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Effectiveness Test of Anti-Inflammation of Ethanol-Extracted Cream of *Graptophyllum pictum* L. Griff on White Male Mice (*Mus musculus*)

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ABSTRACT

Background: *Graptophyllum pictum* L. Griff are commonly used in traditional medications. One of them is as an anti-inflammation. Giving medicine topically may increase bioavailability and medicine efficacy and reduce side effects of conventional anti-inflammatory medicine. The ethanol extract of black pudding leaves had been formulated into cream form. The aim of this study is to investigate the effectiveness of the cream-formed anti-inflammation of ethanol extract of *Graptophyllum pictum* L) Griff on white male mice (*Mus musculus*).

Purpose: The effectiveness of anti-inflammation was tested by using paw edema method. The tested animals used were 25 white male mice divided into 5 groups, namely negative control group (F_0), positive control group (hydrocortisone cream), and treatment group of ethanol-extracted cream of black pudding leaves F_1 (10%), F_2 (15%), and F_3 (20%). The initial paw volume measurement was done on each mouse, and the treatment for each group was administered after inflammatory induction using 0.1 ml of 1% carrageenan solution. The observation of inflammation decrease on each mouse was carried out every 1 hour during 6 hours using plethysmometer.

Result: The data obtained were then analyzed by using one-way ANOVA statistical test followed by Duncan test with 99% confidence level. The result of one-way ANOVA test showed that there was significant difference between treatment groups ($P < 0.01$). Duncan test result showed that the groups that gave the best anti-inflammatory effectiveness were the positive control group and F_3 which contained 20% extract concentration.

Conclusion: Ethanol-extracted cream of *Graptophyllum pictum* L. Griff gave an anti-inflammatory effect to the tested animals white male mice that had been induced inflammation using carrageenan.

Keywords: Anti-inflammation, *Graptophyllum pictum* L. Griff, Cream, Paw edema.

INTRODUCTION

Long-term use of oral anti-inflammation causes inhibition of protective compound of gastric acid so that it can irritate the stomach and trigger ulcer [1]. Change of drug delivery route through topical needs to be done. The drug delivery topically can increase bioavailability and drug efficacy by avoiding first-pass elimination on liver [2]. Widespread trend “back to nature” makes alternative medicinal plants necessary in order to get an effective and low-cost medicine and to reduce the side effects of conventional medications [3].

One of the plants applied in traditional medication was *Graptophyllum pictum* L. Griff. Its leaves were commonly used by the society to treat swelling or inflammation [4,5]. Cream form from ethanol extract of black pudding leaves, but the test of ethanol-extracted cream of black pudding leaves as an anti-inflammation had not been conducted [6]. The ethanol-extracted cream of *Graptophyllum pictum* L. Griff was expected to have anti-inflammation effectiveness so that it could become natural-based alternative anti-inflammatory drug.

METHODS AND MATERIALS

The tools used in this research consisted of analytical scale @Lucky scale, Plethysmometer, and Spuit 1 ml. Aquadest, hydrocortisone cream, carrageenan 1%, ethanol-extracted cream of black pudding leaves, white male mice (*Mus musculus*).

Research procedure

This research was carried out with the actual experimental method (true experimental) using the randomized posttest only control group design.

Tested animal selection

The tested animals used in this research were white male mice in healthy condition with the weight of 20-30 gram. Sample rate calculation or tested animal numbers was determined by using Federer formula.

Tested compound preparation

The tested compound applied was ethanol-extracted cream form of *Graptophyllum pictum* L. Griff with various formulations consisting of F₀ (only containing cream base), F₁ (containing 10% ethanol extract of black pudding leaves), F₂ (containing 15% ethanol extract of *Graptophyllum pictum* L. Griff), and F₃ (containing 20% ethanol extract of *Graptophyllum pictum* L. Griff).

Experimental procedure**Anti-inflammation testing method**

The method used for testing anti-inflammation was paw edema method [7] by injecting carrageenan 1% dissolved in 0.1 ml aquadest on the mice paw sub plantar. The mice were sorted into 5 groups, namely negative control group (F0), tested material group (formula of ethanol-extracted cream of *Graptophyllum pictum* L. Griff F1-F3) and positive control (hydrocortisone cream).

Anti-inflammation testing of ethanol-extracted cream form of black pudding leaves

Initial volume of mice's feet was measured before the treatment was given by using plethysmometer by dipping mice's paw that had been marked until it reached the ankle (reaching the mark) into plethysmometer. After all, received the treatment, the measurement was redone in the hour of 1-6. Inflammation volume was the difference between the mice's feet volume after being injected 1% carrageenan solution and that before being injected the carrageenan solution.

Anti-inflammation effect calculation

Anti-inflammation effect was evaluated by using,

$$a. \text{ Inflammation percentage} = (V_t - V_o) / V_o \times 100\%$$

Notes:

V_t = Inflammation volume after time t

V_o = Mice's feet initial volume

$$b. \text{ Inflammation inhibition percentage} = (a - b) / a \times 100\%$$

Notes:

a = Average inflammation percentage of control group

b = Average inflammation percentage of treatment group of tested materials or comparing drug

$$c. \text{ Anti-inflammation power percentage} = (AUC_K - AUC_p) / AUC_K \times 100\%$$

Notes:

AUC_K = Average udem volume AUC curve toward time for negative control

AUC_p = Udem volume AUC curve toward time for treatment group on everyone

Data collection

Observation of inflammation decrease on each mouse was done every 1 hour during 6 hours after each group was given treatment using the plethysmometer tool. It aimed to investigate the symptom change that occurred after being given treatment by comparing the symptom or behavior before the treatment.

Statistical analysis

The data obtained were analyzed with Kolmogorov-Sminorv test to see the data distribution. If the data were distributed normally, it would apply one-way variant analysis test (One-way ANOVA). Then proceed with Duncam and LSD tests with a 99% confidence level.

RESULT AND DISCUSSION***Result of tested animals' selection***

Based on Federer formula, male mice (*Mus musculus*) was split into two control groups, namely positive control group (hydrocortisone cream), negative control group (F₀), and three treatment groups (F₁-F₃) with 5 samples per group so that 25 mice as the total samples was obtained (*Mus musculus*).

The result of mice's feet udem volume average (Figure 1) and the calculation result of average inflammation percentage (Figure 2) showed the increase of mice's feet volume in the first hour on each treatment group in accordance with the statement of Singh [8] that in the first hour after carrageenan injection edema increase would took place because the carrageenan would induce cell injury so that the cell would release inflammation mediator and excessive prostaglandin production so that inflammation happened and udem appeared.

From both results, it also could be seen there was inflammation decrease gradually on the first hour until the fifth hour till reaching normal condition on each treatment, except the negative control group. There were two phases of udem creation induced by carrageenan. The first phase was the release of histamine, serotonin, and bradykinin occurring in the 1-2 hours after the induction. In the second phase, the prostaglandin release happened in 3-4 hours after the induction, and then the udem grew fast and survived caused by the vasoactive mechanism from prostaglandin [9].

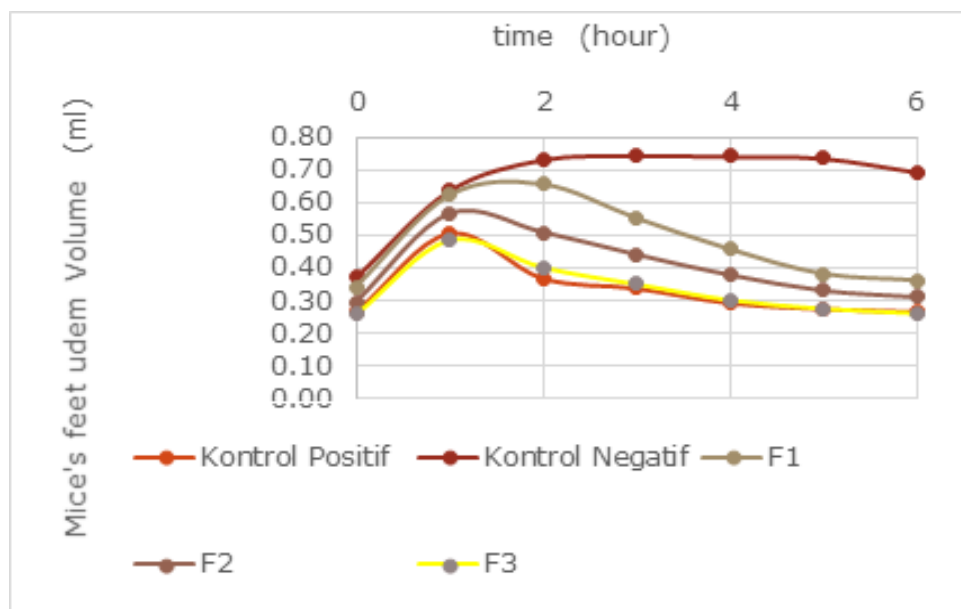


Figure 1. Graphic of Mice's feet udem volume average (ml) toward time (hour).

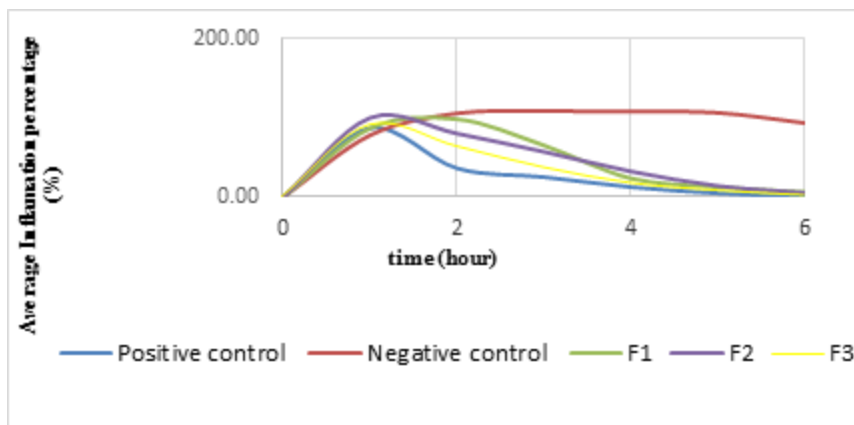


Figure 2. Graphic of average inflammation percentage.

The result of mice's feet udem volume average (Figure 1) and the average inflammation percentage (Figure 2) showed that the positive control group, F3, F2, and F1 experienced the inflammation decrease from the first hour to the second hour after carrageenan induction so that it was suspected that inflammatory inhibition mechanism was caused by the inhibition of histamine, serotonin, and bradykinin. Hydrocortisone cream, according to Katzung [10], was classified glucocorticoid that could cause vasoconstriction if it was directly applied on the skin expected occurring by pressing the mast cell degranulation. Glucocorticoid also reduced the capillary permeability by decreasing the amount of histamines released by basophil and mast cell.

The ethanol-extracted cream of black pudding leaves had not been known for sure if the content of phytochemical compound specifically gave the anti-inflammation effectiveness. According to the theoretical review that researchers had done, the black pudding leaves contained non-toxic alkaloid, glycoside, steroid, saponin, tannin, chlorophyll, flavonoid, and mucus [11]. The research by Elmitra Dan [6] proved that there was flavonoid content in the ethanol extract of *Graptophyllum pictum* L. Griff. According to the several researches that had been conducted, generally the anti-inflammation activity was caused by the flavonoid content. It was supported by the anti-inflammatory mechanism of flavonoid compound that had the similarity with the result obtained from this research, the flavonoid compound as the anti-inflammation could work on the first phase (early phase) by inhibiting the process of serotonin and histamine release which was the chemical mediator to the place where the inflammation occurred [12].

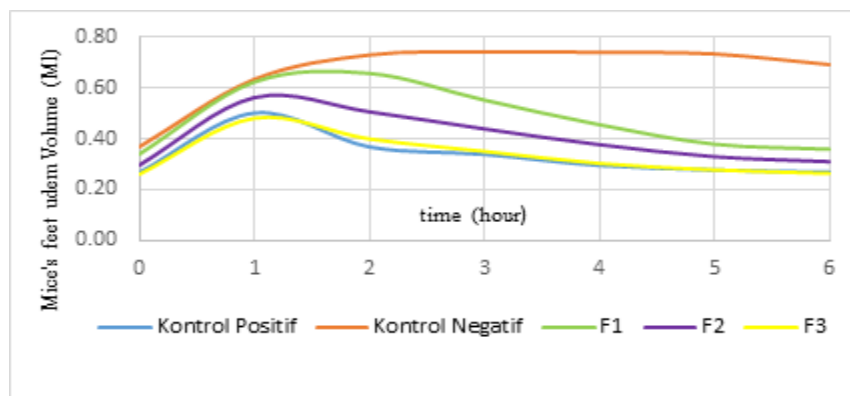


Figure 3. AUC (Area under curve).

Based on the AUC (Area under curve) result (Figure 3), it was acquired that the AUC value from the lowest to the biggest consecutively was the positive control, F3, F2, F1, and F0. The AUC value described the area wide under the curve created between the udem volumes toward the time. The lowest the AUC value, the biggest the anti-inflammation power [13].

Table 1. Calculation result of inflammatory inhibition percentage

No	Groups	% Inhibition
1	Control +	71.96
2	Control -	0.00
3	F1	50.03
4	F2	51.61
5	F3	62.72

Table 2. Calculation result of anti-inflammation power (%).

No	Groups	% DAI
1	Control +	50.24
2	Control -	0.00
3	F1	26.77
4	F2	38.74
5	F3	49.76

The inflammation effectiveness of ethanol-extracted cream of black pudding leaves was analyzed based on the inflammatory inhibition percentage and the anti-inflammation power percentage, and from this two analyzing means the equivalent result was obtained (Tables 1 and 2), namely that consecutively the treatment groups that had the percentages of inflammatory inhibition and anti-inflammation power from the biggest to the lowest was the positive control group, the treatment groups of F3, F2, F1, and lastly the negative control group. The negative control had no anti-inflammation activity. The result obtained from the research was also statistically analyzed in order to determine whether the research finding result was significant on the certain level or not [14]. The statistic test was conducted using SPSS 24 program, namely one-way ANOVA test with 99% confidence level. Before the test was carried out, Kolmogrov-Smirnov test and Levene test was established first to find out the normality and homogeneity from the data which was the prerequisite to perform one-way ANOVA test.

From the normality test result, it was gained that the sig value was 0.777 in which this value was bigger than α 0.01 ($P > 0.01$) which was the requirement for data to be said normal. Whilst, from the homogeneity test result, it was obtained that the sig value was 0.126 in which this value was bigger than α 0.01 ($P > 0.01$) which was the requirement for data to be said homogeny. Both of the test results fulfilled the requirement, so one-way ANOVA test could be carried out (Table 3).

Table 3. One-way ANOVA test result.

AUC	Sum of squares	df	Mean square	F	Sig.
Between groups	14.683	4	3.671	16.896	0
Within groups	4.345	20	0.217		
Total	19.028	24			

From the result of one-way ANOVA test, it was obtained that the sig value was 0.000. This value was lower than α 0.01 ($P < 0.01$) so that there was significant difference statistically amongst each treatment group. Then, to find out which group giving the best anti-inflammatory effect, the post-hoc Duncan test was established (Table 4).

Table 4. Result of post-hoc Duncan.

AUC					
	Treatment groups	N	Subset for alpha=0.01		
			1	2	3
Duncana	Control +	5	2.04		
	F3	5	2.07		
	F2	5		3.016	
	F1	5		3.037	
	Control -	5			4.12
	Sig.	5	0.933	0.944	1

The result of post-hoc Duncan test showed that the positive control and the formulation cream 3 statistically gave better anti-inflammatory effect than each treatment group, and the positive control and the formulation cream 3 gave equivalent anti-inflammatory effect or, in other words, had the same anti-inflammation activity. Then, it was followed by the formulation cream 2 and 1 statistically also gave equivalent anti-inflammatory effect and the negative control gave the worst anti-inflammatory effect.

Overall, the research result stated that ethanol-extracted cream of *Graptophyllum pictum* L. Griff had anti-inflammation effectiveness influenced by the variation of extract concentration contained in cream formula, which was that the anti-inflammatory effect was directly proportional to the concentration amount of ethanol extract of *Graptophyllum pictum* L. Griff in the form of cream. The bigger the extract concentration in the given cream, the bigger the anti-inflammatory effect. This result had a similarity with some previous anti-inflammation researches, namely the research of Felix-Silva [9], which stated that the bigger the dosage used, and then it would show the better result than the lower dosage use [12].

CONCLUSION

Ethanol-extracted cream of *Graptophyllum pictum* L. Griff gave an anti-inflammatory effect to the tested animals white male mice that had been induced inflammation using carrageenan. The anti-inflammation effectiveness on each treatment group from the highest consecutively was the positive control, F3 (20%), F2 (15%), F1 (10%) and F0.

SUGGESTIONS

Next researchers are expected to conduct identification and isolation of the phytochemical substance of *Graptophyllum pictum* L. Griff that give anti-inflammatory effect. They also can create innovation in developing the black pudding leaves potential as natural-based anti-inflammation in various other forms that can make its usage and availability in society.

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