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Research Article

Anti-Inflammatory Activity Test of Ethanol Extract of Kencur Rhizome (*Kaempferia galanga* L.) on Male White Mice (*Mus musculus* L.) Induced Carrageenan

Nisfi Lailatul Khasanah[1], Aldi Budi Riyanta[1], Susiyarti[1]

[1]*Diploma III Pharmacy, Harapan Bersama Polytechnic, Indonesia.*

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***Corresponding Author:**
Nisfi Lailatul Khasanah
Diploma III Pharmacy,
Harapan Bersama
Polytechnic
E-mail addresses:
nisfilaitul2008@gmail.com

ABSTRACT

Inflammation needs to be addressed, considering that inflammation is a local protective response caused by tissue damage. This condition can indeed be overcome by consuming chemical drugs, one of which is in the NSAIDS group, namely diclofenac sodium, but chemical drugs have many negative effects, including nonsteroidal anti-inflammatory drugs and steroids. Therefore, there has been the creation of anti-inflammatory drugs using natural ingredients, especially plants. For generations, Indonesian people have used kencur as an edema medicine. This study aims to examine the anti-inflammatory properties of kencur ethanol extract rhizomes. This study tested anti-inflammatory activity through regen-induced suppression of foot edema in male white mice. In this study, 15 eligible mice aged 2–3 months and weighing 20–30 grams were divided into 5 treatment groups. Three separate doses of galangal rhizome extract—50 mg/kgBW, 150 mg/kgBW, and 250 mg/kgBW—were used in the test. Ethanol extract of kencur rhizomes at a dose of 250 mg/kgBW can reduce edema in male white mice produced by carrageenan by 1% and show possible anti-inflammatory properties, according to the results of studies tested by measuring edema using a digital caliper.

Keywords: Rhizome; anti-inflammatory; diclofenac sodium; carrageenan; male white mice.

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INTRODUCTION

Inflammation is the reaction of body tissues to body processes that have the potential to harm cells. These agents include chemicals, germs, mechanical trauma, and physical trauma (Wijaya et al. 2015). Redness, heat, swelling, discomfort, loss of tissue function, increased permeability, and increased denaturation of proteins and membranes are typical symptoms of inflammation in humans and animals. Atherosclerosis and rheumatoid arthritis can result from this inflammatory process if left untreated (Nafilah et al. 2015). According to data from the Indonesian Ministry of Health (2018) in Indonesia, arthritis is the most common joint disease with a frequency of 7.3%. Ages 15-24 accounted for 1.3% of arthritis cases, and 24-35 year olds accounted for 3.1% of cases. So one of them needs to be overcome with Nonsteroidal Anti-Inflammatory Drugs (NSAIDs). An NSAID often used in the treatment of inflammation is diclofenac sodium. The drug inhibits cyclooxygenase enzymes. Although diclofenac sodium quickly reduces inflammation, it is possible that diclofenac sodium causes dangerous side effects, such as hypersensitivity and problems with the respiratory, circulatory, digestive, and metabolic systems. Considering this, the search for new anti-inflammatory drugs from medicinal plants with fewer side effects is needed quickly for the pharmaceutical industry (Laratmase 2021).

Inflammation can be treated with natural remedies including ginger, cinnamon, turmeric, and curcuma. Ginger can be used as an anti-inflammatory that has been tested by Nailun Nashiroh by topical administration (Nashiroh, Rosidah, and Widyaningrum 2023). Cinnamon can also be used for anti-inflammation with topical administration that has been tested by Rahmalia Yuni Astika (Astika, Sani K, and Elisma 2022). Turmeric also has anti-inflammatory activity with topical administration, which has been tested by Humaira Fadhilah because it contains curcuminoid active compounds (Fadhilah, Rachmani, and Hajaring 2021). One that has the potential to show anti-inflammatory activity is kencur with its advantages because it contains flavonoid compounds, saponins, and tannins in kencur rhizomes (Siburian, 2019). According to research (Andriyono 2019). Kencur has benefits, one of which is as an anti-inflammatory. Flavonoid compounds can lower arachidonic acid by preventing its metabolism, which leads to prostaglandin synthesis. In addition, lysosomal enzymes are mediators of inflammation that can be inhibited by flavonoids. It is possible to stop the spread of the inflammatory process by blocking these inflammatory mediators. Kencur has so far only been used to mix kencur rice drinks, so it is necessary to do an anti-inflammatory test of kencur by giving kencur rhizome extract (*Kaempferia galanga* L.) orally in carrageenan-induced male white mice.

Based on the background, the authors were interested in researching the potential anti-inflammatory properties of the ethanol extract of kencur rhizomes in carrageenan-induced white mice. The use of carrageenan as an inducer was

chosen because it has several advantages, one of which is that it provides a more sensitive response to anti-inflammatory drugs than other irritant compounds and does not also cause tissue damage in mice.

MATERIALS AND METHODS

Materials

The materials used include kencur rhizomes, pro analysis (PA); alcohol 70%, carrageenan 1%, diclofenac sodium tablets 50 mg, CMC Na 0.5%, NaCL, H_2SO_4, CH_3COOH and distilled water. The tools used in this study were a macerator (Maceration Vessel), blender (Miyako), glass funnel (Herma), knife (Dony), rotary evaporator (Scilogex), erlenmeyer (Pyrex), measuring cup (Pyrex), beaker glass (Pyrex), analytical balance (Sojikyo), mortar and stemper (Onemed), porcelain dish, stirring rod (Xuebei), waterbath (One), test tube (Pyrex), test animal digital scales (Scilogex), and digital caliper (Rohs).

Test Animals

Male white mice (*Mus musculus* L.) in good health, 2-month age, body weight 20-30 grams, were used as test subjects in this study.

Sample Collection

Kencur rhizome (*Kaempferia galanga* L.) was taken in Randugunting Village, East Tegal District, Tegal City. The next step is to use running water to clean the dirt that sticks to the skin.

Extraction

Fresh kencur rhizomes (1 kg) were cleaned, washed with running water, and displayed using a knife with a thickness of 1 mm. After that, it is dried in direct sunlight for 3–4 days. After drying, puree it for two minutes in a blender. Then it was sifted with a 100-mesh simplisia sieve (Anief 2007). The extraction of kencur rhizomes was carried out by a maceration method ratio of 1:5 with simplisia as much as 40 grams and 70% ethanol solvent as much as 200 ml and stirred for 5 minutes every 1x24 hours, then filtered with filter paper (Handayani, Ramadani, and Kartika 2018). The filtrate obtained is then evaporated in a water bath at a temperature of 100°C for 40 minutes until the extract thickens. In addition, evaporation is again carried out using a rotating vacuum evaporator for five hours at a temperature of 50°C and a rotation of 60 rpm. Furthermore, the ethanol extract was subjected to anti-inflammatory tests.

Anti-inflammatory Activity Test

In this study, the test animals used were mice adapted to a laboratory environment. Each test animal was treated under conditions similar to feeding and drinking. Before being treated, the test animals were satisfied for 8 days but still given water. To make a 0.5% CMC Na solution, weigh 0.5 g of CMC Na, dissolve it in 100 ml of heated aquadest in a beaker glass, and stir with a stirring rod until well mixed. Negative control used 0.5% CMC Na solution (Cahyaningsih, Yuda, and Susanthi 2018).

The dose of diclofenac sodium for rats in this study was 100 mg/20 gBW dissolved in a 0.5% CMC Na suspension. This was achieved by using a conversion factor of 0.0026 to convert a human dose of 50 mg into a rat dose weighing 20 grams (Priyanto, 2008). As a positive control, we used diclofenac sodium suspension (Cahyaningsih, Yuda, and Susanthi 2018). Three doses of ethanol extract of kencur rhizomes—50 mg/kgBW, 150 mg/kgBW, and 250 mg/kgBW—were prepared and dissolved in 0.5% CMC Na solution, respectively. Ethanol extract suspension of kencur rhizomes will be given to each mouse orally according to the prescribed dose (Cahyaningsih, Yuda, and Susanthi 2018). Carrageenan 1% solution is made by weighing 0.1 g of carrageenan, then dissolved in 10 ml of 0.9% NaCl physiological solution (Cahyaningsih, Yuda, and Susanthi 2018).

Udem is made by injecting 0.1 ml of 1% carrageenan solution intraplantar on the soles of mice. Digital calipers are used to measure edema thickness. Five measurements of edema were taken before treatment and five hours after carrageenan was administered. Furthermore, the thickness of the edema of the feet of mice was assessed, respectively, during the first hour to the fifth hour (Neman, Maarisit, and Karauwan 2022). The variable observed in this study was the thickness of edema in the legs of white mice.

Data Analysis

Descriptive data analysis is done by looking at the percent of inflammatory inhibition power and by using statistical data analysis with the ANOVA (One-Way) method. Percentage of inflammatory inhibition (Neman, Maarisit, and Karauwan 2022).

$$\frac{(Ct - Co)Kontrol-(Ct-Co)\ Perlakuan}{(Ct-Co)\ Kontrol} \text{x } 100\%$$

Information:
Ct = Thickness of the sole of the foot at the nth hour after carrageenan induction
Co = Thickness of the sole of the foot before carrageenan induction (normal)

RESULTS

Udema Measurement

Table 1. The Average Thickness of the Udema of the Sole of the Mouse Foot After Carrageenan-Induced in Each Treatment

Treatment	Foot Thickness (mm)					
	Hour					
	T0	T1	T2	T3	T4	T5
50 mg/kgbody weight	2.53	3.03	2.86	3.1	2.83	3.06
150 mg/kgbody weight	2.4	2.6	2.5	2.5	2.63	2.66
250 mg/kgbody weight	2.3	2.56	2.36	2.6	2.46	2.43
Diclofenac sodium	2.86	3.3	3.26	3.16	3.06	2.96
CMC Negative Control 0.5%	2.6	3.13	3.3	3.4	3.43	3.36

Information:
T0 (A): Edema thickness before treatment
T1 (B): Edema thickness 1 hour after induction
T2 (C): Edema thickness 2 hours after induction
T3 (D): Edama thickness 3 hours after induction
T4 (E): Edema thickness 4 hours after induction
T5 (F): Edema thickness 5 hours after induction

The average results obtained in the table above with the thickness of edema of the soles of mice showed that the smaller the size of the edema thickness of each treatment at hours 1, 2, 3, 4, and 5, the results showed an anti-inflammatory effect, namely by comparing the positive control treatment with diclofenac sodium. Diclofenac sodium tablets are classified as Nonsteroidal Inflammatory Drugs (NSAIDs) and have active pharmacological effects that include anti-inflammatory, analgesic, and antipyretic properties (Agustin and Ratih 2015). Diclofenac sodium works by preventing prostaglandin synthesis, which causes pain.

Table 2. Percentage of Inflammatory Inhibitory Power

Treatment	Percentage (%) of Inflammatory Inhibition Power				
	1st hour	2nd hour	3rd Hour	4th Hour	5th hour
Positive Control	16.98%	42.85%	62.50%	75.90%	87.00%
Dose I (50 mg/kgBB)	5.66%	52.85%	28.75%	63.85%	43.42%
Dose II (150 mg/kgBB)	62.26%	85.71%	87.50%	72.28%	65.78%
Dose III (250 mg/kgBB)	50.94%	91.42%	62.50%	80.72%	82.89%

Based on the results obtained in the table above, it can be seen that there is an influence of anti-inflammatory effects on the dose of kencur rhizome extract at a dose of 250 mg/kgBW, whose percentage of inhibitory power at the 5th hour produces 82.89%, which is almost close to the positive control of 87.00%. So it is said that kencur rhizome extract has an anti-inflammatory effect.

Table 3. Data from ANOVA Analysis One Way

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2.636	4	.659	16.786	.000
Within Groups	.981	25	.039		
Total	3.617	29			

Based on the ANOVA table above, with a confidence level of 95% and a significance of 0.05, the F count obtained is 16.786 greater than F table 3.48, and the sig value. of 0.000 is smaller than alpha 0.05, so Ho is rejected and Ha is accepted, meaning that there is an effect of anti-inflammatory activity on the administration of kencur rhizome extract on decreasing the incidence of udem in male white mice.

DISCUSSION

Research shows the anti-inflammatory properties of the ethanol extract of galangal rhizomes on the soles of white mice. After oral administration of an ethanol extract of kencur rhizomes to produce carrageenan intraplantar, this was shown by a decrease in swelling on the soles of the feet of white mice, and the percentage of inhibition was recorded at each treatment. The experiment was conducted by observing and measuring the percentage of edema decrease in the soles of white mice's feet (Neman, Maarisit, and Karauwan 2022).

The test animals used in this experiment were male white mice. Because the hormonal environment is different and male white mice are more stable than females, male white mice experience less stress than females. In this phase, the physical and mental well-being of female white mice is influenced by hormonal changes that occur periodically, such as in the pregnancy and lactation cycles.

All doses of ethanol extract from kencur rhizomes showed anti-inflammatory effects on treatment test results. This is because kencur rhizomes contain flavonoid components, saponins, and tannins (Siburian 2018).

Flavonoids reduce the amount of prostaglandins produced by blocking the digestion of arachidonic acid. In addition, they reduce the amount of flavonoids by inhibiting the release of lysosomal enzymes, which act as inflammatory mediators. It is possible to stop the spread of the inflammatory process by blocking these inflammatory mediators. Flavonoids play an important role as active ingredients (Arbiyani et al. 2022).

Stating that saponins can do this inhibits the increase in blood vessel permeability, so swelling is one of the symptoms of no inflammation. Saponins are believed to interact with various membrane lipids, e.g., phospholipids, prostaglandin constituents, and other inflammatory mediators (Siregar 2020).

Tannin compounds, which have anti-inflammatory qualities, contain antioxidants that stop neutrophils, monocytes, and macrophages from producing O_2 oxidizers. They also stop the action of direct reactive oxidizing agents such as

hydroxyl radicals (OH) and hypochlorous acid. When the production of O_2 oxidants is inhibited, less H_2O_2 is formed, which in turn inhibits the formation of OH and hypochlorous acid.

The rate of decrease in the anti-inflammatory effect can be seen in the percentage decrease in the thickness of swollen feet in each white mouse test animal in units of time after carrageenan administration. The ability of the test compound to reduce swelling of the feet of test animals after a 1% carrageenan injection can be assessed through the calculation of the percentage of inflammation inhibition compared to the negative control group. By comparing the mean values of the groups given the ethanol extract of kencur rhizomes in the positive control group and the negative control group, the percentage of anti-inflammatory effects can be calculated.

CONCLUSIONS

The percentage of ethanol extract of kencur rhizomes that inhibited inflammation at a dose of 250 mg/kg body weight at the fifth hour gave almost the same results as the positive control treatment of diclofenac sodium, or 82.89%. However, at doses of 150 mg/kg body weight and 50 mg/kg body weight, which showed inhibitory results of 65.78% and 43.42%, respectively, they were still unable to reduce inflammation significantly. A statistical analysis using the one-way ANOVA method was carried out to determine whether there were significant differences in reducing inflammation in test animals between treatment groups.

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