BUKTI KORESPONDENSI

ARTIKEL JURNAL INTERNASIONAL BEREPUTASI

Judul Artikel	The Anti-Inflammatory Activity of Essential Oil of Clove (<i>Syzygium aromaticum</i>) in Absorption Base Ointment with Addition of Oleic Acid and Propylene Glycol as Enhancer
Jurnal	International Journal of Applied Pharmaceutics Vol 11, Special Issue 5 (Sep), 2019
Penulis	Nining Sugihartini*, Rani Prabandari, Tedjo Yuwono, Desty Restia Rahmawati

Tahap revisi 1

Editor mengirimkan artikel yang berisi 7 catatan seperti dalam potongan artikel. Artikel lengkap dengan catatan tersebut terdapat pada lampiran 1

28	Author Quarias ²²²	28
29	Author Queries???	29
30	AQ1:Kindly provide department.	30
31	AQ2:Please check the acknowledgment text part.	31
32	AQ3:Kindly provide corresponding author mail id.	32
33	AQ4:Kindly provide history details	33
34	AQ5:Kindly provide abstract sub heading as per as journal style (Background, Methods, Results, Conclusion)	34
35	AQ6:Kindly review the sentence.	35
36	AQ7:Kindly provide expansion if needed.	36
37		37

Berdasarkan catatan tersebut maka dilakukan perbaikan dengan daftar perbaikan sebagai berikut.

Vol. XX special issue ..XX

Article reference no. IJAP_T0081_RA

Title 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

Name & mail address of corresponding author, Nining Sugihartini (nining.sugihartini@pharm.uad.ac.id)

Highlight	Highlighted Corrections			
S.No.	Asked query no.	Details		
1	AQ1	Department of Pharmaceutical Technology, Faculty of Pharmacy at Ahmad Dahlan University, Yogyakarta, Indonesia. Department of Pharmaceutical Technology, Faculty of Health at Harapan Bangsa University, Purwokerto, Indonesia. Email: nining.sugihartini@pharm.uad.ac.id		
2	AQ2	The authors thank to Ministry of Research and Higher Education (Kemenristekdikti), Republic of Indonesia for financial support via scheme of Hibah Penelitian Timpascasarjana		

Highlighted Corrections

Other corrections

Other	Other corrections			
	Page No.	Column(left or right)/Section /Paragraph/line no or talbe or figure	Incorrect text or matter	Corrected text
1.	1	Department section	¹ Department of ???	Pharmaceutical Technology
2.	1	Department section	Department of ???	Pharmaceutical Technology
3.	1	Corresponding author mail id.	Email: ???	nining.sugihartini@pharm.uad.ac.id
4.	1	History details	Received: ???,	March 2019
5.	1	History details	Revised and Accepted: ???	August 2019
6.	1	Abstract sub heading as per as journal style (Background, Methods, Results, Conclusion)	The optimal concentration of essential oil of clove in absorption base ointment as anti-inflammatory has been studied.	Background: The optimal concentration of essential oil of clove in absorption base ointment as anti-inflammatory has been studied.
7.	1	Abstract sub heading as per as journal style (Background, Methods, Results, Conclusion)	The enhancers that will be used in this study are oleic acid and propylene glycol.	(delete this sentence)
8.	1	Abstract sub heading as per as journal style (Background, Methods, Results, Conclusion)	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).	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).
9.	1	Abstract sub heading as per as journal style (Background, Methods, Results, Conclusion)	Data were analyzed using simplex lattice design method to find the optimum composition of enhancers.	(delete this sentence)
10.	1	Abstract sub heading as per as journal style (Background, Methods,	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	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

		Results, Conclusion)	the composition enhancer provides good anti-inflammatory activity.	epidermal thickness so the addition of the composition enhancer provides good anti-inflammatory activity.
11.	1	Abstract sub heading as per as journal style (Background, Methods, Results, Conclusion)		Conclusion: The increasing concentration of propylene glycol caused the raising activity of essential oil of clove as anti- inflammatory.
12.	1	Citation number	Essential oil of clove has biological activity because it contains high levels of eugenol so can use as an antiseptic and analgesic in the treatment of teeth and mouth [1].	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].
13.	1	The sentence	The mechanism of action of eugenol as an anti-inflammatory through inhibition of prostaglandin synthesis and neutrophil chemotaxis.	The eugenol mechanism of action as anti-inflammatory agent is via inhibition of prostalglandin synthesis and neutrophil chemotaxis.
14.	1	Citation number	which shows its potential as an anti-inflammatory agent [2-4].	which shows its potential as an anti-inflammatory agent [3-5].
15.	. 1	The sentence	The development of a	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
16.	1	Citation number	The material of the penetrating enhancers does not have therapeutic effect, but it can transport drugs from dosage forms into the skin [5].	The material of the penetrating enhancers does not have therapeutic effect, but it can transport drugs from dosage forms into the skin [11].
17.	. 1	Citation number	The research that was conducted by Sugihartini et al. (2015) 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% [6].	The research that was conducted by Sugihartini et al. (2015) 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].
18.	1	The sentence	Based on the potential of active ingredients of eugenol from clove flower essential oil, it was necessary to develop preparations by optimizing the mixture of enhancers to increase	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

			the ability of eugenol as anti- inflammatory by increasing capability to penetrate the layers of skin.	propylene glycol as enhancer to increase the capability of essential oil of clove as anti-inflammatory.
19.	. 2	Expansion	HE	Haemotoxillyn Eosin (HE)
20.	. 2	Citation number	Furthermore, the painting results were observed under a microscope using 400 times magnification [8].	Furthermore, the painting results were observed under a microscope using 400 times magnification [13] .
21.	. 2	Citation number	It means that croton oil can cause irritation and swelling of the skin if it was used topically [9].	It means that croton oil can cause irritation and swelling of the skin if it was used topically [14] .
22.	. 2	Citation number	On histochemical observations using the HE method, croton oil that was administered topically can induce hyperplasia and infiltration of leukocytes [10,11].	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].
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25.	3	Citation number	and activating of oxide-cyclic guanosine monophosphate pathway [12,13].	and activating of oxide-cyclic guanosine monophosphate pathway [18,19].
26.	3	Citation number	LPS and reduced production leukotrienes as mediator inflammation [14,15].	LPS and reduced production leukotrienes as mediator inflammation [20,21].
27.	3	Figure	5 0 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 11 10 11 11 12 12 13 14 15 15 16 17 18 19 10 10 11 11 12 13 14 15 15 16 17 18 18 19 10 10 10 10 10 10 10 10	
28.	3	The sentence	The epidermal thickness, the number of inflammatory cell and cell with COX-2 expression in formula group smaller than formula without enhancer.	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.
29.	3		COX-2 expression. The mechanism	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 oleid acid and propylene glycol as enhancer. This happen when the amount of propylene glycol increased [22,23]. The mechanism
30.	3	Citation number	the amount of drug that passes through the skin can increase [16-21].	the amount of drug that passes through the skin can increase [24,29].
31.	3	The acknowledgme nt text part	This research was conducted with the help of the Dikti Research Grant through scheme of Graduate Team Research Grant in 2016.	The authors thank to Ministry of Research and Higher Education (Kemenristekdikti), Republic of Indonesia for financial support via scheme of Hibah Penelitian Timpascasarjana

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			Technology. 3rd ed. New York: Informa; 2007.	
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52.	. 4	Addition of reference		1. Varghese RE, Ragavan D, Sivara S, Gayathri D, Kannayiram G. Anti- inflammatory activity of Syzygium aromaticum silver nanoparticles : in vitro and in silico study. Asian J Pharm Clin Res 2017;10(11):370-3
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Hasil perbaikan tersebut tertera dalam artikel seperti tersaji dalam lampiran 2.

Tahap revisi 2

Perbaikan yang diminta reviewer berupa waktu revisi, pemberian simbol pada tabel, keterangan tabel dan kalimat pada pembahasan. Artikel dari editor dan perbaikan disajikan pada lampiran 3 dan 4.

Tahap revisi 3

Perbaikan terdapat pada nama penulis keempat dan alamat penulis keempat. Artikel dari editor dan perbaikan disajikan pada lampiran 5 dan 6.

Lampiran 1. Email permintaan perbaikan dari editor dan artikel berisi catatan

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Vol XX, Special Issue X, 2019



INTERNATIONAL JOURNAL OF APPLIED PHARMACEUTICS

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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¹Department of ???, Faculty of Pharmacy at Ahmad Dahlan University, Yogyakarta, Indonesia. ²Department of ???, Faculty of Health at Harapan Bangsa University, Purwokerto, Indonesia. Email: ???

Received: ???, Revised and Accepted: ???

AQ5 ABSTRACT

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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 enhancers that will be used in this study are oleic acid and propylene glycol. 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. 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. In this study, the composition of 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. Data were analyzed using simplex latice design method to find the optimum composition of enhancers. Based on the results of the text, 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.

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 so can use as an antiseptic and analgesic in the treatment of teeth and mouth [1]. The mechanism of action of eugenol as an anti-inflammatory through inhibition of prostaglandin 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 [2-4].

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 [5].

52 The research that was conducted by Sugihartini *et al.* (2015) showed that 53 the optimal concentration essential oil of clove in absorption base ointment 54 which had the best anti-inflammatory activity and met the requirements 55 was 2.5% [6]. Based on the potential of active ingredients of eugenol from 56 clove flower essential oil, it was necessary to develop preparations by 57 optimizing the mixture of enhancers to increase the ability of eugenol as 58 anti-inflammatory by increasing capability to penetrate the layers of skin.

60 MATERIALS AND METHODS

61 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
 Unit of 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 antiinflammatory.

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.

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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

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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 cm2. 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 HE 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 [8].

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

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 66 negative control, @significant difference with healthy control, \$significant

67 difference with negative control, *significant difference with positive control,

68 significant difference with healthy control, significant difference with

69 Formula II

cell, and cell number with COX-2 expression. Data were presented in Tables 2-4 and Fig. 1. Data on Table 2 can be calculated using simplex lattice design method to find the profile of the epidermal thickness, the amount of inflammation cell, and cell number with COX-2 expression with variation composition of enhancer which was shown in Figs. 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 topically [9]. On histochemical observations using the HE method, croton oil that was administered topically can induce hyperplasia and infiltration of leukocytes [10,11]. 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-



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 negative control, @significant difference with healthy control, \$significant difference with negative control, *significant difference with positive control, 'significant difference with healthy control, significant difference with Formula II

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
negative control, [@] significant differe	e control, [#] significant difference with nce with healthy control, ^{\$} significant gnificant difference with positive control, control, 'significant difference with

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Fig. 2: Profile of epidermal thickness in the anti-inflammatory activity of essential oils of cloves (*Syzygium aromaticum*) in absorption base ointments with variation composition of oleic acid and propylene glycol as enhancers





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 [12,13]. 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 [14,15]. There was a significant difference between positive control and formula group. It means that the activity of eugenol was better than natrium diclofenac.



Fig. 4: The graphing relation of the profile expression of cyclooxygenase-2 to the anti-inflammatory power of essential oils of clove flowers (*Syzygium aromaticum*) in preparations of absorbent base ointments composition of oleic acid enhancers and propylene glycol

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 cell and cell with COX-2 expression in formula group smaller than 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. The influence of variation composition of enhancer can be shown from Figs. 2-4.

Profile in Figs. 2-4 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. 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 [16-21].

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.

ACKNOWLEDGMENT

This research was conducted with the help of the Dikti Research Grant through scheme of Graduate Team Research Grant in 2016.

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Research Article

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INTERNATIONAL JOURNAL OF APPLIED PHARMACEUTICS

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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AQ5 ABSTRACT

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The optimal concentration of essential oil of clove in absorption base on the three sential oil of clove in absorption base on the anti-inflammatory has been studied. The development of formulations can be done by adding oleic acid and propylene glycol as enhancers. The enhancers that will be used in this study are oleic acid and propylene glycol. 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 this study, the composition of oleic acid and propylene glycol was 100% oleic acid (FII). The profile of the anti-inflammatory activity of the essential oil of clove in absorption base ointment formula by adding a 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 histopar of ogical preparation. After that, the epidermal thickness, number of inflammatory cells, and cycloxys mase (COX)-2 expression can be measured. Joata were analyzed using simplex latice design method to find the optimum composition of enhancers. Seed 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 on the addition of the composition enhancer provides good anti-inflammatory activity of the composition of enhancers. Seed on the results of the composition enhancer provides good anti-inflammatory activity of the composition of the composition enhancer provides good anti-inflammatory activity of the composition of the composition of the composition enhancer provides good anti-inflammatory activity of the composition of the composition of the composition of the composition enhancer provides good anti-inflammatory ac

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

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INTRODUCTION

Essential if of clove has biological activity because it contains high levels of eugence and use as an antiseptic and analgesic in the treatment of teeth and mouth 4. The mechanism of action of eugenol as an anti-inflammatory through inhibition of prostaglandin 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 aget -4].

43 ne development of a formula for essential oil of clove was continued. 44 One of the ways that can be done to develop a formula is by adding an 45 enhancer to the preparation of formulation. Enhancers or penetrating 46 47 enhancers are ingredients that can increase skin permeability or reduce skin impermeability. The material of the penetrating enhancers 48 does not have the putic effect, but it can transport drugs from dosage 49 forms into the ski 50

The research that was conducted by Sugihartini *et al.* (2015) 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% [1]. Based on the potential of active ingredients of eugenol from clove flower essential oil, it was necessary to develop preparations by optimizing the mixture of enhancers to increase the ability of eugenol as anti-inflammatory by increasing capability to penetrate the layers of skir

60 MATERIALS AND METHODS

⁶¹ Materials and tools

62 This study used essential oil of clove as the material which was 63 obtained from the Center for Essential Oils Studies, Indonesian Islamic 64 University, Sleman, Yogyakarta. The ingredients of ointment with 65 pharmaceutical degree such as adeps lanae, cera alba, stearyl alcohol, 66 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.

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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

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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 cm2. 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 HE 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

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

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 ^{\$+^}			

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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 topicall 7. On histochemical observations using the HE method, croton oil that was administered topically can induce hyperplasia and infiltration of leukocytes [10,11 here was a significant difference between negative control and positive control. It means the activity of natrium diclofenac in Voltaren as active substance for anti-



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 negative control, [®]significant difference with healthy control, ^{\$}significant difference with negative control, *significant difference with positive control, significant difference with healthy control, significant difference with Formula II

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
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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 [12,1] 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.

60 The application of formula can reduce the epidermal of thickness. 61 the number of inflammatory cell, and cell with COX-2 expression. It 62 was supported with the result of statistical analysis that showed the 63 difference significant between negative control and formula group. It 64 shows the activity of eugenol as anti-inflammatory agent in essential oil 65 of clove. The mechanism of eugenol as anti-inflammatory was inhibit 66 the expression of COX-2 in macrophage-stimulated LPS and reduced 67 production leukotrienes as mediator inflammation [14,1] There was 68 a significant difference between positive control and formula group. It 69 means that the activity of eugenol was better than natrium diclofenac.



cyclooxygenase-2 to the anti-inflammatory power of essential oils of clove flowers (Syzygium aromaticum) in preparations of absorbent base ointments composition of oleic acid enhancers and propylene glyco

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 cell and cell with COX-2 expression in formula group smaller than 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. The influence of variation composition of enhancer can be shown from Figs. 2-4

Profile in Figs. 2-4 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 where 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 increas [1] 6-21].

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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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 antiinflammatory 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 research that was conducted by Sugihartini *et al.* (2015) 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

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diclofenac preparation which has been known to be efficacious as antiinflammatory.

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 cm². 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

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	2.5	2.5	2.5
of clove			
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 negative control, "significant difference with healthy control, "significant difference with negative control, "significant difference with positive control, 'significant difference with healthy control, 'significant difference with Formula II each group or five replications in 3 consecutive days. Furthermore, the painting results were observed under a microscope using 400 times magnification [13].

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



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 negative control, "significant difference with healthy control, ^ssignificant difference with negative control, 'significant difference with positive control, 'significant difference with healthy control, 'significant difference with Formula II

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 negative control, "significant difference with healthy control, ^ssignificant difference with negative control, 'significant difference with positive control, 'significant difference with healthy control, 'significant difference with Formula II

cell, and cell number with COX-2 expression. Data were presented in Tables 2-4 and Fig. 1. Data on Table 2 can be calculated using simplex lattice design method to find the profile of the epidermal thickness, the amount of inflammation cell, and cell number with COX-2 expression with variation composition of enhancer which was shown in Figs. 1.

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 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. The influence of variation composition of enhancer can be shown from Figs. 1.

Profile in Figs. 2-4 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 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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International Journal of Applied Pharmaceutics

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Research Article

THE ANTI-INFLAMMATORY ACTIVITY OF ESSENTIAL OIL OF CLOVE (*SYZYGIUM* AROMATICUM ABSORPTION BASE OINTMENT WITH ADDITION OF OLEIC ACID AND PROPYLENE GLYCOL AS ENHANCER

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ABSTRACT

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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 lipoplysaccharide (LPS) stimulated by macrophages. Research has shown that eugenol suppresses TNF signals and COX-2 expression, which shows its potential as an antiinflammatory 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 research that was conducted by Sugihartini *et al.* (2016) 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

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diclofenac preparation which has been known to be efficacious as antiinflammatory.

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 $2 \times 2 \text{ cm}^2$. 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

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±4.6
Formula II	137.75±3.9.
Formula III	131.05±1.93 🔁
*Significant difference with negative of	ontrol "significant difference with

Significant difference with negative control, significant difference with a significant difference with negative control, significant difference with negative control, significant difference with healthy control, significant difference with Formula II

each group or five replications in 3 consecutive days. Furthermore, the painting results were observed under a microscope using 400 times magnification [13].

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



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±2.5
Negative control	7).83±3.66
Formula without enhancer	52.33±8.6
Formula I	= 6.18±3.56
Formula II	35.68±2.4
Formula III	= 30.63±1.79

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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	16±3.65
Positive control	_31.23±2.1
Negative control	4.63±2.41
Formula without enhancer	25.68±1.7
Formula I	102±2.39
Formula II	17.86±2.7
Formula III	1.57±2.59

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cell, and cell numbers with COX-2 expression. Data were presented in Tables 2-4 and Fig. http://www.andlescontenters.com/commons/simplex lattice design method to find the profile of the epidermal thickness, the amount of inflammation cell, and cell number with COX-2 expression with variation composition of enhancer which was shown in Fig.

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 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 vet.

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. The influence of variation composition of enhancer can be shown from Figs. 1

Profile in Figs. 2. Solution 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 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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Research Article

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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