CELASTROL NANOEMULGEL FORMULATION AND IN VITRO PENETRATION TEST

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ABSTRACT

Objective: To obtain formulations of Celastrol (Cst) nanoeulgel via transdermal route. Celastrol is classified in BCS 4 class as an anti-inflammatory drug. These routes are considered to reduce the risk of Celastrol side effects and have the same characteristics as skin morphology.

Methods: Celastrol nanoeulgel was prepared by a high-pressure homogenizer (HPH) technique. To find the optimum nanoeulmulsion area by using the Chemix 7.00 ternary phase program. Celastrol nanoeulgel was evaluated by measuring the particle size, PDI, morphology, zeta potential, stability tests and in vitro using Franz diffusion cell

Results: Results showed the ideal formula based on the ternary phase diagram using chemix 7.00 is smix: water (5:45:50), with particle size 89.9±5 nm, PDI 0.1, and zeta-21 mV. The morphological shape is quite spherical ≤1 00±5 nm. The pH value of this formula is 4.5, which is compatible with the pH of the skin. The highest recovery rate of Celastrol and encapsulation efficiency (EE) were formulas 3 μg/ml and 5 μg/ml, with EE 91.70% and 94.54%, respectively. In vitro test results showed that the formula 3 μg/ml and 5 μg/ml give better penetration results than the formula 2.5 μg/ml. Thus, Celastrol nanoeulgel formula has good potential to be developed as a transdermal anti-inflammatory drug.

Conclusion: Transdermal nanoeulgel containing Celastrol has been successfully developed with particle size ≤200±2 nm.

Keywords: Celastrol (Cst), Transdermal nanoemulsion, Anti-inflammatory, Franz diffusion cells (SDF)

INTRODUCTION

Celastrol (Cst) is a nutritious compound derived from Tripterygium wilfordii Hook F (TWFH) isolates. It can be used to treat several immune system disorders and neuroprotective, as an anti-inflammatory for various causes of inflammation, especially autoimmune disorders [1].

Celastrol is part of the pentacyclic Triterpenoid natural compound from the methyl quinone family. This class of compounds has anti-inflammatory and anti-pain activity. Many studies about Celastrol's pharmacological activity as an anti-inflammatory, found that the compound works at the target receptors that trigger inflammation from various pathways. These include cytokine pathways (TNFα, IL-1, IL-6 and IL-8), as well as the formation of cyclo-oxygenase 1 and 2 pathways (COX 1 and 2), through inhibiting catalytic activity (sPLA2IIA), which produces the eicosanoid formation of arachidonic acid (AA)) [2]. Celastrol's physical and chemical properties make it difficult to dissolve in water, which causes problems with the developing of Celastrol as a formula. Celastrol has a Log P value of 5.63, which, in the pharmaceutical system, is categorized in Biopharmaceutical Classification System 4 (BCS 4). Pharmaceutical drugs, in the BCS 4 category, have very low solubility and permeability.

Therefore, delivering this active substance requires a formula development approach, and this study will use the nanoparticle system. Theoretically, the smaller the nanoparticle size, the more it can increase compound's ability of these compounds to be absorbed or the greater the surface area to penetrate a membrane. Changing the drug administration route from oral to transdermal means the Celastrol compounds can be formulated in line with the development of nanotechnology formulas [3]. Formulations can be developed from to Celastrol with the nanotechnology approach, which aims to improve these compounds' solubility and bioavailability. Among them are the phytosome Celastrol, Celastrol liposomes, Celastrol nanomicel, NLC Celastrol, and the most recent, Niosom Celastrol and Derivatives Celastrol. The method was developed into permal, IP, and topical routes. The study results concluded that Celastrol developed into nanotechnology increased the absorption efficiency (EE) to 98.06% with an average particle size of 89.6±7.3 nm ≤100 nm and zeta potential of 87.8±5.8 mV [4-8].

There are no published studies on the Celastrol compound's potential if it is developed to be administrated via other routes. For example, transdermal routes have similar characteristics to nanoemulsion technology. It is known that nanoemulsion, when developed into the transdermal route, shows optimal work results with promising pharmaceutical dosage forms [9, 10].

The transdermal nanoeulgel has properties in common with the stratum corneum (sc), which is the main obstacle of the transdermal route. The oil base content on the nanoeulgel base will provide its own benefits in therapy.

The Nanoemulsion's ingredients can adhere to the skin's surface and gradually increase its hydration. They can also stretch the skin's permeability, which causes changes in polarity, fluidity, and fat exchange between cells in the skin, ultimately facilitating active substances penetration into systemic tissue.

When developing a transdermal route, the active ingredient must have considered, along with characteristics such as inertness, constant in various temperatures stable in acidic conditions (pH), polarity, toxicity, allergenic, molecular weight < around 450 Da, and low melting point. Further, it should not irritate the skin. Celastrol meets the above conditions so it is worth trying in its development into the transdermal route with the nanoemulgel dosage form [11, 12].

This study aims to develop a transdermal nanoemulgel Celastrol preparation formula by evaluating its anti-inflammatory activity in vitro and in vivo.

MATERIALS AND METHODS

Chemicals and animals

The materials used in this study are; Celastrol standard obtained from X’ian Fengchu Biological Technology Co., Ltd., and Celastrol samples obtained from Xi’an Huisun Bio-Tech Co., L. td (PRC).
Isopropylmirtstat (IPM), Polysorbatum 80 (Tween 80), Propylene glycol (PG), API (Aqua Pro Injection), Ethanol 96%, Methyl Paraben, Propyl Paraben, and 305 seigel, from PT. Bratouk, (Jakarta). Merck methanol pro analyse, Acetonitrile pro analyse merck, formic acid pro analysis. The tools used are; duran and pyrex chemical glass tools, including beaker glass 50 ml, 100 ml, 250 ml, 500 ml, 1500 ml, 2000 ml, 10 ml, 50 ml, 100 ml measuring cups. 7.5 cm amplifier cross mixing rod, measuring flask 2 ml, 5 ml, 10 ml, 50 ml, 100 ml and 250 ml, thermometer, pH meter, Cole Parme® Viscometer, Shimadzu HPLC, Particles size analyzer (PSA) Malvern®, Ultra Turrax 20,000 rpm T25 Digital Homogenizer (120 V USA), GEA Brazilian high-pressure homogenizer (HPH).

Determination of standard Celastrol calibration curves

First, we placed 10 mg of the standard Celastrol in a 10.0 ml volumetric flask and added methanol as a solvent to the mark. Then dilution was made as many as 6 (six) concentrations, namely 5 ppm, 10 ppm, 50 ppm, 100 ppm, 250 ppm, and 500 ppm. Subsequently, an analysis was carried out by HPLC at a wavelength of 230 nm using an injected phase C-18 column with a mobile phase of acetonitrile: 0.1% solution of formic acid (85:15) v/v. The results were calculated to obtain a linear calibration curve [13].

Optimization of nanoemulsion areas with ternary phase diagrams

The optimizing process used the pseudo ternary triangle method, which aims to obtain a stable nanoemulsion area. This process involved mixing surfactant and co-surfactant materials from various concentrations that were suitable for the active substance’s physical-chemical properties. The nanoemulsion optimization was carried out by a high energy method involving stirring using an ultraturrax 20,000 rpm for 15 min. The nanoemulsion area was arranged using Chemix 7.00 software. Previous research used the Chemix 7.00 program to find good, stable nanoemulsion areas in the pseudo ternary triangle to facilitate further work [14].

Nanoemulsion was developed using Isopropylmirtstat (IPM) as oil phase, tween 80 as the surfactant, and propylene glycol as co-surfactant, while distilling water as the water phase. The formulas were compared based on the area of nanoemulsion formed as a result of the Chemix 7.00 program. For example, a mixture of surfactants and co-surfactants called smokes (9: 1 to 1:9) will show stable results. All nanoemulsion formula results were carried out by the desired particle size analysis in the research objective of 100 nm for the transdermal route using a particle size analyzer (PSA) Malvern® [15].

Using pseudoternary models in the manufacture of nanoemulsion formulations was considered the best way to determine the composition of the three constituent elements in nanoemulsion preparations

If the particle size were still bigger than 500 nm, we used a HPH machine with a setting of 600 bars and 8 cycles [16].

Selection of selected formulas from the nanoemulsion area

After obtaining the nanoemulsion area from the Chemix 7.00 program, we selected the best, most stable formula and entered the active Celastrol substance into the nanoemulsion.

Celastrol inserted into the nanoemulsion formula, was divided into 3 (three) forms of Celastrol doses of 2.5 μg/ml, 3 μg/ml and 5 μg/ml respectively.

Manufacture of Celastrol nanoemulgel

We obtained 2 grams of seigel® 305 gel added 20 ml of CO2-free distilled water and then put it into 100 ml of nanoemulsion preparation in each Celastrol dose while stirring using ultra turrax® 20,000 rpm for 15 min until homogeneous [17].

The F1 control, F2 control and F3 control were made with a base of gel seigel® 305. Each Celastrol dose (2.5 μg/ml, 3 μg/ml and 5 μg/ml) was added to a container containing 100 added gel bases. 0.1% DMSO is stirred using ultra turrax® for 15 min with 20,000 RPM [18].

Evaluation of Celastrol nanoemulgel preparations

Organoleptic, pH, and homogeneity testing

Organoleptic testing of Celastrol nanoemulgel preparations carried out by direct observation of the preparations physical apperances, starting from the physical form of nanoemulgel, and the characteristic odor of Celastrol compounds [19].

pH measurement

One gram of Celastrol nanoemulgel preparation was obtained from each formula, put into a 100 ml glass beaker, dissolved with 100 ml distilled water, and stirred using a stirring rod. We measured the pH value using a pH measuring device brand Eutech Instrument pH meter 510 (Singapore). Ideally, the pH that penetrates the skin via the transdermal route is 4.5-6.5. Each Celastrol nanoemulgel preparation that had been formed was tested for homogeneity by weighing 1 gram of the preparation and then sprinkling it on a transparent glass plate. Visual observation was carried out under bright light [20].

Particle size, polarity index (PDI), zeta potential

The test is carried out using each Celastrol nanoemulgel that has been formed in a 1 mg weigh carefully and then dissolved with distilled water up to 9 ml. The solution was taken as much as 100 μl and put into a PSA cuvet container and then analyzed using a Malvern® particle size analyzer at 25°C [21].

Cycling test

Dispersion stability testing is carried out in 6 (six) cycles. Each cycle starts from 4±2°C for 24 h; the preparation is then transferred to another storage area at 40±2°C for the next 24 h [22].

Viscosity

Viscosity was measured using a Cole Parmer® viscometer [23, 24].

Morphology of the preparation

The morphological examination of the preparations was carried out using a JOEL (JEM-1010) JOEL Brand Transmission Electron Microscopy. The procedure was carried out by taking 0.2 μl of a Celastrol nanoemulgel preparation using uranyl acetate dye 2%/ w/v 0.2 μl, which was dripped on the cooper grid plate. Furthermore, observations were made at a magnifications of 30,000, 50,000, and 100,000 times [25, 26].

Test the physical stability of the preparation

The Celastrol nanoemulgel preparations were tested under three different temperatures for 12 w, including odor, color, and pH. Each preparation was stored at a cold temperature at 4±2°C then at room temperature at 28±2°C, and lastly at a hot temperature at 40±2°C [27].

Franz penetration test cell diffusion (SDF)

The penetration test phase of Celastrol nanoemulgel preparation was carried out in vitro at a pH of 7.4 using a phosphate buffer solution. The procedure added 0.2 M potassium dihydrogen phosphate to 39.1 ml of 0.2 N sodium hydroxide supplemented with CO2-free distilled water to a pH of 7.4 [28].

The skin test was conducted on male rats, aged 8 w, with a bodyweight of 200-250 g. The rats were anesthetized using 0.1% ketamine by inhalation. The rat’s skin was cleaned with a hair shaver, and the skin was sliced by removing the fat part slowly. The obtained skin was soaked into a phosphate buffer solution of pH 7.4 for 30 min at 4°C, which can only be used within the first 24 h [29].

From the Franz diffusion cells tool calibration results, a surface area of 1766 cm2 and an average compartment volume of 16.0 ml were obtained. The rat skin position was placed in the donor compartment. Then a 1.0-gram sample preparation is carefully placed over the skin, which contained a phosphate buffer solution at a pH of 7.4 at the receptor site. A sampling of 1.0 ml was carried out at 8 points with a certain time interval of 2, 4, 6, 8, 10, 12, 20, 24 h at 37°C from the receptor compartment. The most important thing in using SDF is to keep the sink well maintained by entering the same amount in the receptor compartment, that is 1.0 ml [30].
First, 1.0 ml of the sample was taken from the receptor compartment channel and put into a 5.0 ml flask, which was sufficient with the methanol pro analyzed solvent (p.a) to the mark limit on the flask. Then 20.0 µl was taken and analyzed by HPLC at a wavelength of 230 nm using a mobile phase of acetonitrile: 0.1% formic acid solution (85:15) flow rate of 1.0 ml. The area from the HPLC analysis results is entered into the calibration curve equation, and the Celastrol content is calculated in the receptor compartment. All HPLC analyses were performed 3 (three) times [31].

**Assay of the Celastrol**

For each dose of 2.5 µg/ml, 3 µg/ml, and 5 µg/ml, 1 gram of Celastrol nanoemulgel preparation was added to a solvent of 5 ml methanol pro-analysis (p.a) to the mark limit on the flask. Then the precipitate obtained was separated from the supernatant, then the precipitate was dissolved with methanol up to 5 ml filtered using a 0.22 µm syringe filter. The obtained filtrate was analyzed by HPLC at a wavelength of 230 nm using a mobile phase of acetonitrile: 0.1% formic acid solution (85:15) flow rate of 1.0 ml. The area of HPLC analysis results obtained was calculated Celastrol levels based on the calibration curve equation [32].

**Determination of Celastrol levels in nanoemulgel preparations**

For each dose of the Celastrol nanoemulgel preparation, 500 mg was added to a centrifuge tube with a methanol pro-analysis (p.a) solvent to 5 ml. The solution was centrifuged 4500 rpm for 30 min. The supernatant formed in a 100.0 µl pipette and analyzed by HPLC at a wavelength of 230 nm using a mobile phase of acetonitrile: 0.1% formic acid solution (85:15) flow rate of 1.0 ml. The area of HPLC analysis results obtained was calculated Celastrol levels based on the calibration curve equation [32].

**Determination of cumulative levels**

All hourly measurements of Celastrol levels obtained in the in vitro Franz diffusion cells (SDF) test, were graphed between the cumulative amounts of Celastrol penetrated per unit time of sampling. So that the cumulative levels of Celastrol, which will penetrate the surface area of the skin membrane of rats, the following equation was used [34].

\[
Q = C_i V + \sum_{i=1}^{t} C_i S_i/A
\]

Information:

\[Q = \text{cumulative number of Celastrol per unit surface area membrane (µg/cm²)}\]
\[C_n = \text{Celastrol concentration (µg/cm²) for each extract Sample}\]
\[V = \text{Franz diffusion cell volume (µl)}\]
\[V_s = \text{sample volume (µl)}\]
\[S = \text{sample volume (µl)}\]
\[t = 1 \text{ for each sample from 1 to n-1}\]
\[A = \text{Surface area of the membrane, which is 1.7666 cm²}\]

Flux is obtained from a graph with steady state conditions based on Fick’s Law

\[J = \text{Flux under steady state (µg cm² h⁻¹)}\]
\[S = \text{Area of diffusion area (cm⁻²)}\]
\[M = \text{Amount}\]

**Determination of Celastrol nanoemulgel type**

Each nanoemulgel type, initially weighed±250 mg nanoemulgel Celastrol preparation, was placed on the object-glass, then dripped Sudan III for the oil phase and methylene blue for the water phase. The object-glass was again placed on the first plate that contained the preparation, and pressed precisely, for improved accuracy. Then the samples on these plates were observed on an Olympus ® electron microscope (maximum enlargement). Microscope examination results showed that the nanoemulgel preparations developed were nanoemulsion type M/A or O/W.

**RESULTS AND DISCUSSION**

The Celastrol calibration curve is linear with a correlation coefficient \((r^2 = 0.9992)\) in the concentration range from 5 to 500 µg/ml as shown in fig. 1.

![Fig. 1: Calibration curve of celastrol](image)

The results of the evaluation of preparations of Celastrol nanoemulgel preparations were obtained each dose, the physical form of nanoemulgel is opaque white color following the PG-NCP transparent color indicator.

The pH measurements obtained with different pH values tended to fall by 2 points from the first day of testing before the 12-day cycle stability test every 24 h. The first time the Celastrol nanoemulgel has just been prepared for direct pH testing using a pH measuring device brand Eutech Instrument pH meter 510 (Singapore). The first pH was 7.19 24 h, the pH value dropped by 2 points to 4.79 and during the next 24 h of the cycle, the pH values were 4.77, 4.79, 4.90, 4.95 and 4.93. The pH value of Celastrol can cause a decrease to around 4.6 [35].
Organolespic homogeneity testing shows that all dosage of Celastrol nanoemulgel are evenly shaped, with no visible layers or forms of damage from nanoemulgel preparations, such as coalescence, sedimentation, flocculation, cremation, or breaking.

Tests with PSA (Particle Size Analyzer) on selected nanoemulsions obtained from particle size intensity analysis, showed an average particle size of 187.7 nm. However, when viewed from the analytical results of D90 intensity, a particle size of 343 nm was obtained, meaning that from the overall formula of nanoemulsion preparation, 90% of the particles are 343 nm. Only 10% have particle sizes below 200 nm. Overall, this cannot be accepted as the chosen base formula because it does not follow the desired transdermal route condition, which is below ≤ 200 nm. The PDI and zeta values obtained were

Table 1: Celastrol nanoemulgel formula

| Composition | Formula |
|-------------|---------|
| µg/ml/%     | F1 (µg/ml) | F2 (µg/ml) | F3 (µg/ml) | F4+DMSO 0.1% (µg/ml) | F5+DMSO 0.1% (µg/ml) | F6+DMSO 0.1% (µg/ml) |
| Celastrol   | 2.5     | 3         | 5         | 2.5             | 3                 | 5                 |
| IPM         | 5       | 5         | 5         | 5               | 5                 | 5                 |
| Smix        | 45      | 45        | 45        | 45              | 45               | 45               |
| Aquadest    | 50      | 50        | 50        | 50              | 50               | 50               |
| Metil Paraben | 0.05 | 0.05      | 0.05      | 0.05            | 0.05             | 0.05             |
| Profip paraben | 0.05 | 0.05      | 0.05      | 0.05            | 0.05             | 0.05             |
| Sepigel 305 | 2       | 2         | 2         | 2               | 2                | 2                |
| GD:Free water | 20   | 20        | 20        | 20              | 20               | 20               |

IPM: Isopropyl Myristate, Smix: Tween 80 and propylene glycol

Table 2: Ternary diagram optimization model

Explanation: 1 = Globul Size (nm)<500, 2 = Polydispersitas (PDI)<0,3, 3 = Diamater Globul 90 (D90) (nm)<200 nm, 4 = Zeta Potensial, (mV)>±20-35.
sufficient to meet the requirements of a fairly good formula, that is, 0.2 and -20.1 mV. In theory, 0.2 in the polydispersity index is a heterogeneity index in a sample medium of a mixture of polymers and colloids or can be said to be the level of uniformity of particle size distribution, while the value of 0.2-0.3 is interpreted as a population distribution of homogeneous sizes which can be accepted as a mixture of polymer-based and colloidal nanoparticle preparations. When compared with other research that does not display particle size based on the developed formula [36]. Then the particle size reduction step was carried out by the high energy method, using a HPH at a capacity of 300 bar and carried out for 10 cycles for all Celastrol nanoemulgel formulations 2.5 µg/ml, 3 µg/ml and 5 µg/ml. Then the particle size was measured again using a PSA (particle size analyzer) to obtain the particle size (z-average, D10, D50, and D90), polydispersity index (PDI), and zeta potential. The results obtained from these tests obtained a particle size for the intensity distribution that was under this study's objectives, and the desired transdermal route was 200 nm (Table 3).

### Table 3: Results of PSA before and after HPH

| Preparations formula | Evaluation | Global size (Nanometers) | Polydispersity index (PDI) | Zeta potential (mV) | D 90 (Nanometers) |
|----------------------|------------|--------------------------|---------------------------|---------------------|------------------|
| Nanoemulsion base non celastrol |            | 187.7                    | 0.2                       | -20.1               | 343              |
| Nanoemulsion Celastrol before homogenized with HPH | F1         | 231.03                   | 0.5                       | -38                 | 672              |
|                       | F2         | 272.9                    | 0.5                       | -35.9               | 698              |
|                       | F3         | 325.8                    | 0.5                       | -27.6               | 496.3            |
| Nanoemulsion Celastrol After homogenized with HPH | F1         | 91.5                     | 0.1                       | -15                 | 158.6            |
|                       | F2         | 99.9                     | 0.1                       | -24.2               | 182.3            |
|                       | F3         | 70.4                     | 0.1                       | -15.3               | 119.3            |

The above results show that the nanoemulgel preparation method involving high energy had very good results and produced very small particle sizes. This phenomenon occurs due to the pressure generated by the HPH. The cycle process was repeated according to the desired particle size. From the experiment of Gotu kola extract nanoemulsion, researchers obtained an average particle size of 198.4 nm with PDI 0.329 and zeta-30.9 mV after using HPH with 600 bar at 8 cycles to reduce the particle sizes of nanoemulsion [37]. Akbas, Soyler and Oztop, 2018 studied capsinin nanoemulsion in chilies, had an average particle size of 65 nm using HPH method with 140 bars in 5 cycles. Other experiments formulated an olive oil nanoemulsion had an average particle size of 275 nm with a pressure of 450 bar in 4 cycles using HPH. High-pressure homogenizer method is very effective for reducing the particle sizes of colloidal dispersions [38, 39].

For the evaluation results of the Celastrol nanoemulgel preparations, organoleptic test, from all doses obtained, pH and homogeneity showed the resulting preparations, in the form of opaque colored nanoemulgel, matched the drug dosage color index, namely NG CNP Transparant White, pH value 7.19, when the preparation was complete. However, the pH decreased after a cycle endurance test for 12 d in a row. The results can be seen in (Fig. 3). The pH reduction in these preparations is due to the Celastrol compound’s physical and chemical properties, which basically have a pH between 4–6.

![Graph of the pH test cycle](image)

The Celastrol nanoemulgel preparations were spread evenly on the transparent clear glass surface for all doses of formula made. The measurement results of the viscosity of nanoemulsion and Celastrol nanoemulgel preparations indicated that, at all dosages, the nanoemulsion viscosity values were 20 cP and 21 cP for nanoemulgel. This shows that the viscosity level of the two is not significantly different because the value is only 1 point difference, which can be interpreted that the preparation can flow well without flow resistance from the material composition of each preparation compiler. The flow rate (rheology) of nanoemulsion preparations seems to follow Newton’s law by using spindle 1, shows a linear straight line. However, in theory, based on dispersion both suspension and colloid drug preparations tend to have a relationship with Newton’s law (Fig. 2). Based on testing the flow properties of celastrol nanoemulgel, using spindle no. 4 (four), the curved graphical shape is generated both up and down (Fig. 4).
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Fig. 4: Viscosity graph of celastrol nanoemulgel

The explanation in the text is that the nanoemulgel Celastrol preparation has flow properties that follow the non-Newton law of pseudoplastic type; it is caused by an increase in the shear rate of a liquid and semi-solid preparation, so the viscosity decreased. The constituent components of Celastrol nanoemulgel may cause this event because they come from the long-chain group of tween 80, and the medium-chain triglyceride of oil. The pseudoplastic type of Celastrol nanoemulgel indicated that the preparation cannot be poured when it is in a storage container. This is shown in the graphic image, which shown that the up and down lines coincide so that each viscosity value is always different since the pseudoplastic type does not have an absolute viscosity value [40].

Fig. 5: The results of the ternary area nanoemulsion triangle optimization using the chemix 7.00 program

The effect of pH on a pharmaceutical preparation's viscosity value is significant, as seen in testing the Celastrol nanoemulgel preparation for formulas 1, 2, and 3. The same viscosity value is 20 cP. However, the pH test on day 0 obtained, respectively pH value 7.19 for F1, 7.15 for F2, and 7.09 for F3. On the 28th day, the pH value changed quite significantly.

The event was also caused by the gel-based material, namely sepigel 305%, which basically consists of an emulsion-based component with an intermediate chain group that form chain bonds that extend into the flow by increasing inflow shear velocity to the next shear pressure. This affects each preparation formula's viscosity (fig. 3 and 4).
Fig. 6: Franz diffusion cells test results graph. F1: NEG 25 µg/ml, F2: NEG 3 µg/ml, F3: NEG 5 µg/ml (NEG Cst: Nanoemulgel), F1: EG 2.5 µg/ml, F2: EG 3 µg/ml, F3: EG 5 µg/ml (+DMSO 0.1%) (EG: Emulgel Cst), cumulative of total Celastrol penetrated. All values were represented as mean±SD (n=3)

Fig. 7: Average penetration flux of nanoemulgel celastrol (Cst) and emulgel Cst+0.1% DMSO (mean±SD, n=3), F1: NEG 2.5 µg/ml, F2: NEG 3 µg/ml, F3: NEG 5 µg/ml (NEG Cst: nanoemulgel), F1: EG 2.5 µg/ml, F2: EG 3 µg/ml, F3: EG 5 µg/ml (+DMSO 0.1%) (EG: emulgel cst)

The results of the determination of Celastrol in the assay test in the nanoemulgel preparations obtained levels of 90.98% for F1, 91.70%, and 74.50%. For example, in formulation 1 with a dose of 2.5 µg/ml if 100 ml of v/v preparation were made into 100.2 grams of the dosage after adding a 305®sepigel basis, the percentage of the 2.5 µg/ml dose that what mixed was calculated in the whole preparation. By weighing a sample of 1.0 gram carefully and analyzing it by HPLC, the percentage of the area on the HPLC was calculated.

To determine the level of Celastrol in nanoemulgel preparations, i.e., how much Celastrol was absorbed, the adsorption efficiency (EE) of the remaining sediment was calculated after centrifuging at 4500 rpm for 30 min. The results, in F1 were 79.46%, F2 85.54% and F3 94.54%.

In vitro penetration tests, using Franz diffusion cells (CDF) (fig. 6,7), show that the formula 2.5 µg/ml Celastrol nanoemulgel has difficulty to penetrate the mouse skin membrane, compared to the concentrated formula 3 µg/ml and 5 µg/ml. Those formulas (3 µg/ml and 5 µg/ml) penetrated very well through the mouse skin membrane. This can be caused by the small cumulative amount of Celastrol content in the donor compartment in formula 1 (2.5 µg/ml), affecting the level of penetration or flux. In addition, the three nanoemulgel formulas considered to have better penetration capabilities than the control formula, which is not a nano formula, even the control formula is added by 0.1% DMSO to facilitate penetration. The results obtained that the nanoemulgel formula was still superior to the control formula.

Fig. 8: TEM Morphology result a. before and b. after HPH
Fig. 9: Homogeneity test and nanoemulgel type
CONCLUSION

Transdermal nanoemulgel containing Celastrol has been developed successfully to obtain a nanoemulsion area that is suitable for the purpose of the transdermal route of ≤200±22 nm.

By using Celastrol as an active ingredient, 5% isopropl myristate (IPM) as the oil phase, tween 80, and propyleneglycol (PG) are both referred to as mix 45% and 50% water, in comparison (5:45:50). The combination of these preparations resulted in the size of the particle size of globules for the average D90 in each preparation formula made was 2.5 μg/ml 17±2 nm, 3 μg/ml 189±2 nm, and 5 μg/ml 121±2 nm. The potential zeta values obtained were -14.6 mV, 24.8 mV, -4.86 mV, and the PDI value for all formulas was 0.1. In vitro test results of all transdermal nanoemulgel Celastrol preparations produced better penetration than control preparations containing Celastrol+0.1% DMSO gel.

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AUTHORS CONTRIBUTIONS

Nur Alam Abdullah conducted a validation method of nanoemulsion formula using Chemx program 7.00, conducted nanoemulsion formulation and evaluation, HPLC analysis, interpreted the results, wrote the manuscript draft. Mahdi Jufri was responsible for supervising the experiments and secured financial support. Abdul Mun’im and Fadlina Chany Saputri reviewed and edited the manuscript. All 4 authors have read the and approved the final version of the manuscript.

CONFLICT OF INTERESTS

The authors declared that they have no conflict of interest.

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Fig. 10: Particles size analyser (PSA)
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