ANTI-INFLAMMATORY ACTIVITY OF PARA METHOXY CINNAMIC ACID (PMCA) IN NANOEMULSION USING SOYBEAN OIL

by Noorma Rosita

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INTRODUCTION

Inflammation is a protective mechanism of the local microcirculation to tissue injuries caused by physical trauma, stimulation by hazardous chemicals, heat, antigen-antibody reaction and the effect of microbial.[2] It is known to be involved in the inflammatory reactions such as release of histamine, bradykinin, prostaglandins, fluid cell migration, extravasations, tissue break down and repair which are aimed at host defense and usually activated in most disease conditions. Para methoxy cinnamic acid (PMCA) known has topical anti-inflammatory effect but only 0.64 compare with Na-diclophenac.[4] Its cause PMCA is a poorly soluble drug substance (BCS II), the solubility in acetate buffer pH 4.2 ± 0.2 was 70.04 ± 0.66 mg/liter.[3] So in this study to increase the solubility PMCA loaded in nanoemulsion using soybean oil. And then the anti-inflammatory activity of PMCA in nanoemulsion was measured by the release rate, penetration rate through rat skin using Franz diffusion cell and histological test on mice's ear skin.

MATERIAL AND METHODS

Research Material:
Para methoxy cinnamic Acid (Sigma Aldrich), soybean oil (PT Kurniajaya), Tween 80 (Sigma Aldrich), Span 80 (Sigma Aldrich), ethanol 96 % (Merck), acetic acid (Merck), sodium acetate (Merck), NaCl (Merck), NaH_2PO_4 (Merck), Na_2HPO_4 (Merck), croton oil (Sigma) and aquademineralisata (PT Brataco)

Animals
Male Wistar rats (150 - 230 gm) and mince (20 - 30 gm) were taken from PUSVETMA Surabaya. The animals were housed under standard conditions of temperature $(25\pm2)^0C$, 12/12 hours light/dark cycles and fed with standard pellets. All animal experiments were conducted with the permission from Animal Care and Use Committee (ACUC) of Veterinary Faculty, Airlangga University, Indonesia. (Reference number; 378-KE).

Research Method

Nanoemulsion formula containing PMCA
PMCA in nanoemulsion produced base on the formula presented in Table 1.

Materials	Concentration (%)
PMCA	0.2
Soybean oil	2.66
Span 80	1.92
Tween 80	18.66
Ethanol 96%	3.42
Acetate buffer solution pH 4.2 ± 0.2	ad 100

Table 1. PMCA Nanoemulsion Formula

Nanoemulsion formula used in this study is the result of a previous study by Erawati et.al, using a combination of surfactant Tween 80 and Span 80 with a ratio of 9:1 (having HLB 14), the ratio of surfactant and co surfactant 6:1, the ratio of oil phase (soybean oil) and water phase (acetate buffer solution pH 4.2 ± 0.2) is 27.5:1. [3]

Solubility test
PMCA solubility test; on the nanoemulsion

using soybean oil (as oil phase) added with 500 mg PMCA, then it was sonicaited for 10 minutes and shaking with 150 rpm for 60 minutes. And then, the amount of PMCA dissolve in nanoemulsion was determined using spectrophotometer. After that the nanoemulsion PMCA characterized include; density, viscosity, pH, droplet morphology, droplet size and polydispersity index.

Release test

Membrane Preparation; the membrane used in the test release of PMCA in nanoemulsion system is a cellophane membrane. Membrane cut to size, then immersed in aqua-demineralization for ± 12 hours. A moment before use, the membrane is drained until no water is dripping, and then mounted on the surface of the receptor compartment of Franz diffusion cell.

Measurement the amount of the PMCA release from the nanoemulsion; receptor compartment of Franz diffusion cell filled with phosphate buffer pH 6.0 ± 0.2 up to full. Then, 2 ml of nanoemulsion PMCA inserted into the donor compartment. Experimental temperature is set and maintained at a temperature of 32 ± 2°C. Magnetic stirrer rotated at a speed of 100 rpm. Samples of 1 ml were taken within a certain time interval, i.e. at 0, 5, 10, 15, 30, 45 minutes, and then 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24 hours. Immediately after sampling medium was replaced with phosphate buffer pH 6.0 ± 0.2 with a volume of samples taken. Subsequently, samples were taken observed with spectrophotometer. PMCA concentration in the sample was calculated using the regression equation of standard curve. Determination of PMCA cumulative amount released per unit membrane area (mg/cm2) was calculated from the concentration obtained each time (μg/ml) which had been corrected with the Wurster's equation. Furthermore, multiplied by the number of medium and divided by the membrane surface area. The results obtained by the cumulative number of PMCA released per unit time. The release profile of PMCA, is done by making a relations curve between the cumulative number of PMCA released (mg/cm2) versus time (minutes). The release rate of (Flux release) PMCA was obtained from the slope of the regression equation in the steady state.

Penetration test

Membrane Preparation; skin male Wistar rats that had been shaved used as the membrane in the penetration test of PMCA in nanoemulsion system.

Measurement the amount of PMCA penetrate through rat skin; receptor compartment of Franz diffusion cell filled with phosphate buffer pH 7.0 ± 0.2 up to full. Then, 2 ml of nanoemulsion PMCA inserted into the donor compartment. Samples of 1 ml were taken within a certain time interval as in release test.

RESULT AND DISCUSSION

The PMCA solubility in nanoemulsion 3.07 ± 0.19 g/liter (presented in Table 2) increased than its solubility in acetate buffer pH 4.2 ± 0.2 is 70.04 ± 0.66 mg/liter. The characterics of nanoemulsion containing PMCA presented in Table 3; the density value was 1.0263 ± 0.0002 is almost equal to the density of water; the viscosity was 5.58 ± 0.05cP; the pH value was 4.47 ± 0.006 it was in the range of human skin pH, so it will not cause irritation; the droplet size was 57.3 ± 7.6 nm and the polydispersity index was 0.548 ± 0.044 it was indicate a moderate particle size distribution.[1] The droplet morphology of empty nanoemulsion and nanoemulsion containing PMCA in saturated solubility by TEM.type JEM-1400 was resented in Figure 1.

Medium	The amount of PMCA dissolved
Nanoemulsion	3.07 ± 0.19 g/liter
Acetate buffer pH 4.2 ± 0.2	70.04 ± 0.66 mg/liter

Table 2. The solubility of PMCA in nanoemulsion and in acetate buffer pH 4.2 ± 0.2

The characteristics of nanoemulsion containing PMCA	
density	1.0263 ± 0.0002
viscosity	5.58 ± 0.05 cP
pH	4.47 ± 0.006
droplet size	57.3 ± 7.6 nm
polydispersity index	0.548 ± 0.044

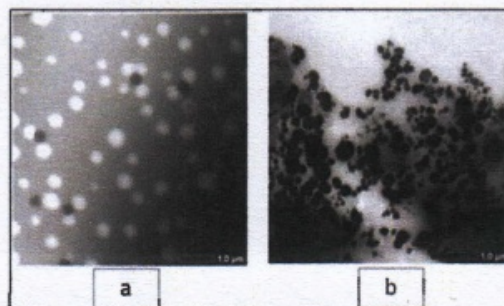Table 3. The characteristics of nanoemulsion containing PMCA

Figure 1. Droplet morphology of a) empty nanoemulsion and b) nanoemulsion containing PMCA in saturated solubility by TEM type JEM-1400

The release profile and the release rate of PMCA in acetate buffer pH 4.2 ⊡ 0.2 and in nanoemulsion were presented in figure 2 and figure 3. After investigate for 24 hours (1440 minutes) the result shows that the release rate of PMCA in nanoemulsion (0.4024± 0.0339 µg/cm2/minute) increased compare with it in acetate buffer pH 4.2 ⊡ 0.2 is 0.0239 ± 0.0003 µg/cm2/minute.
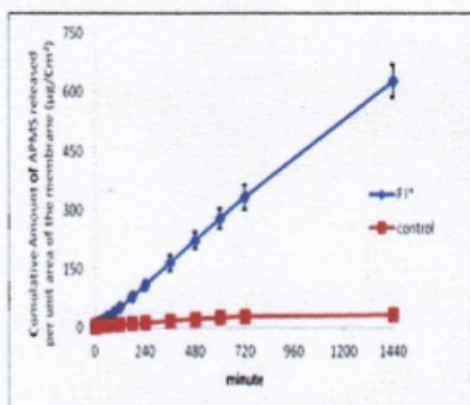
Figure 2. Release profile of PMCA in acetate buffer pH 4.2 ±0.2 (control) and in nanoemulsion (F I*)
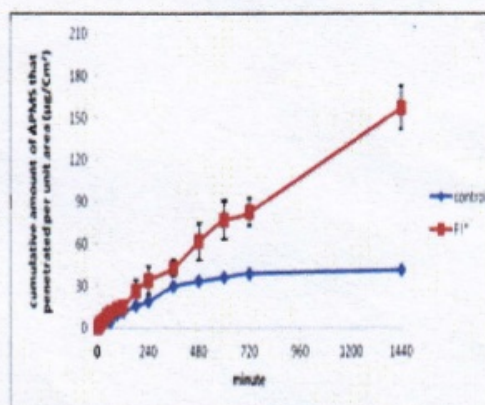
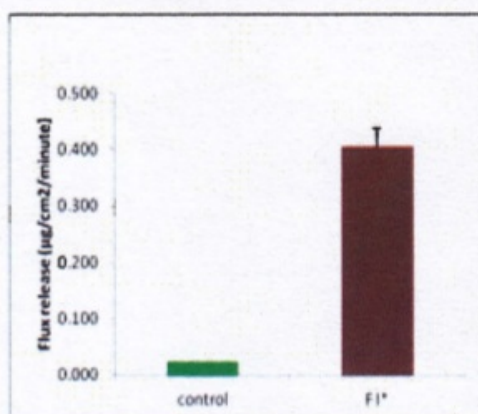**Figure 2.** Penetration profile of PMCA in acetate buffer pH 4.2 ± 0.2 (control) and in nanoemulsion (F I*)

Figure 3. Histogram of release rate of PMCA in acetate buffer pH 4.2 ± 0.2 (control) and in nanoemulsion (F I*)
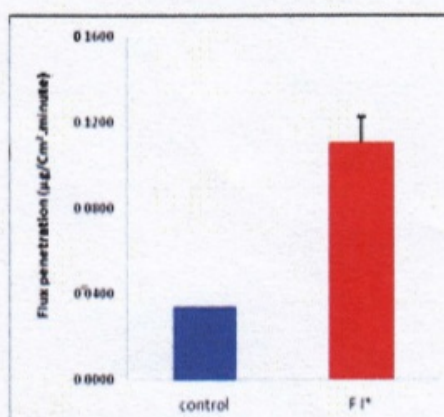

Figure 5. Histogram of penetration rate of PMCA in acetate buffer pH 4.2 ± 0.2 (control) and in nanoemulsion (F I*)

Also the penetration rate of PMCA in nanoemulsion was increased too (0.1513 ⯑ 0.0314 µg/cm2/minute) compare with the penetration rate of PMCA in acetate buffer pH 4.2 ⯑ 0.2 (0.0341 ⯑0.0003 µg/cm2/minute) as presented in figure 4 and 5. The increased of penetration rate of PMCA in nanoemulsion caused by 1) the solubility of PMCA in nanoemulsion increased (more than 43x compare with the solubility of PMCA in acetate buffer pH 4.2 ⯑ 0.2) and 2) the surfactant and co surfactant in nanoemulsion formula can function as enhancers.

From figure 6 and table 4, known that the anti-inflammatory effect of PMCA in nanoemulsion (F I*) higher than the anti-inflammatory effect of PMCA in acetate buffer pH 4.2 ☐ 0.2 (control). The result of statistical test using ANOVA-one way (P = 95%), known there are no significant different between the ears mice skin thickness after treated with PMCA in nanoemulsion (F I*) with health skin (K-) but thinner than inflammation skin (K+) also with the ears mice skin after treated with PMCA in acetate buffer pH 4.2 ☐ 0.2 (control).
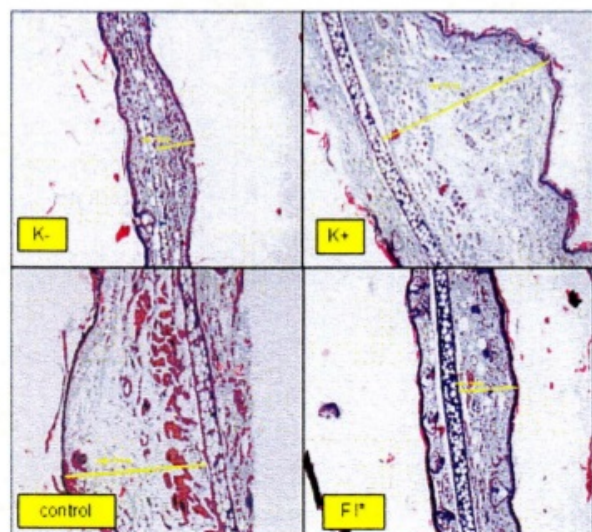

Figure 6. The histology profile of ears mice skin, health skin (K-), inflammation skin (K+), after treated with PMCA in acetic buffer pH 4.2 ⯑ 0.2 (control), and in nanoemulsion (F I*)

Sample	The Average Of Skin Thickness (µm)
K-	282.96 ± 36.80
K+	592.53 ± 59.81
Kontrol	541.69 ± 56.56
F I*	310.56 ± 30.76

Table 4. The average of ears mice skin after treated with PMCA in acetate buffer pH 4.2 ⯑ 0.2 (control), and in nanoemulsion (F I*) compare with health skin (K-) and inflammation skin (K+).

CONCLUSION

The nanoemulsion with soybean oil as drug delivery system can increase the anti-inflammatory activity of PMCA.

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