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Research Article

THE ANTI-INFLAMMATORY ACTIVITY OF ESSENTIAL OIL OF CLOVE (*Syzygium aromaticum*) IN ABSORPTION BASE OINTMENT WITH ADDITION OF OLEIC ACID AND PROPYLENE GLYCOL AS ENHANCER

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ABSTRACT

Background : The optimal concentration of essential oil of clove in absorption base ointment as anti-inflammatory has been studied. The development of formulations can be done by adding oleic acid and propylene glycol as enhancers. The purpose of this study was to determine the anti-inflammatory activity of the essential oil of clove in absorption base ointment formula by adding a mixture of oleic acid and propylene glycol as enhancers.

Methods: In this study, the composition of oleic acid and propylene glycol was 100% oleic acid (FI), 50% oleic acid and propylene glycol (FII), and 100% propylene glycol (FIII). The profile of the anti-inflammatory activity essential oil of clove was carried out using male of mice Balb/C strain which was induced inflammatory with croton oil on back of skin. After treatment, it was sacrificed and then was taken the back of skin to get histopathological preparation. After that, the epidermal thickness, number of inflammatory cells, and cyclooxygenase (COX)-2 expression can be measured.

Results : Based on the results of the test, it shows that FIII has the smallest of the amount of COX-2 expression, the number of inflammatory cells, and the epidermal thickness so the addition of the composition enhancer provides good anti-inflammatory activity.

Conclusion: The increasing concentration of propylene glycol caused the raising activity of essential oil of clove as anti-inflammatory.

Keywords: Absorption base, Anti-inflammatory, Enhancer, Essential oil of clove.

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INTRODUCTION

Essential oil of clove has biological activity because it contains high levels of eugenol [1] so can use as an antiseptic and analgesic in the treatment of teeth and mouth [2]. The eugenol mechanism of action as anti-inflammatory agent is via inhibition of prostalglandin synthesis and neutrophil chemotaxis. In addition, it is also able to inhibit the NF-kB factor in activating the tumor necrosis factor-α (TNF-α) and inhibiting the expression of cyclooxygenase (COX)-2 in lipopolysaccharide (LPS) stimulated by macrophages. Research has shown that eugenol suppresses TNF signals and COX-2 expression, which shows its potential as an anti-inflammatory agent [3-5].

Based on this activity, the study about the activity of essential oil of clove in formulation of cream, lotion and ointment in absorption base has been conducted [6-10]. The development of a formula for essential oil of clove was continued. One of the ways that can be done to develop a formula is by adding an enhancer to the preparation of formulation. Enhancers or penetrating enhancers are ingredients that can increase skin permeability or reduce skin impermeability. The material of the penetrating enhancers does not have therapeutic effect, but it can transport drugs from dosage forms into the skin [11].

The previous study showed that the optimal concentration essential oil of clove in absorption base ointment which had the best anti-inflammatory activity and met the requirements was 2.5% [12]. This study was carry out to develop the formulation of essential oil of clove in absorption base ointment with addition of mixture of oleic acid and propylene glycol as enhancer to increase the capability of essential oil of clove as anti-inflammatory.

MATERIALS AND METHODS

Materials and tools

This study used essential oil of clove as the material which was obtained from the Center for Essential Oils Studies, Indonesian Islamic University, Sleman, Yogyakarta. The ingredients of ointment with pharmaceutical degree such as adeps lanae, cera alba, stearyl alcohol, vaseline white, oleic acid, and propylene glycol. The animal test used male mice of Balb/C strain with 2–3 months of age. The equipment used glassware (Pyrex) water bath (Memmert), analytical weighing (Ohaus), and microscope (Olympus).

All of the research procedures have obtained the ethical approval letter from the Research Ethics Committee numbered 011508062 in 2015.

Research procedure

Preparations of ointment

The essential oil of clove formulation is presented in Table 1. Each formula was varied a concentration of oleic and propylene glycol with 2.5% concentration of essential oil of clove. The preparation of ointment was done using fusion method. The essential oil was added when the base was get cold [7].

Evaluation of anti-inflammatory activity

Anti-inflammatory activity evaluation was carried out on four groups of Balb/C strain mice. The distribution of groups of mice was as follows:

Positive control groups

The positive control group was a group of mice that got induction of inflammatory agents (0.1 ml of croton oil concentration of 4%). After that, they were given a comparison product of 100 mg of topical sodium

diclofenac preparation which has been known to be efficacious as anti-inflammatory.

Negative control group
The negative control group was a group of mice that received induction of inflammatory agents alone without any anti-inflammatory agents.

Healthy control group
Healthy control group was a group of mice that did not get induction of inflammatory agents or the treatment of samples of Formula I, II, or III. This group was also known as the baseline group.

Ointment of essential oil of clove without enhancer
Group of ointment without enhancers was a group of mice that got induction of inflammatory agents and then they were given ointment without enhancers.

Ointment of Formula I, II, and III
The group of Formula I, II, and III was groups of mice that received inflammatory agent induction; then, they were given ointment of Formula I, II, and III.

The inflammatory induction procedures were first cleaning the mouse hair in the back. After 24 h, the back of the mouse was dripped with 0.1 ml of 4% croton oil in an area of 2×2 cm^2. Then, application of 100 mg ointment was done 30 min later. The treatment was given for 3 days. After that, the mouse sacrificed and the back tissue was taken to make the painting of Haemotoxillyn eosin and COX-2 preparation. Microscopic parameter which was observed was epidermal thickness, number of inflammatory cells, and COX-2 expression from each treatment of group FI, FII, and FIII with the control group, healthy controls, positive controls, and groups of formulas without enhancers. The tests were carried out on five animals as the animal testing in each group or five replications in 3 consecutive days. Furthermore, the painting results were observed under a microscope using 400 times magnification [13].

Table 1: Formula essential oil of clove in absorption base ointment with addition of oleic acid and propylene glycol as enhancers

Ingredients	Formula I (%)	Formula II (%)	Formula III (%)
Essential oil of clove	2.5	2.5	2.5
Adeps Lanae	2.61	2.61	2.61
Cera alba	7.11	7.11	7.11
Stearyl alcohol	2.61	2.61	2.61
White vaseline	75.17	75.17	75.17
Oleic acid	10	5	0
Propylene glycol	0	5	10

Formula I (FI) with composition of 100% oleic acid and 0% propylene glycol
Formula II (FII) with composition of 50% oleic acid and 50% propylene glycol
Formula III (FIII) with composition of 0% oleic acid and 100% propylene glycol

Table 2: The results of epidermal thickness test of essential oil of clove in absorption base ointment with the addition of oleic acid and propylene glycol as enhancer

Treatment groups	Epidermal thickness (μm)
Healthy control	81.9±26.88*
Positive control	107.2±8.42*
Negative control	228.0±12.95
Formula without enhancer	167.3±16.43
Formula I	151.71±4.67*+@
Formula II	137.75±3.95*+@
Formula III	131.05±1.93*+@

*Significant difference with negative control, @significant difference with healthy control, +significant difference with positive control, -significant difference with Formula I

Data analysis
Data were analyzed using simplex lattice design method to find the profile of epidermal thickness, the number of inflammatory cells, and the number of COX-2 expression. The differences between formulas were analyzed using one-way ANOVA with 95% level confidential.

RESULTS

Parameter to evaluate the activity of dosage form was microscopic observation based on epidermal thickness, the amount of inflammation cell, and cell number with COX-2 expression. Data were presented in Tables 2-4.

The results of statistical analysis showed the significant difference between healthy control and negative control in all parameters. It means that croton oil can cause irritation and swelling of the skin if it was used

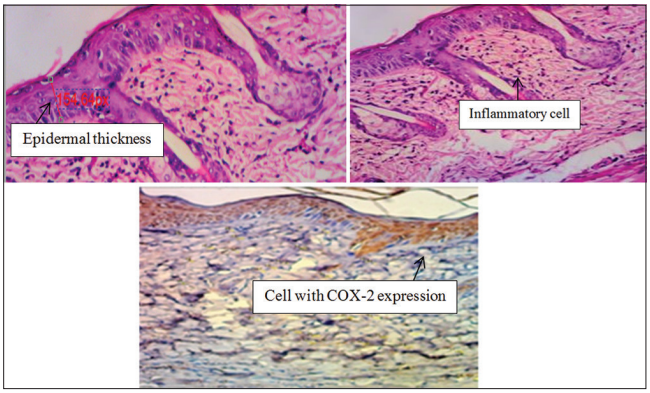

Fig. 1: The microscopic picture of epidermal thickness, inflammatory cells, and cells with cyclooxygenase-2 expression at ×400

Table 3: The result of the number of inflammatory cell test in MABC absorbent base ointment with the addition of oleic acid enhancers and propylene glycol

Treatment groups	Number of inflammatory cells
Healthy control	13.17±2.32*#+
Positive control	59.67±2.50*#@
Negative control	70.83±3.66#+@
Formula without enhancer	52.33±8.69*+@
Formula I	36.18±3.56*+#-
Formula II	35.68±2.49*#+
Formula III	30.63±1.79*#+@

*Significant difference with negative control, #significant difference with formula without enhancer, @significant difference with healthy control, +significant difference with positive control, -significant difference with Formula III

Table 4: The results of statistical analysis of cyclooxygenase-2 expression in MABC absorbent base ointment with the addition of oleic acid enhancers and propylene glycol

Treatment groups	Number of inflammatory cells
Healthy control	18.16±3.65*#+
Positive control	31.23±2.10*+@
Negative control	43.63±2.41#+@
Formula without enhancer	25.68±1.73*+@
Formula I	18.02±2.39*+#-
Formula II	17.86±2.73*#+
Formula III	11.57±2.59*#+@

*Significant difference with negative control, #significant difference with formula without enhancer, @significant difference with healthy control, $significant difference with negative control, +significant difference with positive control, ^significant difference with Formula III

topically [14]. On histochemical observations by using the HE method, crotton oil that was administrated topically can induce hyperplasia, infiltration of leukocytes, edema, neutrophil infiltration, a prostaglandin production and an increase in vascular permeability [15-17]. There was a significant difference between negative control and positive control. It means the activity of natrium diclofenac in Voltaren as active substance for anti-inflammatory. The mechanism of diclofenac was by inhibiting of the activity of COX-1 and COX-2 enzyme, thromboxane prostanoid receptor that influenced to release and uptake of arachidonic-acid, lipoxygenase enzyme, and activating of oxide-cyclic guanosine monophosphate pathway [18,19]. However, there was a significant difference between healthy control and positive. It was probably due to the duration of the application of Voltaren as positive control just for 3 days so the effect was not effective yet.

The application of formula can reduce the epidermal of thickness, the number of inflammatory cell, and cell with COX-2 expression. It was supported with the result of statistical analysis that showed the difference significant between negative control and formula group. It shows the activity of eugenol as anti-inflammatory agent in essential oil of clove. The mechanism of eugenol as anti-inflammatory was inhibit the expression of COX-2 in macrophage-stimulated LPS and reduced production leukotrienes as mediator inflammation [20,21]. There was a significant difference between positive control and formula group. It means that the activity of eugenol was better than natrium diclofenac. However, there was still significant difference between healthy control and formula group. It was probably due to the duration of application of formula just for 3 days so the effect was not effective yet.

The activity of eugenol as anti-inflammatory increased with the addition of enhancer in the formula. The epidermal thickness, the number of inflammatory cells and the number of cells with COX-2 expression in the formula group were smaller than in the formula without enhancer. Enhancer could increase the capability of eugenol to penetrate the layers of skin so it can reach the area of inflammatory to give its activity.

Data showed that the increasing composition of propylene glycol caused the decreasing of epidermal thickness, the number of inflammatory cell, and cell with COX-2 expression. This result similar with the previous study. The amount of cell with COX-2 expression, inflammatory cell and epidermal thickness was decline after the application of formulation of essential oil of clove in water soluble base ointment and lotion that contain mixture of oleic acid and propylene glycol as enhancer. This happen when the amount of propylene glycol increased [22,23]. The mechanism of propylene glycol as an enhancer was by dissolving the keratin layer of the stratum corneum, interacting, and disrupting the arrangement of intracellular lipids in the stratum corneum. In addition, propylene glycol can increase drug solubility in the stratum corneum so the amount of drug that passes through the skin can increase [24-29].

CONCLUSION

Based on the result, it can be found that the activity of eugenol in essential oil of clove in absorption base ointment can be increased with the addition of enhancer. Its activity was better than natrium diclofenac in positive control. The formula containing propylene glycol needs to be evaluated for its anti-inflammatory activity for a longer duration to ensure its effectivity.

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