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RESEARCH ARTICLE

Encapsulation of Progesterone-Like Compounds in 10% Liposome Increases Their Concentration in Rats Administered an Injectable Dosage Form of These Compounds

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Abstract

The use of herbal medicine to fill the void in synthetic medicine is very necessary for health lifestyle. Progesterone-like compounds (PLCs) from the extract of the leaves of *Dendrophthoe pentandra* L. *Miq* from the subspecies, *Benalu Duku* (BD), are known to contain beneficial compounds that contain anti-cancer and androgenic substances. The slow release of active compounds can be achieved using slow release vehicles. Liposomes that are small unilamellar vesicles (SUVs) are one of the excipient substances that can be reliably used as a vehicle to achieve timely release of bioactive substances. This study was conducted to demonstrate that encapsulating PLCs in 10% liposomal SUV enables the gradual release of bioactive compounds. Three single doses of 3; 5; 7 mg PLCs-Liposome SUV/100 g per body weight of rats were injected into rats in the trial groups (15 of rats). Thereafter, the plasma concentrations of PLCs were assessed using liquid chromatography electrospray ionization mass spectrometry (LC-ESI MS). The concentrations of PLCs in the trial groups were found to be 1.20 to 2.40 fold higher than those in the control group. Such findings indicate that encapsulating drugs in 10% liposomes can result in a higher drug level in blood than that obtained without drug encapsulation (P<0.05).

Keywords: *Androgenic, Health Lifestyle, Mistletoe plant, Progesterone-like compounds, Small unilamellar vesicle*

Progesteron-Benzeri Bileşiklerin %10 Lipozom İçerisinde Kapsüllenmesi ve Bu Bileşiklerin Enjektabl Dozaj Formunun Uygulandığı Sıçanlardaki Konsantrasyonlarını Artırır

Öz

Sentetik tıptaki boşluğu doldurmak için bitkisel ilaçların kullanılması sağlıklı yaşam tarzları için oldukça gereklidir. *Benalu Duku* (BD) alt türü olan *Dendrophthoe pentandra* L. *Miq*'in yaprak ekstraklarından elde edilen progesteron benzeri bileşiklerin (PLC), anti-kanserojenik ve androjenik maddelerden oluşan faydalı bileşikler içerdiği bilinmektedir. Aktif bileşiklerin yavaş salınımı, yavaş salınan araçlar kullanılarak elde edilebilir. Küçük tek katmanlı veziküller (SUV) olan lipozomlar, biyoaktif maddelerin zamanında salınımını sağlamak için güvenilir bir şekilde kullanılabilen eksipiyan maddelerden biridir. Bu çalışma, PLC'lerin %10 lipozomal SUV içinde kapsüllenmesinin biyoaktif bileşiklerin kademeli olarak salınmasını sağladığını göstermek için yapılmıştır. 100 g vücut ağırlığı başına üç farklı doz (3; 5; 7 mg) PLC-Lipozom SUV deneme gruplarındaki ratlara (15 rat) enjekte edildi. Daha sonra PLC'lerin plazma konsantrasyonları, sıvı kromatografi-elektrosprey iyonizasyon kütle spektrometrisi (LC-ESI MS) kullanılarak değerlendirildi. Deneme gruplarındaki PLC konsantrasyonlarının, kontrol grubundakilerden 1.20 ile 2.40 kat daha yüksek olduğu saptandı. Bu bulgular, %10'luk lipozomlarda kapsüllenen ilaçların, ilaç kapsüllenmesi olmadan elde edilene göre kanda daha yüksek bir ilaç seviyesi ile sonuçlanabileceğini göstermektedir (P<0.05).

Anahtar sözcükler: *Androjenik, Sağlıklı yaşam tarzı, Ökseotu bitkisi, Progesteron benzeri bileşikler, Küçük tek lamelli kesecik*

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Introduction

Many bioactive compounds, such as amino acids, phytohormones, plant enzymes, alkaloids, flavonoids, and other substances, can be found in mistletoe plants, especially their leaves [1]. According to current reports, more than 60% of the bioactive compounds in mistletoe plants have been used in pharmaceutical products. As a result, these compounds have had a positive impact on the healthcare system owing to their use as treatments for diseases associated with hormone deficiency disorder [2]. The subspecies of *Dendrophthoe pentandra L. Miq, Benalu Duku* (BD) produces a small number of diverse bioactive compounds, namely progesterone-like compounds (PLCs), that can be used to treat cancers and androgenic hormone disorders so make a better performance to health lifestyle [3,4]. High concentrations of progesterone, which may protect against libido disorders, accumulate in the crude methanol extract of BD leaves [5-7].

Plants containing beneficial PLCs that are used as herbal medicines may supplement the needs of the human body through mechanisms involving normal physiological hormones [8]. Generally, treatment using hormones as bioactive compounds requires the administration of a high dose. An alternative option to reduce the high dose required is to produce the drug as an injectable dosage form. Nanoparticle technology or ultra-fine technology can help to accelerate the pharmacodynamic action of injectable dosage forms for applications to non-vascular administration [9]. The effect of the drugs will be better if the concentrations of the bioactive compounds can be maintained for a long period. These concepts can be modified to produce new concepts that vehicle as an excipient to control the release time of the active substance. Small unilamellar vesicle (SUV) is a phospholipid vesicle consisting of one or more concentric lipid bilayers enclosing discrete aqueous-oil spaces [10,11]. The unique ability of liposomal systems to entrap both lipophilic and hydrophilic bioactive compounds enable a diverse range of drugs to be encapsulated by these vesicles [12,13]. This ability causes the pharmacodynamic action of the bioactive molecules to be increased through a controlled dispensing system and enables the time interval of drug administration to be reduced [14]. The gradual release concentrations of PLCs encapsulated of SUV can be observed after comparing to concentrations of Certified Reference Material (CRM) progesterone [15]. Thus, the availability of drugs in the test group is expected to be longer than control group. The explored model, which enabled the gradual release of drugs from liposome employed as a vehicle, was identified as a suitable test to prove the pattern of drug dispensing to produce long-acting effects. Such test will be significant if the drug will be dispensed via non-vascular parenteral routes (intramuscular, sub-cutaneous, intra-peritoneal etc.). It is known that injections do not exhibit the first-pass phenomena, and small particles will cause the surface area of the particles to become larger, facilitating the absorption and adsorption processes. This strategy will be easily realized if the drug is designed in the form of injection and uses a slow-release vehicle [16]. Some substances that can be used as vehicles include fats or oils, one of which is liposomes. However, this strategy has yet to be investigated.

Based on the above information, this research will be carried out to determine the benefits of encapsulating PLCs in liposomes for administration as an injectable dosage form. The hypothesis of this research is that encapsulated liposomes of active compounds, namely, PLCs will increase the concentration of progesterone in blood plasma. The present study was designed to estimate the concentrations of bioactive PLCs in rat plasma after encapsulation in SUV.

Material and Methods

Chemical

The reagent and chemicals used to extract and analyze PLCs by HPLC or LC-ESI MS and to produce the injectable dosage form were of CRM grade or pro analysis (PA) grade. SUV was obtained from Merck Corp. (Germany) [17]. The liposome component as small unilamellar vehicles on injection dosage form at levels sterile, pyrogen free, isotonic, free from ion compounds was used at compositions as follows; cholesterol 9 µmol, L-α-phosphatidylcholine 63 µmol, and stearylamine 18 µmol. Samples of BD were collected from the Muara Enim District of Palembang, South Sumatera, Indonesia.

Extraction and Isolation of BD

Leaves of BD were manually separated, washed, shade-dried, and ground to fine powders. To remove the impurities, hydrolysis was performed. Briefly, 20 g of each powdered plant sample was mixed with 100 mL of 0.5 M acidified (HCl) methanol (w/v) and agitated at room temperature for 24 h [18]. The total hydrolyzed extract was separated from other impurities that were not dissolved in methanol:water (70:30) by SPE with a C_{18} cartridge. First, reverse phase activation of the C_{18} SPE cartridge was carried out by adding 5 mL methanol to the cartridge. Thereafter, the hydrolyzed plant extract dissolved in 5 mL methanol was added to the cartridge. Clean-up was carried out with the addition of 5 mL of water to SPE followed by drying for 10-15 min. The pure BD extract containing PLCs was eluted using fractions of methanol:water (70:30) 5 mL and all elution results [19].

Purifying PLCs From the Hydrolyzed Powders of The BD Leaf Extract

HPLC was performed using a Shimadzu LC-6AD pump, DGU-20A5 degasser, type 20A communication bus module (CBM), UV-visible type SPD-M20A photodiode array detector, and FRC-10A fraction collector. A C_{18} RP LiChrospher

100 column was employed. For HPLC, isocratic elution was carried out at wavelength, 254 nm; flow rate, 0.5 mL/min; column temperature, 20°C; mobile phase, methanol:water (70:30); and stop-time, 11.00 min [18].

Encapsulation of Liposomes

A total of 500 mg of dried liposomes was weighed for combination with 5 g of PLCs, melted at 70°C, and added to hot water until a volume of 5 mL was obtained. The mixture was shaken well for 30 min and sonicated for 30 min. Analysis of particle PLCs and particle encapsulate of liposome were using by Scanning Electron Microscope (SEM) of Zeiss MA 10 installed at year 2010 producing from One North Broadway, White Plans, NY 10601 US. The SEM were adjusted as follows; Electron High Tension (HET) at about 15.000 to 30.000 kv, Working Distance (WD) approximately 5 to 10 mn, Signal A at SE 1 as secondary detector, Magnified (Mag) 700 to 10.000 times and scale of object at about 1 to 50 µm. Operating of SEM was used for object particle dried and stable at range of HET workflow.

Injectable Dosage Form

Substances containing PLCs and 10% liposomes were prepared in clean bench room as follows; filtered with a porous membrane filter (20 µm), then adjusted to a pH value between 6.80 and 7.00 using sodium hydroxide.

Animal Experiment

Rats *(Rattus norvegicus*; healthy, adult, male (150-200 g) were employed for the animal experiments. Rats were obtained from the Veterinary Pharma Research Centre, *(http://pusvetma.ditjenpkh.pertanian.go.id/layananpenunjang)*. Before treatment, an ethics test was first carried out using rats at the Faculty of Veterinary Medicine, Universitas Airlangga (Certificate No: 2.KE.102.11.2020). Experimental animals were cared for and maintained in accordance with the animal welfare guidelines, and housed in a comfortable place.

Experimental Design

Research designs were used as an *in vivo* model by post test only control group design in groups as follows; research groups, negative control groups and positive control groups. The sample size for this experiment was calculated using the equation below [20].

$$N = \frac{\left[\left(Z_1 - \frac{\alpha}{2}\right) + Z_\beta\right]^2}{\frac{(d)^2}{(S_a)^2 + (S_b)^2}}$$

Where (Z1- α/2) = 1.96, with a significance of 0.05; Z_β = 1.645 with an error limit of 5%; d = 3.62, Sa = 1.7; and Sb = 1.4. As the N value was rounded to 5, the positive control group should consist of 15 rats, the negative control group should contain 15 rats, and the test groups should also contain 15 rats. The 15 rats allocated to the test groups were further divided into three sub-groups (for each dose) consisting of five individuals.

Each sub-group of test rats was injected intraperitoneally with PLCs encapsulated in 10% liposome; the sub-groups received 3 mg/100 g BW, 5 mg/100 g BW, and 7 mg/100 g BW. The rationale for assigning three doses was based on research reports that the three doses provided minimal pharmacological response of BD as androgenic effect [18,20]. Rats in the positive control group were injected with a dose of pure progesterone. At 30 min after the injection, 1 mL of blood was withdrawn from the heart. After 60 min, more blood was withdrawn. A total of 1 mL of blood was centrifuged for 30 min and plasma (0.5 mL) was obtained. Blood sampling was done by first giving ketamine 1 mg/kg BW intramuscularly.

LC-ESI MS

Acetonitrile was added to the plasma and the mixture was shaken well for 10 min. The supernatant was collected, centrifuged, and separated using a C_{18} SPE column, which was first activated by the addition of methanol. The SPE elution material was then dried and added to the mobile phase for injection into the LC-ESI MS system. PLCs encapsulated in 10% liposomes were subjected to liquid chromatography coupled with mass spectrometry-mass spectrometry (LC/MS-MS) using an Accela TSQ Quantum Access apparatus (Thermo Fisher Scientific, San Jose, CA, USA). To achieve chromatographic separation, vial samples were injected through an auto sampler (Surveyor auto-sampler plus) into the rheodyne system (Surveyor) equipped with a Hypersil GOLD (0.2 µm particle size, length 10 cm) for gradient elution. The elution was carried out at a flow rate of 0.5 mL/min. Solvent A consisted of a mixture of water:acetonitrile:formic acid 90:10:0.1% (v/v), while solvent B was water:acetonitrile:formic acid 10:90:0.1% (v/v). Elution was performed using the following gradient: Solution B increased from 35% to 70% at 20 min. The identification of PLCs was conducted in full scan mode in the range of 100–600 m/z.

Statistical Analysis

The analyzed data between the trial groups compared to the control groups were assessed using the Statistical Package for Social Sciences (SPSS) 24.0, at 5% significance by independent t-test.

Results

The chromatogram peaks of PLCs separated from the crude extract of BD leaves by HPLC appeared at retention times between 6.50 to 8.00 min. In contrast, the pure substance of progesterone was observed at a retention time of 7.119

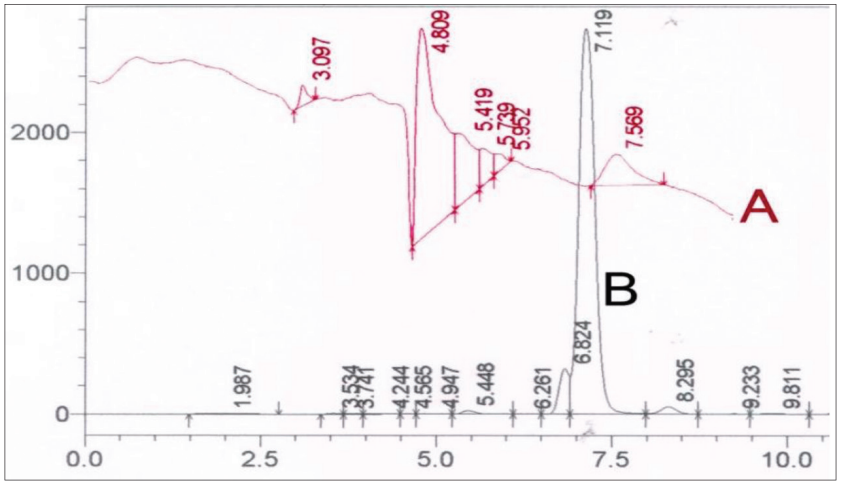

Fig 1. Red line represents the methanol extract of *Benalu Duku* leaves, with peak for the PLCs appearing at a retention time of 7.569 min **(A)**. Black line represents pure progesterone in the mobile phase, with its peak appearing at a retention time of 7.119 min **(B)**

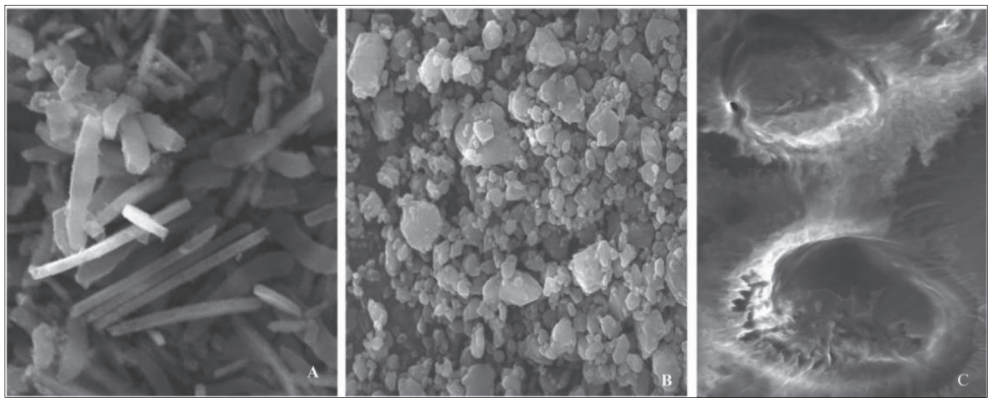

Fig 2. Particle for progesterone-like compounds at working distance 8.0 mm, magnification 10.000x, scale of object 1 µm **(A),** particle for progesterone as a certified reference material at working distance 7.5 mm, magnification 3000x, scale of object 3 µm **(B),** and particle for progesterone-like compounds after encapsulation in 10% liposome for administration as an injectable dosage form at working distance 7.5 mm, magnification 700x, scale of object 10 µm **(C)**

min. In *Fig. 1*, PLCs (red line, A) appeared at a retention time of 7.569 min while the CRM of pure progesterone (black line, B) appeared at a retention time of 7.119 min. The PLCs were collected at a retention time of 7.569 min and obtained at a weight of ±1600 mg. The particles of PLCs before and after encapsulation in 10% liposome (w/w) are shown in *Fig. 2-A,B,C* at 500x to 10.000x magnification.

The optimizations carried out to analyze the progesterone standard after encapsulation in 10% liposome are presented in *Fig. 3* and *Fig. 4*. A gradient elution was carried out on the LC-ESI MS system using acetonitrile: water (30:70) with 0.1% formic acid. *Fig. 4* shows that PLCs encapsulated in 10% liposomes could be identified by MS/MS, with a ratio of ionized mass molecule (m/z) of 326.50-327.50. LC-ESI MS targeted a special molecule mass of progesterone. The peaks for impurities in the biological matrix were found to disappear at the retention time and molecular mass of the analyte. After encapsulation in 10% liposome, the PLCs were identified at a retention time of 4.35 min, and the ESI of the ionic molecular mass spectrum was 326.50. The drifts of retention time and m/z between 0.5 to 1 min or m/z at 0.1 to 1 were indicated precision.

The linearity between concentrations and the area abundance ion between concentrations remained stable. Employing LC-ESI MS analysis also proved satisfactory as the MS detector was very sensitive. Based on the fact presented in the serial concentrations of progesterone as follows; 0.05 µg/mL with 139911 AA, 0.10 µg/mL with 279831 AA, 0.50 µg/mL with 1399155 AA, 0.70 µg/mL with 1958811 AA and 0.90 µg/mL with 2518480 AA, the system suitable test (SST) of the MS detector at square of the correlation coefficient (R^2) 0.961 by equation of Y = 64361 X - 67159.

The coefficient of variation (CV) values for recovery and accuracy are presented as follows; concentrations

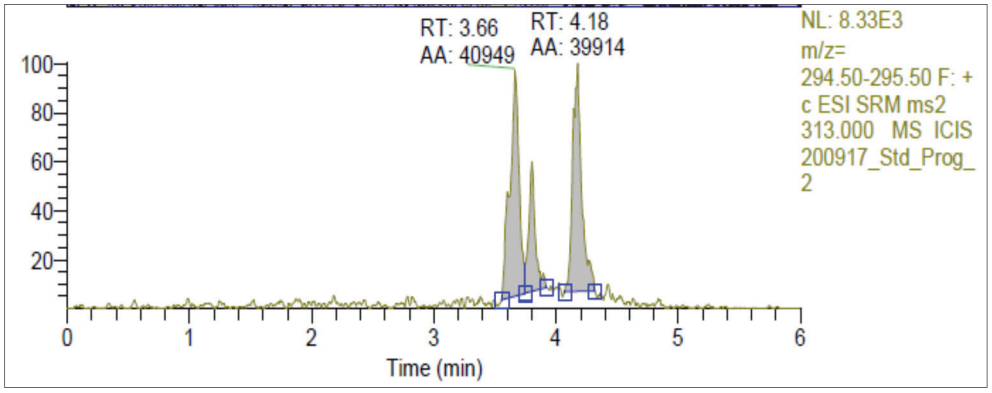

Fig 3. Mass spectrum of the progesterone standard (0.100 ppm) at a retention time of 4.18 min, m/z = 294.50 - 295.50, and MS-MS at 313.000 by LC-ESI MS

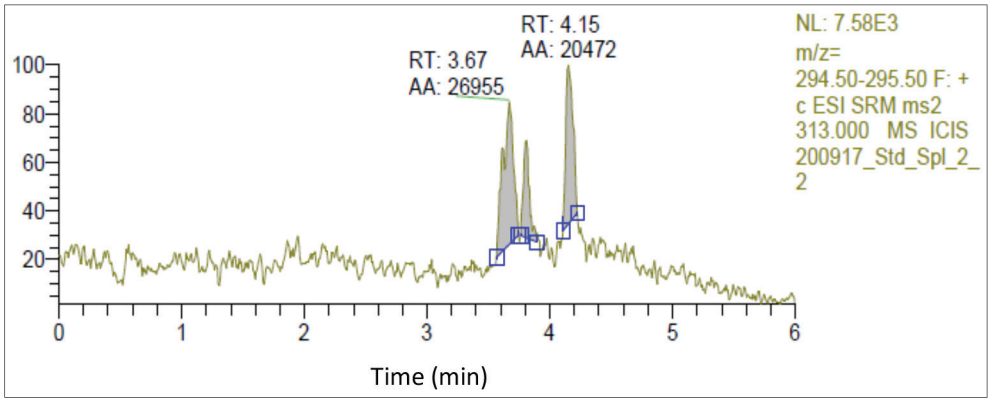

Fig 4. Mass spectrum of progesterone-like compounds after encapsulation in 10% liposome in rat plasma at a retention time of 4.15 min, m/z of 294.50-295.50, and MS-MS of 313.000

Table 1. *The means concentration of PLCs in rats plasma when encapsulated in 10% liposome (trial groups) and not encapsulated in 10% liposome (control group)*

Single Dose Administration Intraperitoneal	Concentration of Progesterone-Like Compounds (µg/mL)				P
	Control (Mean ± SD)		Trial (Mean ± SD)		
	30 min	60 min	30 min	60 min	
3 mg/100 g BW	0.111±0.028[a]	0.245±0.039[a]	0.333±0.015[b]	0.757±0.024[b]	0.08
5 mg/100 g BW	0.250±0.015[a]	0.518±0.009[a]	0.548±0.022[b]	0.936±0.034[b]	
7 mg/100 g BW	0.541±0.013[a]	0.746±0.042[a]	0.928±0.019[b]	2.161±0.042[b]	

[a,b] Values within a row with different superscripts differ significantly at P<0.05

0.05 µg/mL in 98%, 102%, 104% at mean 3.015 %CV; concentrations 0.1 µg/mL in 101%, 111%, 112% at mean 5.632% CV, concentrations 0.9 in 101.3%, 101.4%, 101.6% at mean 0.151%CV. The method had good accuracy as the mean of the values was less than 10% CV. In biological matrices, such as rat plasma, the separation of analytes from impurities is difficult to achieve. However, the analysis can become easier assessing a mass spectrum. All samples had good recoveries, ranging from 80% to 120%. The correlation between serial concentrations and area abundance (AA) at means±SD is presented as follows; 0.03 µg/mL at 83125±876.74 AA; 0.05 µg/mL at 139844±88.255 AA; 0.1 µg/mL at 283111±5.733 AA; 0.9 µg/mL at 2569639±47.137 AA. According to the result at above, the method had good precision as the % CV was less than 3%. This result was used to generate a standard curve to determine the levels of PLCs in the plasma of rats by equation of Y = -2803 + 2858270 X.

The lowest concentrations could be observed in 0.03 µg/mL (83125 AA), then by other concentrations of 0.05 µg/mL (139844 AA) resulted linear regression equation as follows; Y = -1953.5 + 2835950 X. The calculated Limit of Detection (LOD) by inserting the smallest area abundance free from a noise peak (3 × 1000) was resulted 1.746 x 10^{-3}

µg/mL, then was calculated Limit of Quantification (LOQ) from three times of LOD, resulted 5.240 x 10^{-3} µg/mL.

The results showed that the average concentration of progesterone in the trial group administered 3 mg/100 g BW in 30 min and 60 min was 0.333 µg/mL and 0.757 µg/mL, while the control group was 0.111 µg/mL and 0.245 µg/mL. Progesterone concentrations in the trial group administered 5 mg/100 g BW in 30 min and 60 min was 0.548 µg/mL and 0.936 µg/mL, while the control group was 0.250 µg/mL and 0.518 µg/mL. Progesterone concentrations in the trial group giving 7 mg/100 g BW in 30 min and 60 min were 0.928 µg/mL and 2.161 µg/mL, while the control group was 0.641 µg/mL and 0.846 µg/mL. *Table 1* shows the results of the analysis of PLCs encapsulated in liposome correlation to progesterone in the trial and control groups.

Discussion

A new separation technique was used in this study. Briefly, hydrolysis and separation were carried out using 0.5 M HCl in methanol to reduce the interfering substances bound to PLCs, including polyphenols, anthraquinones, and alkaloids, which have terpenoid structures. Many of these substances bind to the stalk of BD, which binds to the leaves. *Fig. 1* shows that the progesterone CRM appeared at a retention time of 7.119 min. Accordingly, the PLCs were collected between retention times of 6.500-8.000 min, and the peak for the BD extract appeared at a retention time of 7.569 min. These findings indicate a change in the pattern of the appearance of progesterone, which originally appeared at a retention time of 6.107 min according to a previous report [18]. As the previous researchers did not perform hydrolysis and separation techniques using SPE, this may justify the differences in the results between the two studies. In the new technique employed in the present study, nuisance materials that often appear between 6.100 and 6.500 min were separated. The removal of these interfering substances yielded very pure PLCs. A new technique, such as that employed herein, requires a large amount of crude extract of BD compared to previous techniques; this is because there are more steps in the separation process than in the process used by previous researchers. *Fig. 2-A,B* shows that the PLCs particles (A) are relatively smaller than the particles for the CRM progesterone (B). *Fig. 2-C* shows that encapsulation in 10% liposomes was ideal to cover the bioactive compounds in the PLCs. Sonification also affected the uniform distribution of particles encapsulated in the liposomes. Thus, the nanoparticle technology using liposome encapsulation encourages agonist bioactive action and induces a controlled release pattern. In this research, very small particles were obtained through nanotechnology, confirming the potential for development on an industrial scale. The fine particles of PLCs obtained are known to be very lipophilic; thus, through liposome encapsulation, the outer part of the envelope component can be hydrophilic [21]. Under such circumstances, the initial action of the injectable drug was almost equivalent to that of the solution dosage form (categorize easy to dissolve at 1:1 as w/v). *Fig. 4* shows that the structure of progesterone in PLCs with 10% liposome could still be observed, with a molecular mass (m/z) between 294.50 and 295.50, which is based on the molecular mass of CRM progesterone as presented in *Fig. 3* white m/z at ESI MS-MS 313.000. Another observation that the active substance progesterone on PLCs is RT at 4.15 min, that is shifted more slowly than standard progesterone at RT 4.18 min. The following parameters of the LC-ESI MS method were assessed to obtained result of the research at SST levels. The precision and accuracy by recovery analysis as a limit of detection (LOD), and limit of quantification (LOQ) were shown sensitive, so that it was very suitable for assaying with small concentrations. Based on our findings, this method was satisfactory for determining drug concentration total at level nanoparticle encapsulated by liposome. In this study, the presence of progesterone in the plasma matrix of rats was determined using MS-MS. The levels of progesterone were also determined using the area and retention time obtained from the chromatogram. Using both chromatograms and molecular mass measurements can demonstrate that the compound being examined is compatible with CRM progesterone [22,23]. As shown in the % recovery ranged from 98.0% to 101.6% with CV ranging from 0.151% to 5.632%. Recovery is defined as the ratio between the observed analysis results and the true value. For example, in samples of biological matrix that contain thousands of metabolites, which serve as impurities, the recovery rates strongly depend on the quality of the sample preparation technique. The lower concentrations of PLCs will yield the lowest precision and a greater uncertainty in the recovery rates. In biological matrices, such as blood plasma, the recovery was found to range from 80% to 120%, with a CV of <7%. Correlations between concentrations versus area abundance were indicated strong relationship on the range of concentrations 0.030 µg/mL to 0.900 µg/mL at equation $Y = -2803 + 2858270\ X$ ($R^2 = 1$). Based on the LOD and LOQ assessed as described at above, the analysis method using LC-ESI MS indicate sensitive to detection at small concentration, especially for metabolite. Concentrations lower than 1.746×10^{-3} µg/mL was not detected, although a signal could still be read by the instrument. By assessing the PLCs encapsulated in 10% liposome, we found that the concentrations of the encapsulated PLCs at 30 min and 60 min were higher in the trial groups than the concentration of pure progesterone in the control groups ($P<0.05$ as described in *Table 1)*. The level of PLCs in each dose (3 mg/kg BW, 5 mg/kg BW, and 7 mg/kg BW) was one-fold higher than the level in the control, indicating that the elimination rate at each time point (30 min and 60 min) differed from that of the control. This is because the peak of PLCs at each time point was higher than that of the control. Such finding aligns with the concept of linear pharmacokinetics, where the rate of elimination is highly dependent on the kinetic

pattern of the drug. Thus, the higher the level of drug in the body, the deeper the spread of the drug, and the longer the drug exists, the lower the clearance value [24]. In such conditions, the drug can remain in the body for a long time and exert an agonist action for an even longer time. This agonist role can occur because of the use of liposomes as a vehicle, which enables the gradual release of bioactive compounds [25,26]. The part of the liposome molecule that is associated with water is hydrophilic while the part that binds to the drug is lipophilic, thereby enabling the quick distribution of the drug preparation in the body. Liposomes protect the molecular structure of progesterone, which is part of cyclopenta[a]phenanthrene that is at risk of rupture when bound by strong electronegative ions from body electrolytes. Thus, PLCs does not have a first pass effect phenomenon due to the body's electrolyte reaction. This novelty proves that the structure of progesterone that is not protected by liposomes is ultimately easily conjugated with electrophilic substances groups, which in turn can decrease the bioavailability of progesterone. Another structure of the progesterone molecule that is also susceptible to electrophilic bonding from body ions is -tetradecahydro bound to cyclopenta-phenanthren-. In conclusion, the encapsulation of PLCs in 10% liposomes increased the spread of progesterone deep within the body of rats. Further, a longer release of progesterone can be achieved with liposomes than without liposomes.

Availability of Data and Materials

The data sets during and/or analyzed during the current study available from the corresponding author (M. Lauzardi) on reasonable request.

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Competing Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

ML: Project manager, preceptors, research coordinators, research analysis, drafting manuscript and writing the manuscript. SS, CHC, JLH, CWL and CKP: writing and editing the manuscript, BH: analysis LC-ESI MS and HPLC; MS: analysis statistic, RS: preparations animal experimental, LM: extraction leaf of *Benalu Duku*.

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