthen the hair growth effect has never been reported. The problem of hair loss and thinning remains a global challenge, and conventional therapies as well as natural products have not provided effective solutions. For this reason, the researchers are interested in developing nanoliposome technology to increase the penetration of LEO, which has not been widely studied in the context of hair growth. Nanoliposomes are expected to improve the effectiveness of LEO for stimulating hair growth. This study used Sprague-Dawley male white rats as test animals to evaluate the effectiveness of LEON gel topical preparation as an innovation in hair care therapy.

Materials and Methods

Materials

The LEO used in this study was obtained from Young Living® Essential Oils, Made in USA (Batch Number 357517V1, LOT 135339, Manufacturing Date 10.2023, Expiry Date 10.2026). Other materials used are including Phospholipon® 80H (Lipoid GmbH, Germany), PhytoSolve® 4021 (Lipoid GmbH, Germany), Methyl Paraben (UENO Fine Chemical Industry Ltd., Japan), Propyl Paraben (UENO Fine

Chemical Industry Ltd., Japan), Propylene glycol (Brataco, Indonesia), Chloroform (Merck, Germany), Methanol (Merck, Germany), Tween 20 (Merck, Germany), Phosphate-Buffered Saline (PBS) (Oxoid Ltd., United Kingdom) pH 7.4, Carbomer 940 (Spectrum Chemical Mfg. Corp., New Jersey), Triethanolamine (TEA) (Petronas, Malaysia), Natur Hair Tonic Ginseng™ (Gondowangi Tradisional Kosmetika, Indonesia), Plate Count Agar (PCA) medium (Oxoid Ltd., United Kingdom), Peptone Water (Oxoid Ltd., United Kingdom), Water For Injection (WFI) (Otsuka, Indonesia), Ketamine (Troy Laboratories PTY Ltd., Australia), Xylazine (Interchemie Werken "De Adelaar" B.V., Holland), and Aquadest (Pharmaceutical Technology Laboratory, Pharmacy Program of Indonesia Islamic University, Indonesia). This study used Sprague-Dawley male white rats with the inclusion criteria: aged 2-3 months with an average weight of 200-300 grams, healthy and active.

Preparation of Lavender Essential Oil Nanoliposomes

The nanoliposome preparation process used the thin-layer hydration method. The preparation was carried out by mixing Phospholipon® 80 H, PhytoSolve® 4021, methanol, and chloroform until homogeneous for 1-3 minutes using a vortex mixer (type 37600 mixer, Barnstead/Thermolyne, United States), followed by evaporation of the solvent using a rotary evaporator (Heidolph Hei-VAP Precision, Heidolph Instrument GmbH & Co., Germany) at 50°C with a pressure of 200 bar at a speed of 125 rpm to form a thin layer on the surface of the round bottom flask and a thick phase.[12] After that, 300 mg of lavender essential oil, tween 20, and PBS pH 7.4 were added to the round bottom flask. The sample was again subjected to a rotary evaporator for 30-60 minutes with the exact mechanism without being conditioned using a vacuum. Afterwards the liposome size was reduced by using an ultrasonic homogenizer (model 300 V/T, BioLogics Inc, United States) for three cycles with a power of 50 for 3 minutes and a pulse of 70.[12,14] The nanoliposome formulation can be seen in Table 1.

Preparation of Lavender Essential Oil Nanoliposomal Gel

The preparation of LEON gel base began with mixing carbomer 940 into aquadest until it expanded, and then dissolving methyl and propyl parabens with pre-warmed propylene glycol until homogeneous. After that, all the ingredients were mixed and homogenized using a homogenizer (T 25 Digital ULTRA-TURRAX®, IKA, Malaysia) to form a gel. When homogeneous, LEON and TEA are added gradually until a gel with the desired consistency is formed. The LEON gel formulation can be seen in Table 2.

Evaluation of the Physical Characteristics of Lavender Essential Oil Nanoliposomes

The determination of the particle size, PDI, and ZP was done using PSA (Nano Particle Analyzer SZ-100, Horiba Ltd., Japan). The samples were taken as much as 1 mL and then diluted into 10 mL of water for injection and put into a cuvette; then, the cuvette containing the samples was put into a holder for particle size and ZP measurements. The morphology testing was carried out by using a TEM (model JEM-1400, JEOL Ltd., Japan). A total of 5 mL of samples was placed at the grid and absorbed using filter paper with the help of a vacuum and then observed.[12,15]

Evaluation of the Physical Characteristics of Lavender Essential Oil Nanoliposomal Gel

The organoleptic tests were carried out physically through the senses of sight and smell for color, shape, and odor of the preparation.[16] Furthermore, the homogeneity test was applied to 1 mg of the sample placed evenly on one object glass and covered with another followed by observation of the presence or absence of coarse grains.[17] The pH was determined by using a digital pH meter (LAQUA PH2000, Horiba Ltd., Japan), which was previously calibrated with a standard solution of pH 4 buffer standard (acid), pH 10 buffer standard (base), and pH 7 buffer standard (neutral). The electrode was then dipped in the sample until the device showed the pH value of the preparation.[18] The viscosity testing was done using a Brookfield DV-1 Prime Viscometer (Brookfield Engineering Laboratories Inc., United States) with spindle number S63. The spindle was dipped into 100 mL of preparation in a beaker glass at a speed of 30 rpm.[19] The spread ability test was done by weighing 0.5 g of nanoliposomal gel preparation, placing it between two object glasses and giving a weight of 0 to 2 kg. It was allowed to stand for 1 minute, and then the diameter of the spread area was recorded. The adhesion testing was done by applying 0.5 g of nanoliposomal gel preparation on one object glass, which was then covered with another. Next, a load weighing 500 g was placed on top and allowed to stand for 5 minutes. The object glass was then placed at the test device and a load weighing 100 g was released, while the time required to release the object glass was noted.[17]

Table 1: Lavender essential oil nanoliposome formulation

	Material	Quantity	Function
1.	Lavender essential oil	300 mg	Active substance
2.	Phospholipon® 80 H	362 mg	Phospholipid
3.	PhytoSolve® 4021	500 mg	Phospholipid
4.	Chloroform	5 mL	Solvent
5.	Methanol	5 mL	Solvent
6.	Tween 20	1 g	Flexibility enhancer
7.	PBS	19 mL	Buffer solution

Table 2: Lavender essential oil nanoliposomal gel formulation

	Material	Quantity		Function
		F1	F2	
1.	LEON	0 mL	20 mL	Active substance
2.	Carbopol 940	500 mg	500 mg	Gelling agent
3.	Methyl paraben	20 mg	20 mg	Preservative
4.	Propyl paraben	20 mg	20 mg	Preservative
5.	Propylene glycol	10 mL	10mL	Humectant
6.	TEA	q.s	q.s	Alkalizing agent
7.	Aquadest	ad 100 mL	ad 100 mL	Solvent

Description:
F1: Formulation without LEON (gel base only)
F2: Formulation with LEON

Microbial Contamination Test

Using the Total Plate Count method, the test began with sterilizing the test tubes, Erlenmeyer flasks, and Petri dishes by using an autoclave at 121°C for 15 minutes. Then, the Plate Count Agar was weighed as much as 5.6 g, dissolved in 1 L of distilled water, and sterilized for ± 2 hours. After that, the agar medium was molded into a petri dish. The materials and dilution were put in Laminar Air Flow to avoid contamination. Next, the sample was weighed for 10 g, poured into a sterile bag and then 90 mL of peptone water was added and homogenized for ± 1 minute. A total of 1 mL of sample was taken from the 10^{-1} dilution and put into 10 mL of peptone water to obtain 10^{-2} dilution, and the same steps was performed until 10^{-3}, 10^{-4}, and 10^{-5} dilutions. Then, 1000 µL of solution was taken from each dilution and transferred to the agar medium. The samples were spread using a sterilized spreader. Then, they were allowed to solidify, wrapped using parchment paper, and incubated at 35-37°C for 24 hours. The number of growing colonies was then counted.[5]

Preparation of Animal Tests

Before the study was conducted, the ethical clearance was obtained to ensure that the test animals were treated according to the applicable ethics, which are based on the seven 2011 WHO standards, and was granted to this research through an approval letter No.159/KEP-PKU/X/2023 by research ethics committee of PKU Muhammadiyah Gamping Hospital. To prepare the rats for the research, they were acclimatized before testing to ensure they were accustomed to living in a new environment and the treatment given during the study. The acclimatization was carried out for ± 7 days at a laboratory room temperature of ± 20-25°C, 30-70% humidity, 12-hour light cycle, and 12-hour dark cycle. The rats were placed in a plastic box of 60 cm x 40 cm x 20 cm with a wire lid and rice husks as the base. The rats were fed twice a day and given drinks ad libitum, and the husks were replaced every three days.

Irritation Test

The irritation test was carried out on 15 Sprague-Dawley male white rats. The treatment of the research subjects was performed by applying 0.5 g of each preparation, which was then covered with sterile gauze and plaster, then the symptoms were observed after 24 hours.[20]

Hair Growth Activity Test

The Number of test animals used in the study was determined based on the Federer formula,[21] which resulted in 6 test animals that were used in 1 group, and the total number of test animals used in the hair growth test was 30 rats for 5 treatment groups. The rats were taken randomly (simple random sampling), and they were divided into the standard control group (without treatment), base control (gel base), T1 (Natur Hair Tonic Ginseng™), T2 (LEO gel), and T3 (LEON gel).

Before being shaved, the rats were anesthetized using a combination injection of ketamine 0.2 mL and xylazine 0.02 mL taken using a syringe and injected into the thigh of the rats and waited until they passed out. The shearing was performed on the rat's back with an area of 2.5 cm x 2.5 cm. After that, each rat's back was shaved using scissors, and fine hair was cleaned using a razor blade until it was cleaned.[22] The hair growth activity test was carried out on the research subjects consisting of 30 male Sprague-Dawley white rats. The treatment was performed by applying 0.5 g of each preparation twice daily every 12 hours. Then, the hair was plucked every week for one month. The growing hair was plucked on different sides for approximately ten strands. Then, the length and thickness of the plucked hair was observed on the thickest side. This observation was done using an electron microscope (ZEISS SteREO Discovery.V8, Carl Zeiss AG., Germany).[23]

Statistical Analysis

The results obtained from the characterization of the LEON and evaluation of the LEON gel preparation were analyzed qualitatively to identify the characteristics of the formulation that had been made and compare the results with previous research journals, then quantitative analysis was carried out by processing the observation data using the Microsoft Excel (Microsoft Office 365, version 2501, 2025) software to find the average results and standard deviation (SD) from the test results.

Besides that, the results obtained in the irritation test and hair growth activity test were analyzed quantitatively. In the irritation test, the data obtained were then analyzed to determine the Primary Irritation Index (PII), followed by scoring of the erythema and edema based on rat's skin severity. In addition, data from the hair growth activity test were analyzed statistically using the One-Way ANOVA method and continued with the post-Hoc test (IBM, SPSS Statistics 26, 2024) to determine the significance of the differences in the length and width of the hair shaft that grew in each treatment group.

Results and Discussion

Physical Characteristics Evaluation of the Prepared Lavender Essential Oil Nanoliposome

The nanoliposome particle was analysed by using PSA to determine the size and particle size distribution of the nanoliposomes formed. The particle size test, as can be seen in Table 3., shown promising results of 83.0 ± 3.2 nm in size, which is within nanoliposome size range of 10-200 nm.[24] The nanoliposome formation was carried out from the basic raw materials by gradually building the structure based on the bottom-up technique, which involved the construction of vesicles to form a liposome structure as a protection base and a carrier that encapsulated LEO. The particle size distribution of LEON can be seen in Figure 1A., which illustrates that the LEON preparation produced has a uniform particle size and even particle distribution. The even distribution of particles is confirmed by the particle strain, which can be seen from the start of peak formation from each test replication with good strain. This result was also confirmed by the PDI value of 0.4 ± 0.003 Table 3. A PDI represents particle size distribution, which value is between 0 (for a sample with a very uniform particle size) and 1 (for a highly polydisperse sample with a large population of particle sizes).[12]

The ZP test value of LEON was performed to measure the electrical charge on the surface of particles in suspension, and this value is crucial in determining the stability of colloidal systems. The ZP value obtained was -14.4 mV ± 0.3, indicating surface charge characteristics and stability of the liposomes. A negative ZP value suggests that the nanoliposomes have a negative charge on their surface. Typically, ZP values outside the range of -30 mV to +30 mV indicate good stability in suspension. However, the obtained ZP value of -14.4 mV ± 0.3 may be because the formulation involves a mixture of various lipids; the less charged lipids may reduce the total ZP value.[12]

The TEM observations were made to investigate the morphology and distribution of the nanoliposomes. As shown in Figure 1B, the observed globules were spherical, and no aggregation was observed. These results confirmed the shape of LEON as a small unilamellar vesicle type with a size range of 20-200 nm. This was also strengthened by the particle size result obtained, 83.0 nm ± 3.2, for the monolayer spherical shape.[25]

Table 3: Particle size, PDI, and ZP test results

	Particle Size (nm)	PDI	ZP (mV)
Test Results*	83.0 ± 3.2	0.4 ± 0.003	-14.4 ± 0.3

Description: *Average result of 3 replications ± SD

Evaluation of the Physical Characteristics of Lavender Essential Oil Nanoliposomal Gel

The organoleptic test of the preparation of LEON gel is a milky yellow color with a distinctive lavender odor, slightly fat, and somewhat dense texture. There was no phase separation or uneven color after the preparation was stored for 24 hours as shown in Figure 2. The homogeneity testing obtained homogeneous LEON gel with the observations showing no clumpy or uneven gelling in the preparation.

The pH value obtained was 5.76 ± 0.05, indicating that the pH of the LEON gel formula falls into the acceptable human scalp pH range of 4.5-6.5.[26] In this pH range, the LEON gel preparation is expected to not irritate the skin, which causes discomfort when used.[27] Meanwhile, the viscosity value obtained was 3988 cP ± 6.11. A good viscosity value of gel-based preparation is between 2,000-4,000 cP.[28] The viscosity of gel-based preparations usually follows the concentration of the gelling agents and other ingredients such as TEA. The higher the concentration of the gelling agent and TEA, the higher the viscosity value.[29] The spread ability test results in Table 4 show that the spread ability obtained ranged from 5 to 12 cm. The greater the spread ability obtained, the wider the ability of the active substance to spread and contact with the skin to provide maximum effect;[30] when applied to the skin surface, it can affect the absorption and release of active substances

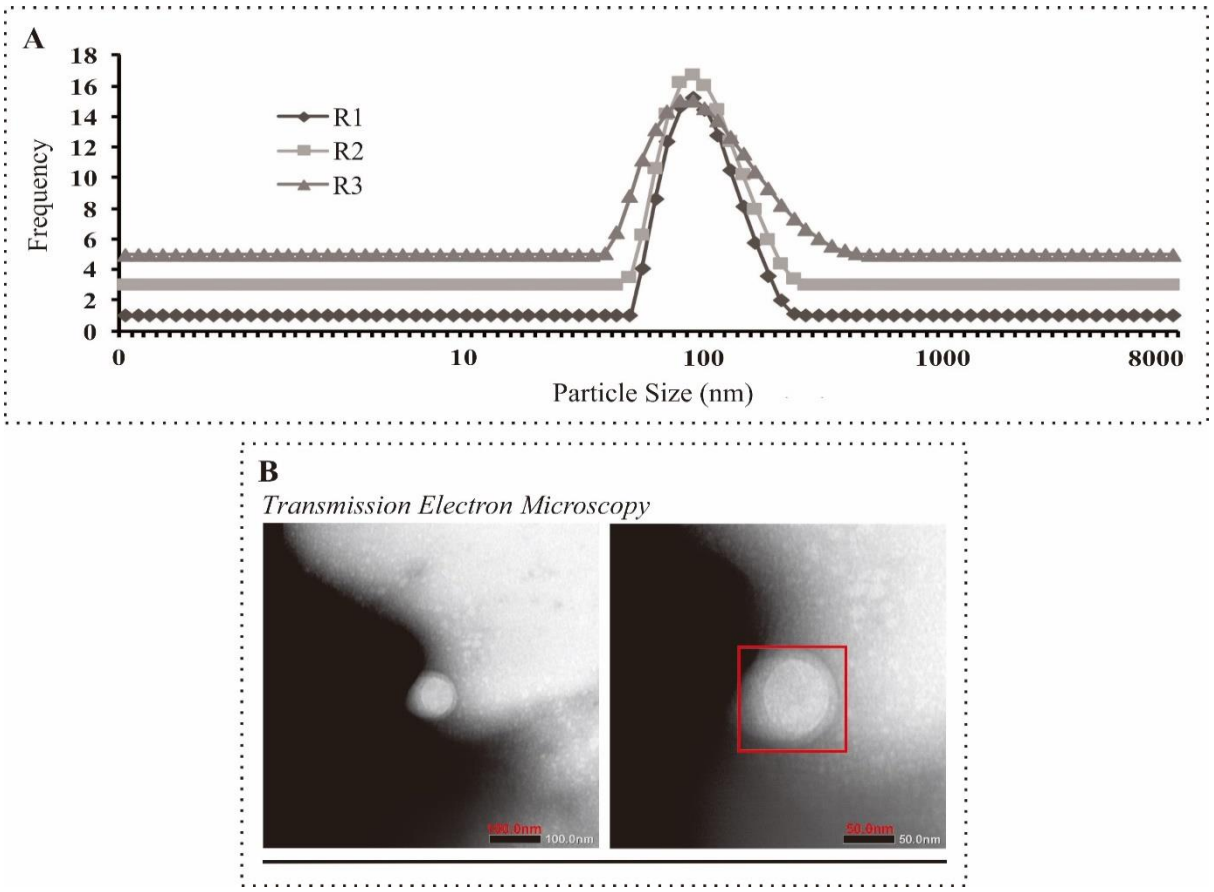

Figure 1: Evaluation results of the physical characteristics of LEON. **(A)** Particle size distribution graph of three readings, shown uniform and even particle size distribution; **(B)** Morphology test of the preparations observed by using TEM at 50 to 100 times magnification, resulted shown spherical globules without aggregation observed.

Description:

R1: Replication 1; R2: Replication 2; R3: Replication 3

and can ensure user comfort.[31] The adhesion test resulted in 1.81 seconds, showing that the nanoliposomal gel preparation has met the requirements of more than 1 second of adhesion. These results indicate that the nanoliposomal gel preparation can adhere well to the skin. Adhesion is directly proportional to viscosity; the higher the viscosity, the higher the ability to adhere. Adhesion that is too strong will block the skin pores, while adhesion that is too weak will reduce the effectiveness of the active substance.[29]

Microbial Contamination Test of Lavender Essential Oil Nanoliposomal Gel

The number of colonies obtained was 1-7 colonies throughout all the dilution as shown in Figure 3., while it should meet the calculation rules. The Total Plate Number can be calculated and analyzed when the number of colonies ranges between 30-300 colonies. This is because agar media with more than 300 colonies is not valid for calculation. Therefore, the possibility of miscalculation is enormous, whereas if the number of colonies produced is less than 30, it is not valid when calculated statistically.[32] Therefore, the resulting preparation is free from contamination, this is because the preparation already contains formulated antimicrobials.

Lavender Essential Oil Nanoliposomal Gel Irritation Test

The results of the irritation test of lavender essential oil nanoliposomal gel preparations based on the PII value can be seen in Table 5. Based on these results, both the control and treatment groups did not cause irritation as shown in Figure 4., characterized by the absence of erythema and edema on the skin of rats, making it safe to use. This is likely because the pH of the nanoliposomal gel preparation matched the acidity of the skin, thus preventing its use from being irritating. The ingredients of the LEON gel formulation are within the appropriate range determined in the Handbook of Pharmaceutical Excipients guidelines.[23]

Figure 2: LEON gel serum in the same formula, no separation/uneven color observed

Hair Growth Activity Test

The hair growth activity test results are shown in the rat hair graph in Figure 5A. The observation in the first week showed no significant hair length growth in all the groups compared to the standard group without treatment.

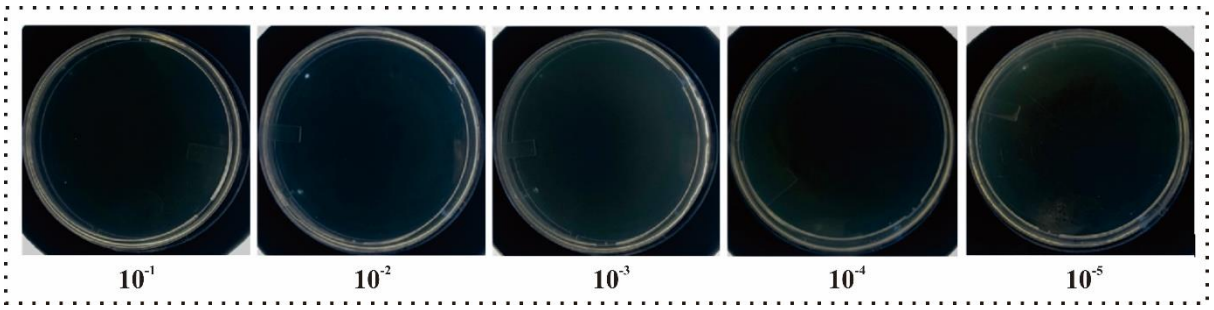

Figure 3: Total Plate Number Test Results. Dilution 10^{-1} with colony count = 7, 10^{-2} with colony count = 1, 10^{-3} with colony count = 1, 10^{-4} with colony count = 1, 10^{-5} with colony count = 0

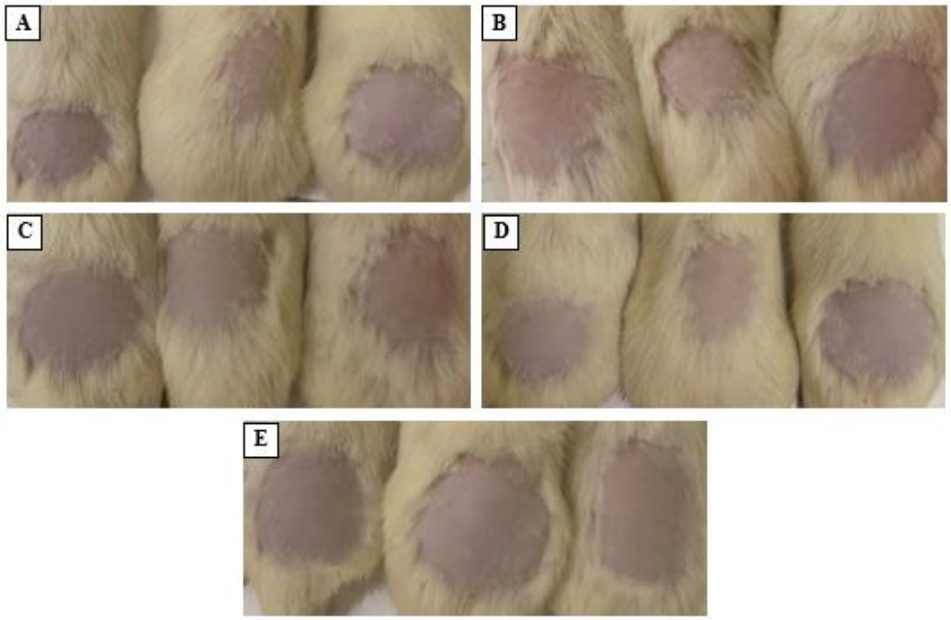

Figure 4: Observation after irritation test treatment which shown no irritation effect in all groups: (A) Normal control (no treatment); (B) Base control (gel base only); (C) Positive control (Natur Hair Tonic Ginseng™); (D) Treatment 1 (LEO gel); (E) Treatment 2 (LEON gel).

The growth of rat hair width also showed no significant differences in the first week from the negative control group (gel base only), positive control (Natur Hair Tonic Ginseng™), and treatment group compared to the standard control group.

Table 4: Results of the Spreadability Test

Addition of Load	Spread ability	Weight of Covered Glass
0	5.3 cm	165.140 g
50 g	6.2 cm	
100 g	7.2 cm	
150 g	8.3 cm	
200 g	9.7 cm	
500 g	10.4 cm	
1 kg	11.1 cm	
2 kg	12.4 cm	

The Treatment Group 2 showed a significant difference in the hair fur width growth, with $p<0.01$, compared to the standard control group shown in Figure 5B. This is because the nanoliposomal gel delivery system is proven to accelerate hair growth. The small size of nanoliposomes can increase the surface area, which will increase solubility and allow good penetration into the scalp, thus enabling that LEO to reach its target better than conventional formulations.[33]

The observation results in the second and third weeks showed no significant difference in the growth of rat hair length from the negative control group (gel base only), positive control group (Natur Hair Tonic Ginseng™), and treatment group when compared to the standard control group without treatment. The Treatment Group 2 showed a significant difference in the hair length growth $p<0.0001$ compared to the standard control group in Figure 5A. At the same time, the results of the growth of rat hair width in the second and third weeks showed no significant growth in all the groups compared to the standard group without treatment shown in Figure 5B. This is likely because the growth of hair width in rat does not last forever. After reaching a particular stage, hair growth will stop, and the hair will reach the maximum length and width due to genetics and other factors, such as hair growth cycle, environmental, nutritional health, and rat's age.[34,35]

The observations in week four showed that the growth of rat hair length in the negative control group (gel base only) was significantly different $p<0.05$ compared to the standard control group without treatment in Figure 5A. The negative control group was treated using a gel base only, which might not have ingredients to stimulate hair growth. Therefore, the group that was only given the gel base have not experienced significant changes in hair growth. However, there may be natural variability in this case, whereas in a group that does not receive special treatment, there will be natural variation in each rat's hair growth. Some rats can experience faster growth than others, leading to different results.

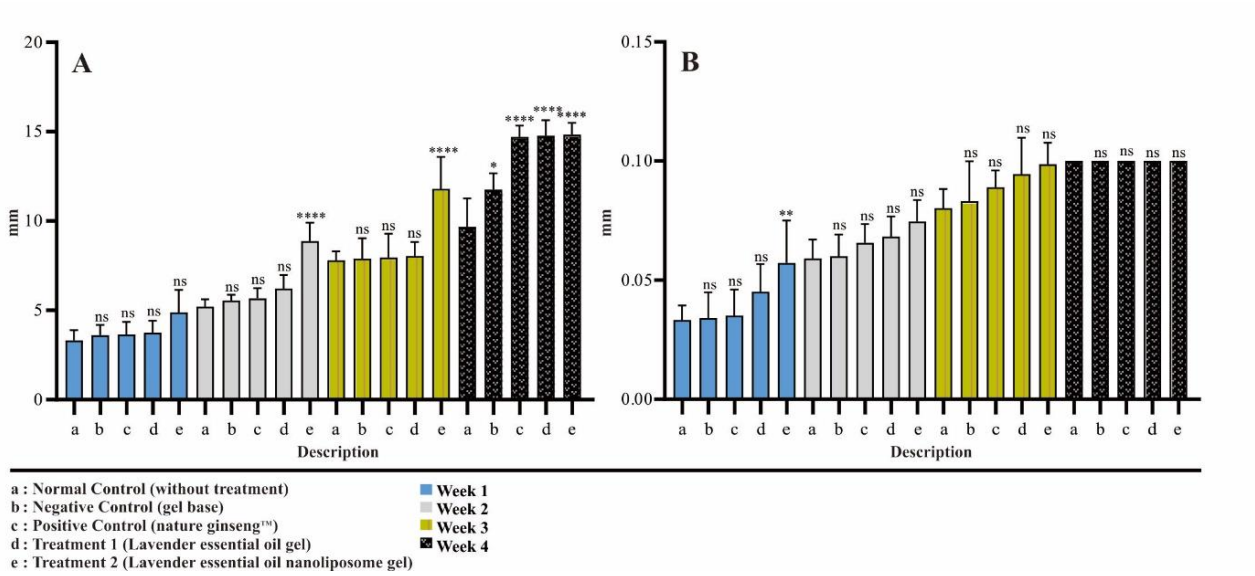

Figure 5: Graph of rat hair growth activity test. **A:** Rat hair length growth chart, **B:** Rat hair width growth chart. The samples were analyzed by using One-Way ANOVA with 95% confidence and a post-Hoc test to determine significant differences in each group. Description:
n: 5; ns: not significant; *: p<0.05; **: p<0.01; ***: p<0.001; ****: p<0.0001

Table 5: Irritation test results

Treatment	Erythema Value			Edema Value		
	TR1	TR2	TR3	TR1	TR2	TR3
Normal control (no treatment)	0	0	0	0	0	0
Base control (gel base only)	0	0	0	0	0	0
Positive control (Natur Hair Tonic Ginseng™)	0	0	0	0	0	0
T1 (LEO gel)	0	0	0	0	0	0
T2 (LEON gel)	0	0	0	0	0	0
Total		**0**			**0**	
***Primary Irritation Index* (PII)**			**0**			

Description: TR1: Test rat 1; TR2: Test rat 2; TR3: Test rat 3

At the same time, the results in the positive control group showed a significant difference (p<0.0001) as shown in Figure 5A, when compared to the standard control group without treatment. The positive control group was treated with Nature Hair Tonic Ginseng™, a preparation available in Indonesia market, with active ingredients that can activity to accelerate hair growth. From these results, Natur Hair Tonic Ginseng™ showed a significant difference compared to the standard control and base control groups in terms of the hair length growth evaluated. The Treatment Group 1 showed a significant difference (p<0.0001) as shown in Figure 5A, compared to the standard control group because the Treatment Group 1 was given LEO gel. This shows that LEO has active compounds that function to accelerate hair growth. This is confirmed by Batiha et al., who state that lavender essential oil has linalool and linalyl acetate chemical content, which is productive in accelerating hair growth by helping increase scalp blood circulation.[8] The increased blood circulation will bring more oxygen and nutrients to the hair follicles, thus supporting better hair growth. This also shows a significant difference compared to the favorable treatment group. Figure 5A shows that the hair growth accelerating agent of Treatment Group 1 is better than that of the positive control group. Meanwhile, the results for Treatment Group 2 showed a significant difference (p<0.0001) compared to the standard control group because the Treatment Group 2 was treated with LEON gel. Figure 5A also shows a significant difference between the positive control and treatment groups. This indicates that the nanoliposome delivery base is better than the conventional one for topical drug use, indicating that the physical form of liposomal nanoparticles has excellent penetration ability. Therefore, there is a positive correlation in the delivery technique using a nanoliposome base, which results in faster growth than conventional delivery. These results indicate that nanoparticle encapsulation of drug preparation can indirectly affect the penetration speed at the drug preparation into penetrate the membrane barrier.[36] This is because nanoliposomes are useful as a carriers of LEO with flexible properties to control and deliver the drug to penetrate the membrane barrier, especially in the challenge of passing through the stratum corneum into the dermis to the circulatory system or through the route into the hair follicles.[37]

Therefore, LEON gel accelerates the growth of rat hair length, whereas, in the second week, there was significant growth in the hair length until the fourth week. This is also proven by Lee et al., in a study of female rat test animals that compares lavender oil at concentrations of 3% and 5%, with a positive control in the form of 3% minoxidil, and a standard group in the form of Saline.[14] The study reports that LEO has shown to have an effect as a hair grower compared to the standard control group observed morphologically and histologically. When modified in the form of nanoliposomal gel, it has been shown to the ability to further accelerate hair growth. The small nanoliposome size can increase the surface area, thus increasing solubility and allowing good penetration

into the scalp, enabling the essential oil to reach its target better than conventional formulations.[38]
The results of the growth of rat hair width in the fourth week in each group showed no significant difference between the standard control group without treatment and the negative control group (gel base only), positive control group, Treatment Group 1 and Treatment Group 2 (Figure 5B.). This is likely because lavender only has the activity to accelerate the growth of rat hair length, but not to accelerate the growth of rat hair width. In addition, the growth of hair width in rats does not last forever. After reaching a particular stage, hair growth will stop, and the hair will reach the maximum length and width based on genetics and other factors, such as the hair growth cycle, environmental, nutritional health, and rat's age.[34,35] Therefore, a significant difference in hair width is complicated to observe.

Conclusion

The LEON produced in this study met the requirements of good physical characteristics of nanoliposome preparations with a single layer spherical morphology without any aggregation with a particle size of 83.0 nm ± 3.2, categorized as a small unilamellar vesicle. The resulting preparation has a slightly fatty thick consistency, a distinctive lavender aroma, a milky yellow color, and good homogeneity. In addition, the LEON gel has fulfilled the acceptability requirements, including the pH, viscosity, spread ability, adhesion, and microbial contamination. It does not show irritating effects for topical use, while also shows graphical representation of the resulted data correlates the time per week with the accelerated growth in the rat hair length and width that is higher than in the other groups. The promising outcomes of this research on nanoliposome-encapsulated lavender essential oil gel underscore its significant potential in therapeutic and dermatological applications. The improved stability and enhanced activity of LEO through nanoliposome delivery systems demonstrate clear advantages over conventional formulations. The gel's efficacy in promoting hair growth without irritating effect is a valuable candidate for further clinical development.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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