

Anti-Inflammatory Effects of *Kaempferia galanga* L. Rhizome Extract in Carrageenan-Induced Female Rats

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Abstract— *Kencur* (*Kaempferia galanga* L.) is a plant that is traditionally used for the treatment of various diseases including inflammation. This study aimed to know the comparison of the effectiveness of *kencur* extracts with diclofenac sodium as an antiinflammatory agent. Fifteen female rats aged 2-3 months (200±50 gram) were divided into 5 groups: rats in negative control group only given a 2% suspension of PGA, rats in positive control group given 20 mg/Kg diclofenac sodium, and rats in three different dose groups given galanga rhizomes extract of 45, 90, and 180 mg/Kg, respectively. All drugs were given 1 hour orally before carrageenan injection of 1% as induction of edema. Edema volume was measured every 30 minutes to 390 minutes to calculate the percentage of edema inhibition. The data were analyzed using one-way ANOVA and followed by LSD test to see differences among groups. Ethanol extract of galanga rhizome in the 5th hour at doses of 45, 90 and 180 mg/kg showed significant differences in a positive control group. Each Sig. value was 0.002; 0.004; 0.002. This showed that the ethanol extract of *kencur* had anti-inflammatory activity at all dose levels.

Keywords: *Kaempferia galanga* L., anti-inflammatory, carrageenan

I. INTRODUCTION

Inflammation is linked to certain diseases that can be found in the community, usually characterized by swelling, pain, redness, and heat. The inflammation can be caused by a normal protective response to tissue injury from physical trauma, hazardous chemicals, or microbiological agents. Inflammation is a response of the body's efforts to activate or destroy invading organisms, remove irritants and prepare phases of the tissue repair process [1].

Kencur (*Kaempferia galanga* L.) is one of the herbal plants of the Zingiberaceae family which has many pharmacological activities [2]. Society in Indonesia traditionally uses *kencur* as medicine for inflammation of the stomach (gastritis), inflammation of the child's ear (ear infection), influenza in infants, colds, headache, cough, diarrhea, sore eyes, sprains, and fatigue. It is also used to

get rid of dirty blood and get through periods smoothly. Previous studies reported that the extract of *kencur* has analgesic and anti-inflammatory effects [3], nematicidal agents [4], mosquito repellent and larvicidal effect [5], vasorelaxants [6], antineoplastics [7], antioxidants [8], and antimicrobials [9].

One of the plants that has an anti-inflammatory effect is *kencur* (*Kaempferia galanga* L.). The choice of appropriate extraction solvent is also important to obtain the best result of extraction efficiency and to obtain compounds that have pharmacological activities. The best solvent is ethanol or its mixture with water because it is a good extraction solvent for almost all low-molecular-weight compounds such as saponins and flavonoids. The type of extraction solvent also affects the number of active compounds in the extract based on the principle of "like dissolve like", in which polar compounds will dissolve in polar solvents and non-polar compounds will dissolve in non-polar solvents [10]. Ethanol is often used as a solvent in the laboratory because it is an inert solvent in which it does not react with other components [11].

In this study, the animals tested were rats because metabolism in the rats' body and humans are physiologically more similar and the structure of the tissue in the rats' feet is easier to be treated with subcutaneous induction compounds. Methanol and ethanol are both alcohol derivative solvents which have hydroxyl (OH) groups and have carbon chains (atomic C). The number of C atoms in ethanol is greater (two C atoms), and thus this compound can dissolve secondary metabolites that are non-polar in purslane plants. Other studies also mentioned that *kencur* rhizome extract at a dose of 45 mg/kg was able to show a very high percentage of inhibition compared to other dose groups and positive control (51.27%) [12]. In this case, society has lack of information about *Kaempferia galanga* extract that functions as an antiinflammatory drug. This extract has a great potential, then society should exploit it. Based on this study, it is necessary to test various methods and comparisons.

II. METHODS

Anti-inflammatory test

A. Preparation for Animal experiment

Before being used, rats were acclimatized for 1 week in a cage to adapt to a new environment. All rats were kept in the same conditions, given the same food and drink, and observed their general condition regularly, and weighed. Before the experiment, rats were deprived of food for ± 18 hours but they still received water *ad libitum* [13]. Rats that were sick which had standing fur, were less active and had unclear eyes were excluded in this study. Fifteen rats were used and randomly divided into 5 groups consisting of 3 rats of each (referring to WHO provisions). The following is the description of the treatment given to each group of rats:

TABLE 1. GROUP ANIMAL EXPERIMENT

| Group | Number of rats | Treatment |
|------------------------|----------------|---|
| Negative control group | 3 | Have given the 2% PGA suspension + induced with 1% carrageenan as much as 0.1 mL |
| Positive control group | 3 | Have given sodium diclofenac at a dose of 20 mg/Kg in 2% PGA + induced with 1% carrageenan as much as 0.1 mL |
| Dosage I | 3 | Have given a suspension of test compound extract in 2% PGA (dose 45 mg/Kg) + induced with 1% carrageenan as much as 0.1 mL |
| Dosage II | 3 | Have given a suspension of test compound extract in 2% PGA (dose 90 mg/kg) + induced with 1% carrageenan as much as 0.1 mL |
| Dosage III | 3 | Have given a suspension of test compound extract in 2% PGA (dose 180 mg/kg) + induced with 1% carrageenan as much as 0.1 mL |

B. Anti-inflammatory Activity Test using Carrageenan-Induced Rats Paw Method (Winyard & Willoughby, 2003; Winter et al, 1962; Morris, 2003)

The followings are the procedure of the experiment.

1. Rats were weighed and grouped randomly into 5 groups. Each group consisted of 3 rats.

2. Rats were acclimatized for 1 week to adapt to the experimental environment.
3. Before the experiment, rats were deprived of food for ± 18 hours but still given drink.
4. At the beginning of the research, all rats were marked with markers at the ankle and therefore the foot was always the same when entered into the mercury.
5. The initial volume of rats' feet (paw) was measured before being treated and it represented the base paw volume (V0).
6. The negative control group was given a 2% suspension of PGA, while the positive control group was given a suspension of diclofenac sodium at a dose of 20 mg/kg. The other three groups were given a suspension of the compound test according to the planned doses given orally.
7. One hour later rats were injected 0.1 ml of 1% carrageenan by subplantar injection in the rats left leg. Before the carrageenan was injected, the area of the rats' paw was wiped with alcohol swabs.
8. Furthermore, edema volume was measured in the 30th, 60th, 90th, 120th, 150th, 180th, 210th, 240th, 270th, 300th, 330th, 360th, and 390th minute after induction using plethysmometer represented as the final volume (Vt).
9. The percentage of edema and average (mean) percentage of edema inhibition was calculated using the following formula:

Description :

Vt: paw volume of rats at time t (after carrageenan-induced) V0: paw volume of rats at time 0 (before carrageenan-induced)

$$\%Edema\ inhibition = \left(\frac{a - b}{a}\right) \times 100\%$$

Description :

a : % edema in negative control group b : % edema in test group [14]

III. RESULTS AND DISCUSSION

In this study, the anti-inflammatory activity test of *kencur* rhizome extract was done by the Winter method (carrageenan induction). Carrageenan induction is the possible method to be analyzed after given a single dose of nontoxic in one day. Besides, this method is the most widely used as it is simple, easy, and suitable for testing the anti-inflammatory activity of a compound in small amounts [15].

The use of carrageenan as edema induction in rats' feet has been widely used in testing the anti-inflammatory activity of a drug compounding [16; 17]. Edema formed by carrageenan induction is in the form of acute inflammation [18; 19]. Carrageenan was chosen in this study because it can trigger the release of prostaglandins after being injected in rats and therefore this compound can be used to find

antiinflammatory drugs to inhibit prostaglandin synthesis [15]. Rats weighed 150-250 grams.

In this experiment, the animals tested were grouped into 5 in which each consisted of 3 rats (referring to WHO provisions). The experimental group consisted of a negative control group (2% suspension of PGA), a positive control group (diclofenac sodium suspension at a dose of 20 mg/kg), and dose test groups with varying doses of 45 mg/kg, 90 mg/kg and 180 mg/kg.

The choice of dose varied referring to the research of Hasanah [12], in which the extract of *kencur* rhizome has the most optimal anti-inflammatory activity at a dose of 45 mg/kg. Tests were carried out using 3 dose variations, indicating the greater the dose given, the greater the antiinflammatory activity produced. The results showed a significant percentage of inhibition in treatments at a dose of 45 mg/kg and the percentage of inhibition was 51.27%. Before the treatment, the basic volume of rats' foot (V₀) was measured first and furthermore, rats' foot volume was measured every 30 minutes for 6.5 hours and it represented V_t.

The anti-inflammatory activity of a compound can be seen from its ability to inhibit the formation of edema in the rats' paw [19]. This was assessed from the increase or decrease in edema volume every 30 minutes. The edema volume was measured using a plethysmometer. The use of this tool required accuracy in the measurement because the volume of mercury always have to be the same in every test. Rats' feet were marked on the ankle joint and therefore the immersion of rats' feet into the mercury remained the same every 30 minutes. The edema volume of rats' feet was measured every 30 minutes for 6.5 hours. After carrageenan induction, the mean volume of edema was obtained and it can be illustrated in the following figure:

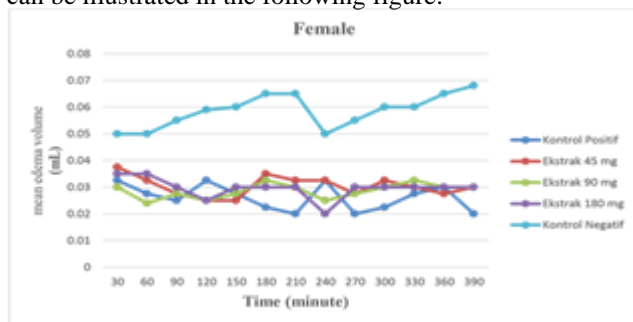


Figure 1. The edema volume in the 30th to 390th minutes.

From the graph of mean edema volume above, it can be seen the difference between the negative control group and the treatment group. The negative control group that was not given extracts and drugs has a significant increase compared with other treatment groups. The group which was not induced by carrageenan did not form edema at all and rats' feet do not show an increase or a decrease in edema volume. Unlike the group with no induction, the groups with carrageenan induction show an increase or a decrease in edema volume after induced by carrageenan. This shows that 0.1 mL of 1% carrageenan-induced could form

significant edema and there were differences among test groups. The increase in edema volume was caused by the release of inflammatory mediators such as prostaglandins, histamine, bradykinin, and serotonin in tissues after carrageenan induction. Besides, the decrease in edema volume of each test group at the fifth hour was due to the effect of carrageenan which had begun to decrease. The formation of edema by carrageenan only lasts for 5-6 hours and gradually decreases within 24 hours after injection [20].

Furthermore, the percentage of edema formation from the edema volume of the rats' paw can be calculated to see the differences in edema between groups by using this formula: $(V_t - V_0) / V_0 \times 100\%$, which is V_t = Volume of edema every hour and V₀ = volume rats' paw before the treatment. The results of the calculation of the mean percentage of edema on the rats' paw can be described as follows:

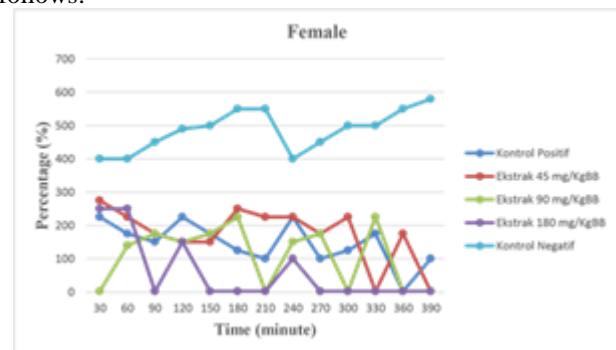


Figure 2. The graph of the relationship between mean percentages of edema and time (minute)

From the graph above, it can be seen that the negative control group has a very high percentage of edema formation and has a significant increase continuously to the 3rd hour. Then it begins to fall at the 4th hour and rises again to the 6.5th hour. In this case, animals tested were only given a suspension of PGA that was unable to inhibit the formation of edema and the response to edema only relies on rats' immunity. Compared to the positive control group, the dose I, dose II and dose III of the edema volume were lower. Based on the results of the statistical tests, the four groups were significantly different compared to the negative control group ($p > 0.05$). Thus, it can be concluded that these four groups have antiinflammatory activity in inhibiting the formation of edema in the rats' paw. Furthermore, the mean percentage of edema formation inhibition in each group can also be calculated to see the anti-inflammatory activity. It can be illustrated in figure 3.

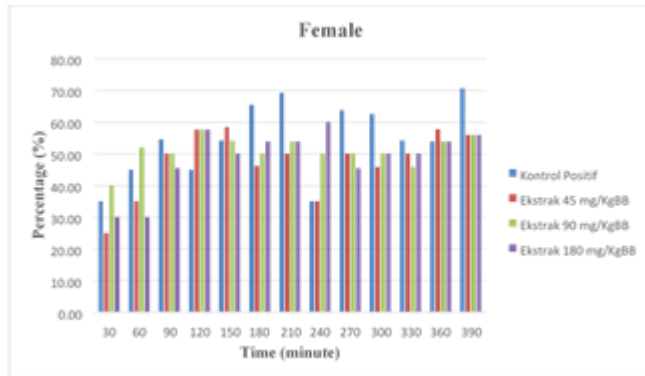


Figure 3. The graph of the relationship of the mean percentages of edema inhibition and time (minute)

From the graph above, it can be seen that the percentage of edema inhibition of each group is very different. It appears that the positive control group with diclofenac sodium drug increases continuously, although the effects in the first 60 minutes are still low.

Based on the graph, the dose I group is lower compared to the positive control in the 30th minute to the 390th minute, although in the 120th and 360th minute the dose I group is higher. The dose II group has a fluctuating percentage of edema inhibition from the 30th minute to the 390th minute. The dose II group decreases in the 120th to the 330th minute, then increases to the 390th minute. Furthermore, the dose III group has a fluctuating percentage of inhibition from the first to the last minute. The highest average percentage of inflammation inhibition is diclofenac sodium starting in 30 minutes after treatment and reaches its peak at 390 minutes. The smallest percentage of inflammation inhibition is at a dose of 45 mg/kg. *Kencur* ethanol extract at a dose of 90 mg/kg has a potential effect on edema inhibition. Certain ingredients can have anti-inflammatory effects. It can be proved that swelling reduced by 50% or more when experimental animals were induced by carrageenan 1% [21].

The edema inhibition in each group was tested for its normality using the Kolmogorov Smirnov test. The data normality test was normally distributed. The homogeneity test was also applied which shows the value ($p > 0.05$). This means the data varied homogeneously. The ANOVA test was performed to find out significant differences between the five groups. The ANOVA test result showed that Sig. value at the 5th hour was 0.006. It can be concluded that there were significant differences between the dose treatment groups and the positive control group. a further test was conducted using LSD to determine the differences

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in each group. The result showed a significant difference between the positive control group and the 45, 90, and 180 mg/kg dose groups. This shows that the ethanol extract of *kencur* with doses of 45, 90, and 180 mg/kg has an anti-inflammatory.

The edema formation process induced by carrageenan occurs in two phases and involves a variety of inflammatory mediators [22]. The first phase occurs for 3 hours after carrageenan induction in which histamine, serotonin, bradykinin are released and have an increase in prostaglandin synthesis around the injured tissue. The second phase occurs from the fourth hour to the fifth hour and there is an absorption of prostaglandins, proteases and lysosomes [22; 23; 24]. Generally, this second phase is sensitive to anti-inflammatory drugs [25].

In this study, the drug used in positive control was diclofenac sodium at a dose of 20 mg/kg showing the best percentage of edema inhibition. This is consistent with the literature that diclofenac sodium as an NSAID inhibits the nonselective cyclooxygenase enzymes, thereby it inhibits the release of prostaglandins [26]. Of the three dose groups, the dose II group (90 mg/Kg) has a high average percentage of inhibition compared to the other dose groups. In addition, the dose I and III (45 mg/kg and 180 mg/kg) have the same average percentage of inhibition. From this study, a decrease in the dose of *kencur* rhizome extract compound increased edema inhibition ability in the rats' paw. This is because actually several types of drugs in high doses cause the release of histamine directly from mast cells causing blood vessels to be more permeable to plasma fluid and causing inflammation processes (immunological processes occur) [27].

IV. CONCLUSIONS

Ethanol extract of *kencur* rhizome extract in the 5th hour at doses of 45, 90 and 180 mg/kg showed significant differences in the positive control group and each Sig. value was 0.002; 0.004; 0.002. This showed that the ethanol extract of *kencur* rhizome had anti-inflammatory activity at all dose levels.

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