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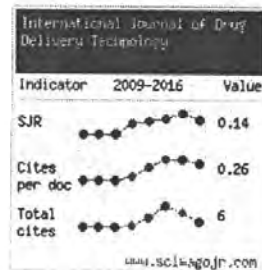
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
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Anti-Inflammatory Evaluation of NLC (Nanostructured Lipid Carriers) Meloxicam In-Vivo

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ABSTRACT

Objective: The aim of this research study was to investigate the anti-inflammatory effect of NLC meloxicam. NLC contains solid and liquid lipid. Monostearin as solid lipid and Miglyol 808 as liquid lipid. **Methods :** NLC meloxicam was prepared using emulsification method with three different lipid ratio. NLC meloxicam was prepared and characterized for measuring the pH, viscosity, particle size, and entrapment efficiency. The rat paw edema test was performed to evaluate the anti-inflammatory activity of three formulations NLC meloxicam. **Results :** based on research result shows that the smaller the solid lipid concentrations, particle size is the larger, the greater viscosity, thus increasing occlusive NLC to the skin. The third formula has the greatest solid lipid concentration shows the smallest AUC value but once in a statistical test known to be significantly different from the three formulas. **Conclusions :** NLC meloxicam showed that it had anti-inflammatory effectiveness.

Keyword : NLC, meloxicam, antiinflammatory, paw edema.

INTRODUCTION

Transdermal application of NLC is regarded as an attractive strategy since the adhesion of nanosized NLC to the skin surface provide an occlusive effect to the skin. The occlusive effect can eventually lead to an increase in skin hydration and promote the deposition of drugs into the viable skin by reducing corneocytes packing and widening inter-corneocytes gaps¹. Additionally, the components of NLC such as lipid and surfactants can also function as permeation enhancers by reducing the barrier properties of SC and consequently increasing the permeation of drug through the skin². Since NLC dispersion possesses low viscosity and is consequently inconvenient to use on the skin, they must be converted into gel to ease the application and to prolong residence time on the skin.

Meloxicam, a non-steroidal anti-inflammatory drug (NSAID), is widely used in the symptomatic treatment of joint disorders (osteoarthritis and rheumatoid arthritis). Its use is associated with various gastrointestinal side effects similar to other NSAIDs³. Transdermal administration of meloxicam can be specifically advantageous for the management of arthritic conditions as it would bypass the gastrointestinal tract and would allow an increased level of drug locally. Contemplating all these points, we aimed to develop NLC-based gel for the transdermal delivery of meloxicam. NLC gel was suitably characterized for particle size, viscosity, pH, entrapment efficiency, and in vivo pharmacodynamic activity.

MATERIAL AND METHOD

Materials

Meloxicam, Monostearin (PT BRATACO) as a solid lipid, Miglyol 808 (Sigma Aldrich), Tween 80, 6 ± 0.05 pH buffer made of NaOH (sodium hydroxide) and KH₂PO₄ (potassium dihydrogen phosphate) pro analysis (Merck), carrageenan, aquades, male Wistar rats (aged 6-8 weeks and weighed 150-200g), food and drink rat.

Methods

Preparation of nanostructured lipid carriers (NLC)

The method used in this research was emulsification. NLC system meloxicam was made by melting the lipid phase of solid and liquid (Monostearin and Miglyol 808) using different ratio (6: 4, 7: 3, 8: 2), and meloxicam at temperature of 65°C. At the same time, a solution of a surfactant (Tween-80 and phosphate buffer pH 6.0 ± 0.05) was prepared and heated at temperature of 65°C. Then the hot surfactant solution was dispersed into the hot lipid phase using ultra-turax with the speed of 3400 rpm for 30 minutes. Further cooling was done at room temperature, 25°C, for about 25-30 minutes and then stirred using a magnetic stirrer at 100 rpm until the temperature of 25°C was reached.

Physicochemical characterization

The physicochemical characterization consist of measurement of particle size and polydispersity index by using Delsa™ NanoSubmicron Particle Size Analyzer, measurement of pH using a pH meter, measurement of viscosity using cone and plate viscometer.

Entrapment Efficiency

1 g of NLC was weighed, then was added with phosphate buffer pH 7.4 ± 0.05 up to 10 mL. Measurement of percent entrapment efficiency was done using UV

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Table 1: Lipid ratio used.

Material	Function	Concentration (%-b/b)		
		Formula 1	Formula 2	Formula 3
Meloxicam	Active ingredient	1	1	1
Monostearin	Solid lipid	6	7	8
Miglyol 808	Liquid lipid	4	3	2
Tween 80	Surfactan	5	5	5
Phosphat buffer pH 6 ± 0.05	until 100			

Table 2: Results of physicochemical characterization NLC meloxicam.

Parameter	Formula 1	Formula 2	Formula 3
Particle analyze (nm)	645.7 ± 60.5	681.4 ± 159.7	867.9 ± 61.6
Polidispersity index	0.283 ± 0.03	0.372 ± 0.04	0.320 ± 0.06
pH	4.85 ± 0.05	4.83 ± 0.02	4.93 ± 0.1
Viscosity (cPs)	21.0 ± 1.3	42.2 ± 0.0	82.1 ± 0.1
Entrapment efficiency (%)	95.58 ± 0.31	91.98 ± 0.96	84.78 ± 1.58

spectrophotometry. Separation result, which was meloxicam that was trapped in the NLC system, would precipitate after separated using centrifuge at 1000 rpm for 2 minutes. Meloxicam that was not stuck in the NLC system would be dispersed in phosphate buffer at pH 7.4 ± 0.05 as the supernatant. We then measured the free drug concentration in the water phase of the NLC dispersion. The blanko was NLC system without addition of meloxicam and test samples were prepared in accordance. Furthermore, was calculated using the formula:

$$EE(\%) = \frac{[Ct - Cf]}{Ct} \times 100\%$$

Information

Ct: The amount of drug used

Cf: The number of drug ingredients are water phase

Anti-inflammatory evaluation

Carrageenan-induced rat paw edema volume model was used to assess the anti-inflammatory activity of formulation. The study protocol was approved by the Ethical Committee of Veterinary, University of Airlangga, Indonesia. The rats (150–200 g) were randomly divided into six groups (3 groups of control, 3 treatment groups), five rats each. Six groups consist of control formula 1, formula 1, control formula 2, formula 2, control formula 3, formula 3. Fifty milligrams were applied on the subplantar region of the left hind paw of each rat. An hour after the application, subplantar injection of 0.1 ml of a 1% w/v freshly prepared carrageenan in normal saline was given into the left hind paw of each rat. Measurements of the paw volume were performed before and after NLC application. The changes of hind paw thickness were measured using a caliper. The formula used to calculate the hind paw thickness was:

$$\% \text{ hind paw thickness} = \frac{ht - h0}{h0} \times 100 \%$$

ht is the hind paw thickness was measured every half hour after carrageenan was injected. While h0 is the hind paw thickness before carrageenan was injected. Therefore, the anti-inflammatory effectiveness of the NLC was observed using the formula of the trapezoidal area under the curve (AUC) from the increase in percentage hind paw thickness profile.

Data analysis

One way ANOVA was used for statistically analyze the data.

RESULT

Manufacture of the NLC meloxicam in this study conducted using emulsification methods. In this study we used three formulas using monostearin solid lipid and liquid lipid miglyol 808 with ratios contained in Table 1. The results of physicochemical characterization in this study shown in Table 2.

Data reported is the average of n = 3

Anti-inflammatory evaluation

Determination of the anti-inflammatory effectivity tests on each of the control formula and formulas can be seen in Figure 1, 2, and 3.

DISCUSSION

The particle size is one of the characteristics of the NLC. The higher liquid lipid used can reduce the particle size, so that increase the release of the active ingredient⁴. NLC has small particle size, causing contact between the surface of the NLC with a greater skin surface and cause their adhesive properties to the skin. This helps improve the adhesive properties of the release of active ingredients through the stratum corneum and provide an occlusive effect which enables penetration of active ingredients on the skin⁵. After the one-way ANOVA statistical analysis on the measurement of particles, obtained p (sig) of 0.083 which was greater than p table (0.05). It showed no significant difference in the particle size of the third formula.

Measurement of polydispersity index (PI) aims to determine the particle size distribution⁶. The more homogeneous the distribution of particle size, the value of its polydispersity index will be closer to zero⁷. On the measurement of polydispersity index (PI) after one way ANOVA statistical analysis, obtained p value (sig) 0.132 greater than p table (0.05). This shows there was no significant difference in the PI of the third formula.

pH evaluation is also related to the acceptability of the application. Based on results of the pH measurement it is known that pH of NLC meloxicam is within skin's pH

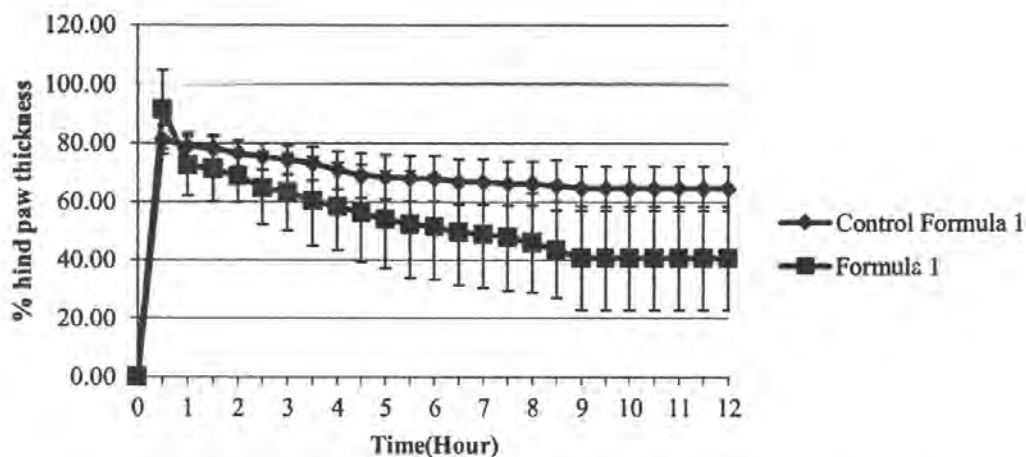


Figure 1: The curve of relation between time (hours) and % thick hind paw, mean \pm SD of control formula 1 and formula 1.

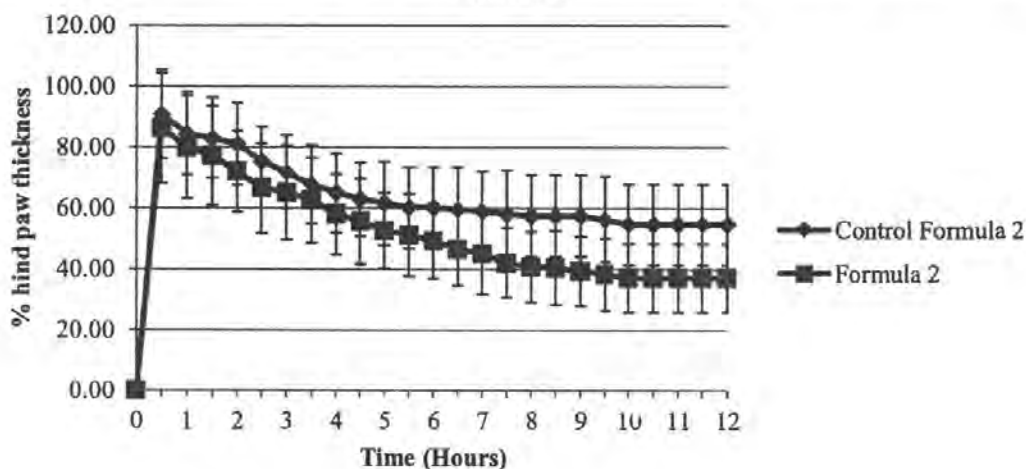


Figure 2: The curve of the relationship between time (hours) and % thick hind paw, mean \pm SD of control formula 2 and formula 2.

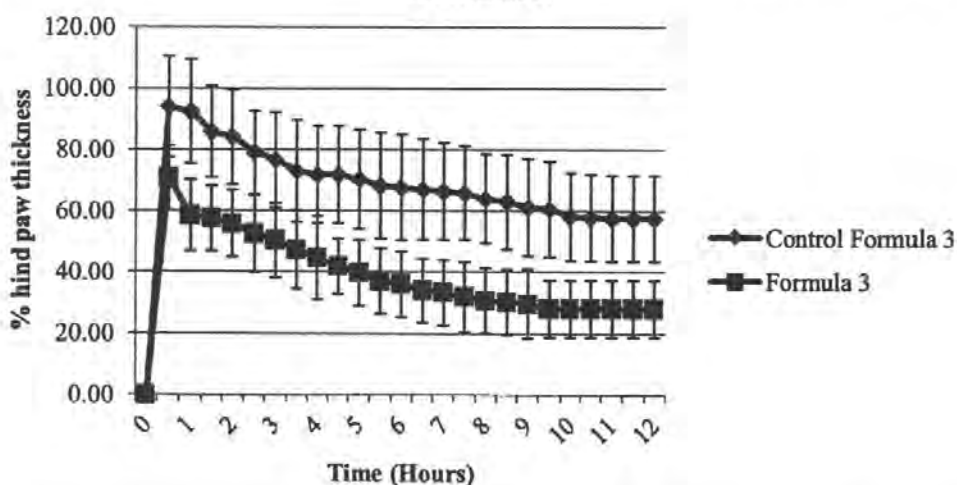


Figure 3: The curve of the relationship between time (hours) and % thick hind paw, mean \pm SD of control formula 3 and formula 3.

range 4.5-6.5⁸. Based on the results of one-way ANOVA statistical analysis on meloxicam NLC pH evaluation, the

value of p (sig) of 0.689 was greater than p table (0.05). This shows that there was no significant difference

between pH NLC meloxicam Formula 1, Formula 2 and Formula 3.

Viscosity associated with application preparation and release of the drug. Preparations should have an optimum viscosity to be spread and attached to the skin in sufficient time⁷. Based on the results of one-way ANOVA statistical analysis, obtained value of *p* (sig) of 0.000, which was smaller than *p* table (0.05). It showed no significant difference between the formulas 1, 2, and 3. To find out which formula was different, then proceed with the analysis of multiple comparison test using Post Hoc Multiple Comparisons. Produced comparative test *p* value less than $\alpha = 0.05$ in the formula 1, Formula 2 and Formula 3 so that it can be concluded that there were significant differences in viscosity Formula 1 and Formula 2, Formula 2 and Formula 3 and Formula 2 and Formula 3. These results proved that greater amount of solid lipid than the liquid in the formation of NLC lipid capable of increasing the viscosity of dosage¹.

From the result of the determination entrapment efficiency percentage, known that the higher liquid lipid meant the higher the amount of active ingredient that was trapped in the system. This is possibly because the active ingredient dissolved in the liquid lipid. In addition, the incorporation of lipid liquid on solid lipid can cause disruption of the crystal lattice in solid lipid so as to improve the efficiency of entrapment the active ingredient⁴. Results of entrapment efficiency is in line with the results of Yuan et al. showing that trapping of progesterone, as an active ingredient, the highest in oleic acid composition, as a liquid lipid, at most⁹. Based on the one-way ANOVA statistical analysis of trapping efficiency test of meloxicam in NLC system, the value of *p* (sig) 0,000, smaller than *p* table. This showed that there were significant differences between the three formulas To find out which formula was different, then proceed with the analysis of multiple comparison test using Post Hoc Multiple Comparisons. Produced comparative *p* value less than $\alpha = 0.05$ for all comparisons formula so that it could be concluded that there is a difference between the trapping efficiency formula.

Evaluation of the effectiveness of anti-inflammatory in vivo study was conducted using experimental animals, Wistar, male rats were divided into six groups, each consisting of 5 rats. Six of the group include 3 negative control group and three treatment groups. The treatment group consisted of Formula 1, Formula 2 and Formula 3. The negative control group was used to compare the thick edema between the formula and the group base without the active ingredient.

Parameter used in this study was Area Under the Curve (AUC). AUC is the area under the curve. AUC showed change of hind paw thickness was the effect of anti-inflammatory¹⁰. The lower the value of AUC indicated more effective inhibition provided. This was shown by the narrower % hind paw thickness given. The anti-inflammatory effect of NLC meloxicam was known by comparing the % AUC hind paw thickness treatment group and control group.

It was known that the control group Formula 1, Formula 2 and Formula 3 which does not contain meloxicam had

greater % of the average AUC \pm SD (816.75 ± 72.88 ; 758.47 ± 153.98 ; 839.88 ± 137.45 respectively), while the treatment group formula 1, formula 2 and formula 3 which contain the active ingredient meloxicam had lower % of the average AUC \pm SD than the control group (631.55 ± 183.05 ; 618.52 ± 149.59 ; $468.23 \pm 124.50\%$ respectively). Based on the study results, revealed that meloxicam NLC system was able to provide anti-inflammatory effect. The results showed that % hind paw thickness that formula 3 had was the best with the most minimal yield AUC values. Formula 3 had the least liquid lipid and the most solid lipid so that the active ingredients were less entrapped because the character of the active ingredient was more likely liquid lipid soluble. This was indicated by the value of % entrapment efficiency, formula 3 was the lowest. The active ingredients which were not entrapped might be able to penetrate in advance so as to achieve anti-inflammatory receptors that could provide a faster onset of action. Determination of % entrapment efficiency of the active ingredient in the NLC system can be used to predict the release of the active ingredient. The release profile of the active ingredient of NLC occurred as biphasic system. The first phase is the phase of initial burst release followed by prolong release of the active ingredient gradually. Burst release phase occurred on the active material which was not entrapment in the system. Then followed by the slow release of the drug entrapped in the system¹.

Percentage of AUC third edema formula was analyzed statistically using one-way ANOVA, the result showed no significant difference between the mean AUC of Formula 1, Formula 2 and Formula 3 ($\alpha = 0.082$).

CONCLUSION

Combination of Monostearin and Miglyol 808 at ratio of 6: 4 (formula 1), 7: 3 (Formula 2), 8: 2 (formula 3) does not affect the anti-inflammatory effect of NLC meloxicam.

CONFLICT OF INTEREST

Declared None

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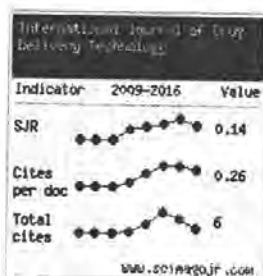
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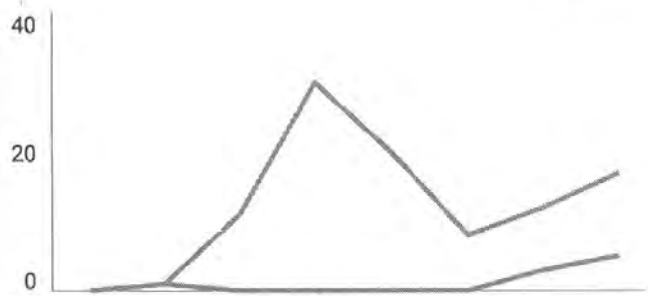
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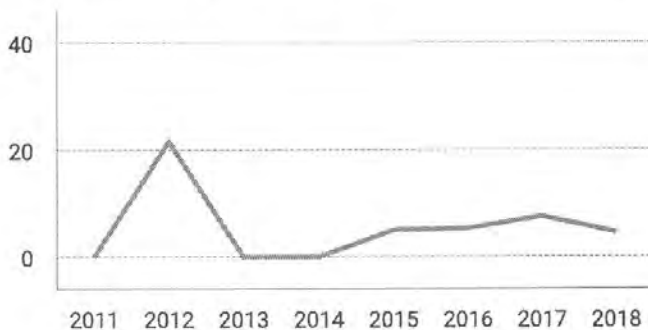
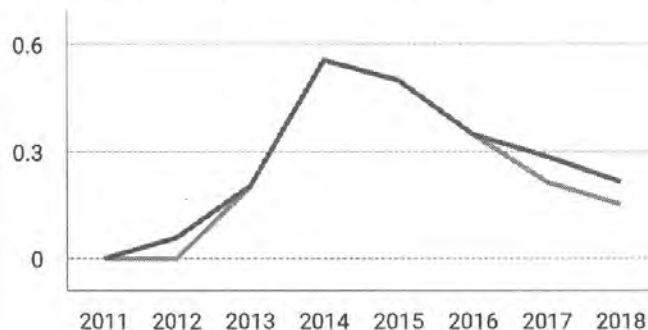


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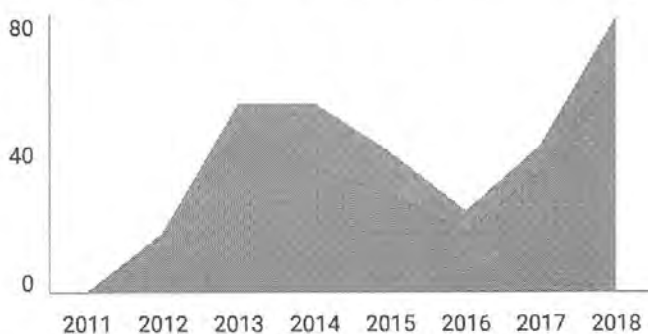
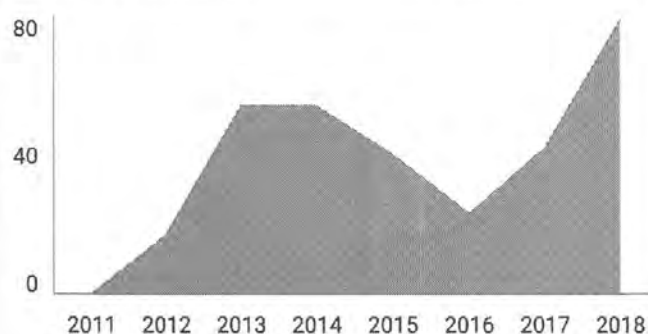
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