

Anti-inflammatory activity of essential oil of clove (*Syzygium aromaticum*) in O/W and W/O Creams

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Previous studies have shown that the formulations of clove essential oil with a concentration of 5% in W/O cream and 2.5% in O/W cream have anti-inflammatory properties. The formulations are developed by adding enhancers, namely oleic acid and propylene glycol. The optimum compositions of oleic acid and propylene glycol are 50%:50% in W/O cream and 30%:70% in O/W cream. This study aimed to determine the effects of different compositions of creams on their anti-inflammatory activities. The creams were evaluated for their anti-inflammatory properties by using mice with croton oil-induced inflammatory. The mice were sacrificed, and the back skin was removed to make histopathologic preparations at the end of the three-day inflammatory induction to obtain data of epidermal thickness and the number of cells with COX-2 expression. The results showed that the addition of oleic acid and propylene glycol as enhancers increased the anti-inflammatory activity of both O/W and W/O creams. Since the O/W cream decreased the thickness of the epidermis and the number of cells with COX-2 expression more than the W/O cream, the formulation of essential oil of clove in O/W cream is concluded as a better anti-inflammatory than that of W/O cream.

Keywords: clove essential oil, cream, oleic acid, propylene glycol, anti-inflammatory

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INTRODUCTION

Clove essential oil (CBEO) contains 70-80% eugenol as its main active ingredient (Nurdjanah, 2004). Eugenol has two biological activities, namely as antioxidant and anti-inflammatory (Alma *et al.*, 2007). The mechanism of its anti-inflammatory action involves inhibiting prostaglandin synthesis and neutrophil chemotaxis (Ma and Kineer, 2002) and blocking the transmission of nerve impulses useful for pain reduction in pulpitis (Barkin, 2015).

Based on these activities, several preparations have been formulated to optimize the extensive use of CBEO in the community. Some examples include two types of preparations, namely W/O and O/W creams. Previous research has found that optimum concentration of CBEO in W/O creams and O/W cream are 5% and 2,5%, respectively (Sugihartini *et al.*, 2015). In the next stage of their development, the formulation includes enhancers. Enhancers are additives that can increase the ability of active substances to penetrate the skin (Songkro, 2009). Penetration-enhancing ingredients do not have a therapeutic effect; instead, it can help to transport drugs from dosage forms into the skin (Kumar and Priyanka, 2015).

In the case of CBEO, the most common enhancers are oleic acid and propylene glycol. The mechanism of oleic acid to increase percutaneous absorption relies on its ability to change the fluidity of stratum corneum lipids and, subsequently, increase the permeability of this layer (Darijanto *et al.*, 2004). As for propylene glycol, it works by increasing the permeation of dosage forms into the skin membrane. Previous studies have identified the optimum compositions of oleic acid and propylene glycol in different types of creams, namely 50%:50% in W/O creams (Tuldjanah, 2016) and 30% oleic acid:70% propylene glycol in O/W creams (Yuliastuti, 2016). Referring to these findings, the research evaluated the anti-inflammatory effects of both W/O and O/W creams using two parameters, i.e., epidermal thickness and the number of cells with COX-2 expression, to determine the best formula to deliver CBEO as an anti-inflammatory.

MATERIALS AND METHODS

Materials

The research materials included clove essential oil (CBEO) standardized by the Center for Essential Oils Studies (CEOS) in Universitas Islam Indonesia. The ingredients (pharmaceutical grade) of the W/O and O/W creams were cetaceum, Cera Alba, liquid paraffin, sodium tetraborate, white vaseline, methylparaben, propylparaben, sodium lauryl sulfate, Aquadest, oleic acid, and propylene glycol. The research used croton oil (Sigma) to induce inflammatory in test animals, i.e., male BALB/c mice (weight 20-30 g, age 2-3 months). Other materials were physiological NaCl solution and 10% formalin solution.

Methods

Formulation of CBEO Creams

The formulas of the clove essential oil (CBEO) in W/O and O/W creams are described in [Tables I and II](#).

Table I. The formula of W/O creams of clove essential oil (Pranawati *et al.*, 2016; Tuldjanah, 2016)

Ingredients	Formula (%)
Standardized CBEO	5
Oleic acid	5
Propylene glycol	5
Cetaceum	11.1
Cera alba	8.9
Liquid paraffin	49.8
Sodium tetraborate	0.5
Aquadest	14.7

Table II. The formula of O/W creams of clove essential oil (Haque dan Sugihartini, 2015; Yuliasuti, 2016)

Ingredients	Formula (%)
Standardized CBEO	2.5
White Vaseline	20
Methylparaben	0.025
Propylparaben	0.015
Propylene glycol	5
Stearyl alcohol	20
Na lauryl sulfate	1
Distilled water	41.46
Oleic acid	3
Propylene glycol	7

Both W/O and O/W creams were created with fusion method. The preparation of the W/O cream was preceded with base melting. The oil-soluble base (liquid paraffin, Cera Alba, cetaceum, and oleic acid) and water-soluble base (propylene glycol, distilled water, and sodium tetraborate) were melted at the same temperature, 70°C, and mixed until homogeneous, creating a cream product. When the cream was finally cooled down, they were added with CBEO and stirred until homogeneous (Tuldjanah, 2016).

The same procedure was applied to the preparation of the O/W CBEO cream, which started with the fusion of solid stearyl alcohol at 75°C. The oil-soluble ingredients (i.e., white vaseline, propylparaben, stearyl alcohol, Na lauryl sulfate, and oleic acid) were mixed in a warm mortar until homogeneous. The water-soluble materials (i.e., propylene glycol, distilled water, and methylparaben) were also mixed until homogeneous. Afterward, the two mixtures were stirred until homogeneous and left to cool down. Finally, the CBEO was added to the mixtures and stirred until homogeneous (Haque and Sugihartini, 2015).

Anti-inflammatory activity screening test of CBEO creams

The screening test for anti-inflammatory properties of CBEO creams first divided the 42 test mice evenly into seven groups. These groups included one healthy control group that was not given any treatment, while the other six groups received croton oil to induce inflammatory. The six groups were then divided based on the treatment they received after the application of croton oil, namely negative control (no treatment), positive control (treated with comparative product Voltaren® gel), test

formula group (cream consisting of base, CBEO, and a mixture of enhancers—oleic acid and propylene glycol), no-enhancer group (cream consisting of base and CBEO), no-CBEO group (cream consisting of base and a mixture of enhancers—oleic acid and propylene glycol), and base group (cream base).

The induction of inflammation began with shaving the hair on the back of the mice that had been previously smeared with Veet, a commercial hair removal cream. Then, this back skin was marked with a 2x2 cm² grid. After 24 hours, this skin was dripped with 4% croton oil as an inflammation inductor. In the groups that received treatments, 100 mg of the preparations were applied to the skin after 30 minutes of induction. The induction of inflammation by croton oil and the administration of the preparations were carried out for three days. Afterward, the mice were sacrificed, and the back tissue was removed to create specimens using HE and COX-2 antibody staining methods. From the staining results, the epidermal thickness and the amounts of COX-2 expression were measured (Sugihartini *et al.*, 2015).

RESULTS AND DISCUSSION

The anti-inflammatory test results are represented with epidermal thickness and the number of cells with COX-2 expression, as summarized in Table III.

Table III. The anti-inflammatory test results based on epidermal thickness and the number of cells with COX-2 expression

Groups	Epidermal Thickness (µm) (n=6)		The Number of Cells with COX-2 Expression (n=6)	
	W/O Cream	O/W Cream	W/O Cream	O/W Cream
Healthy Control	3.68±0.32 *	3.68±0.32 *	6.03 ±0.86 * [#]	6.03 ±0.86 * [#]
Negative Control	25.94±4.60	25.94±4.60	71.22±9.88	71.22±9.88
Positive Control	6.49±1.42* [#] @	6.49±1.42* [@]	22.20±1.06* [@]	22.20±1.06* [#] @
Test Formula	4.80±0.66* [@]	4.85±0.91* [@]	21.82±5.74* [@]	17.44±2.83* [@]
No-Enhancer	7.62±0.83 [#]	6.06±0.91	22.75±3.85	18.47±2.22
No-CBEO	6.99±0.88 [#]	6.99±0.88	28.94±9.05 [#]	26.93±2.59 [#]
Base	9.25±1.83 [#]	8.34±1.30 [#]	26.93±2.59 [#]	26.18±4.09 [#]

Notes: * significant difference with negative control

@ significant difference with healthy control

significant difference with test formula group

The statistical analysis revealed significant differences in epidermal thickness and the number of cells with COX-2 expression between the healthy control and the negative control in W/O and O/W creams, as shown in Figures 1 and 2. This finding indicates that croton oil indeed causes inflammation in the test animals, confirming its extensive utilization as inflammation inductor (Lan *et al.*, 2012). It contains phorbol esters and 12-0-tetradecanoylphorbol-13-acetate that exert an irritating mechanism—leading to inflammation and swelling, and increase NF-κB that can induce papilloma in the skin of mice, hyperplasia, and infiltration of leukocytes (Santos *et al.*, 2018; Boligou *et al.*, 2017; Orra *et al.*, 2015; Subramanian and Vellaichany, 2014).

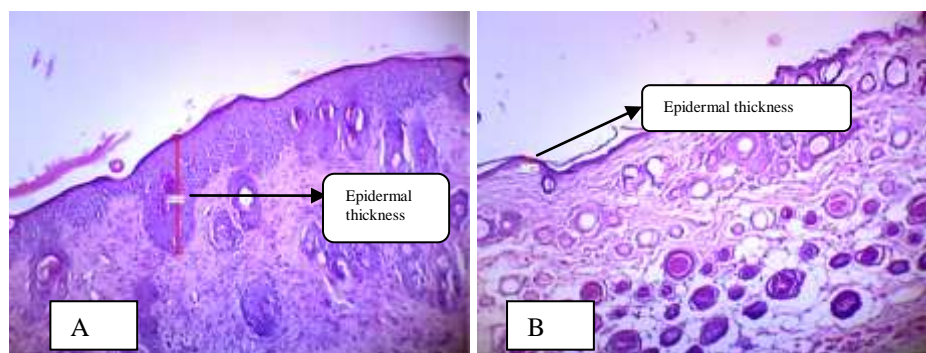


Figure 1. Microscopic image of skin tissues at 400x magnification after Hematoxylin-Eosin (HE) staining; the arrows mark the epidermal thickness of (A) negative control and (B) healthy control group

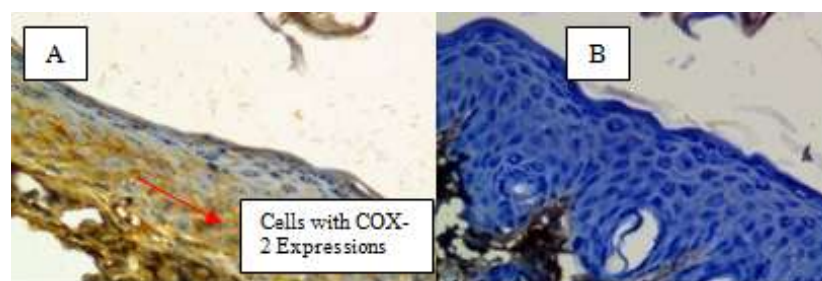


Figure 2. Microscopic image of skin tissues at 400x magnification, as identified with an immunohistochemical technique using COX-2 antibody; the red arrows mark COX-2 expressions in (A) negative control and (B) healthy control group

The statistical tests also proved that the epidermal thickness and the number of cells with COX-2 expression in negative control and positive control were significantly different. The positive control was treated with Voltaren® Gel. This gel contains diclofenac sodium that works as an anti-inflammatory by inhibiting COX-1 and COX-2 and, as a result, prevents prostaglandin synthesis (Anonymous, 2010). Therefore, Voltaren® Gel can decrease both epidermal thickness and the number of cells with COX-2 expression significantly. The statistical test in this study found that the healthy control and the positive control had a significant difference, meaning that the publicly circulated dosage form, i.e., Voltaren® Gel, is a drug with anti-inflammatory properties that reduce epidermal thickness but cannot restore it to a healthy condition.

The results of the statistical tests showed that the negative control and the test formula (W/O and O/W creams) were significantly different ($p < 0.05$). This finding indicates that the test formula can reduce the epidermal thickness and the number of cells with COX-2 expression in the skin of mice. This effect is caused by the anti-inflammatory activity of eugenol as the main component (87.36%) of CBEO that involves blocking TNF signals and preventing COX-2 expression so that the production of thromboxane B₂, prostaglandin synthesis, and neutrophil chemotaxis are inhibited (Murakami *et al.*, 2003; Ma and Kineer, 2002; Chainy *et al.*, 2000). This data is supported by the significant difference between the test formula group and no-CBEO group (receiving cream without CBEO). The latter had thicker epidermis and a higher number of cells with COX-2 expression than the former.

In this study, the anti-inflammatory capacity of CBEO cream is improved by adding enhancers. The test formula group, containing a mixture of two enhancers—oleic acid and propylene glycol, had

Anti-inflammatory Activity ... (Sugihartini et al.,)

thinner epidermis and lower number of cells with COX-2 expression than the no-enhancer group. The significant difference lies in the epidermal thickness caused by the W/O cream. The two enhancers contained in the cream formulation can increase the penetration of eugenol in the skin to reduce the thickness of the epidermis. Oleic acid can change the fluidity of lipids in the stratum corneum and, consequently, increase the permeability of this layer (Darijanto *et al.*, 2004). Meanwhile, propylene glycol works directly on the structure of epidermal proteins and increase protein solubility and denaturation. This mechanism leads to the loss of secondary, tertiary, and quaternary structures, an opening to the stratum corneum, and an improvement of drug solubility in this layer (Jiang *et al.*, 2000). Also, propylene glycol has been reported to be able to reduce the defense function of the skin by dissolving keratin and disrupting the lipid arrangement in the cervical strata (Ginting, 2014; Mohammed *et al.*, 2014; Remon, 2007; Lane, 2013; Santos *et al.*, 2012; Duracher *et al.*, 2009). The addition of enhancers, i.e., a mixture of oleic acid and propylene glycol, can raise the permeability and flux of eugenol contained in CBEO emulsion gels (Kurniawan *et al.*, 2018). Previous research has confirmed that it can increase the anti-inflammatory effects of CBEO formulated in a water-soluble base (Rahmawati *et al.*, 2017) and a lotion base (Iriani *et al.*, 2017). The mixture of oleic acid and propylene glycol provides a synergistic effect in facilitating the penetration of eugenol into the skin layer and increasing its anti-inflammatory capacity.

Table III shows that the formulas of the W/O and O/W CBEO creams administered to the test formula group result in a thinner epidermis and a smaller number of cells with COX-2 expression than the positive control. In other words, these formulas generate activities better than the products that are already in the market. The statistical test results affirmed that the reduction in the epidermal thickness due to the application of W/O creams was significant but not in the case of O/W creams. This effect is probably due to the different concentrations of CBEO in W/O and O/W creams. The CBEO level in W/O creams is higher than in O/W creams, and, consequently, more active substances can be released from W/O creams.

Different results were shown by the number of cells with COX-2 expression. There was a significant difference between the test formula and the positive control in O/W cream. Although the CBEO content in O/W cream was lower, it had more water that reduced the affinity of CBEO to the base and, therefore, released more amount of essential oil. The W/O and O/W creams generated a higher difference in the number of cells with COX-2 expression than the difference in the epidermal thickness. This result indicates that the release of CBEO from the cream base is more influenced by a variety in the compositions of the formula, which determine affinity, instead of CBEO concentrations. Previous research has proven that the formulation of CBEO in O/W cream has higher viscosity and adhesion than W/O cream (Safriani *et al.*, 2017). A prolonged duration of adhesion allows more CBEO released from the cream and, accordingly, lowers the number of cells with COX-2 expression. Another influencing factor is different enhancer compositions. The enhancer of the W/O cream contains 50% oleic acid and 50% propylene glycol (Tuldjanah, 2016), while the one in the O/W cream has 30%:70% ratio (Yuliastuti, 2016). The addition of more propylene glycol generated better penetration-enhancing activity. This finding is in line with Rahmawati *et al.* (2017) and Iriani *et al.* (2017) that have proven how increasing the proportion of propylene glycol in the enhancer mixture can improve the anti-inflammatory effects of CBEO in water-soluble base and lotion.

The addition of CBEO and mixed enhancers has been shown to exhibit anti-inflammatory effects. The test formula reduced the epidermal thickness and the number of cells with COX-2 expression more significantly than the base. However, based on the results of the statistical tests, the epidermal thickness and the number of cells with COX-2 expression in the healthy control and the test formula groups (W/O and O/W creams) were significantly different ($p < 0.05$). The test formula reduced the epidermal thickness and the number of cells with COX-2 expression but not as much as the healthy control. The same case applies to the statistical analysis of healthy control and positive

control. These results are likely because the new cream was administered only for three days so that its healing or anti-inflammatory effect was not maximal yet (Sugihartini *et al.*, 2017).

The statistical results of the epidermal thickness and the number of cells with COX-2 expression in W/O and O/W creams did not show any significant differences. The resultant epidermal thickness in the positive control and the test formula groups was significantly different in W/O cream, while the number of cells with COX-2 expression in both groups was insignificantly different. The concentrations of CBEO in W/O and O/W creams were 5% and 2.5%, respectively. Therefore, considering the efficiency, the level of CBEO in O/W cream is favorable. The enhancer in the O/W cream consists of 30% oleic acid and 70% propylene glycol. A higher proportion of propylene glycol can deliver a more effective CBEO penetration, which is in line with the results of Iriani *et al.* (2017) and Rahmawati *et al.* (2017).

CONCLUSIONS

The penetration of clove essential oil (CBEO), as an anti-inflammatory, into the skin is more effective when formulated in O/W cream than in W/O cream.

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