ANTIINFLAMMATORY EFFECT OF 96% ETHANOL EXTRACT OF *Catharanthus roseus* LEAF IN CARRAGEENIN-INDUCED MALE RAT

Rifda Naufa Lina, Dian Arsanti Palupi*, Hasty Martha Wijaya, Annis Rahmawaty, Ike Vera Dwi Nur Azis Slamet

Health Technology Institute Cendekia Utama Kudus, Indonesia

*Corresponding Author: Dianarsanti68@gmail.com:

Abstract. Inflammation is the process of the body's response to adverse stimuli caused by various harmful agents such as infections, antibodies, or physical injuries. Catharanthus roseus plant is one of the plants that is thought to have an anti-inflammatory effect because Catharanthus roseus Leaf contains flavonoids, saponins, alkaloids, and tannins. This study aims to determine the anti-inflammatory effect of ethanol extract Catharanthus roseus Leaf on foot edema of male white rats. This study was an experimental study using 25 male white rats divided into 5 groups. Mice were induced using 1% carrageenan subplantar. In the treatment group doses I, II, and III were given ethanol extract of Catharanthus roseus Leaf at doses of 100 mg, 200 mg, and 400 mg/Kg BW, negative controls were given 0.1% NaCMC and positive controls were given Diclofenac Sodium. The treatment was carried out for 3 hours and the treatment was administered orally with a volume of 5 ml. The research results were analyzed using SPSS with One Way ANOVA and the results obtained were p=0.000. Then the LSD Post Hoc test was carried out which showed no significant difference between the positive control and the 400mg/KgBB group. Therefore it can be concluded that the effective dose of ethanol extract from Catharanthus roseus Leaf which has an anti-inflammatory effect is 400 mg/KgBW.

Keywords: [*Ethanol extract of* Catharanthus roseus *Leaf, anti-inflammatory, Edema, Carrageenan*]

INTRODUCTION

Inflammation is an indicator of the immune system that fights disease and functions to destroy, reduce, and localize injurious agents and injured tissue. The characteristics of acute inflammation are edema, redness, pain, and heat. The inflammatory process is caused by the release of various chemical mediators, such as leukocyte products, plasma proteases, vasoactive amines, and arachidonic metabolites (Khotimah & Muhtadi, 2014).

Signs of an inflammatory response or acute inflammation include swelling/edema, redness, heat, pain, and changes in function. Things that occur in the acute inflammatory process are largely made possible by the release of various chemical mediators, including vasoactive amines, plasma proteases, arachidonic acid metabolites, and products (Hasanah et al., 2011).

The mechanism of inflammation begins with a stimulus that damages the tissue, resulting in the dilation of the blood vessel walls. Furthermore, there is a change in blood volume in the capillaries so that the blood vessel cells stretch against each other and cause plasma proteins to come out. This results in the accumulation of fluid in the tissues and the release of histamine and prostaglandin mediators which cause an inflammatory process. Meanwhile, the process of pain begins with pain stimulation in the form of chemical and thermal substances which cause damage to cell membranes so that the tissue is damaged and releases pain mediators prostaglandins. Prostaglandins are released into the blood circulation and are delivered to the brain as pain (Rahayu, Dewi & Ayu, 2016).

In Indonesia, the prevalence of diseases involving inflammatory processes is quite high. Some of these diseases, namely diabetes mellitus 2.1%, asthma 4.5%, dermatitis 6.8%, acute respiratory infections 25.50%, pneumonia 2.13%, joint disease 24.7%, tumors/cancer 0 .4%, hepatitis 1.2% (Riskesdas, 2013).

According to Pramitaningastuti & Anggraeny (2017), inflammation is usually treated using steroid-class anti-inflammatory drugs and non-steroidal anti-inflammatory drugs. Chemical anti-inflammatory drugs are widely used by the public because they have a fast effect in eliminating inflammation but have dangerous side effects, including disturbances in the gastrointestinal tract, blood, respiration, metabolic processes, hypersensitivity, and Reye's syndrome. According to Priamsari & Krismonikawati (2019), one of the inflammatory drugs comes from the Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) group which works by inhibiting the cyclooxygenase (COX) enzyme so that the formation of arachidonic acid into prostaglandins is inhibited. In addition, NSAIDs also have therapeutic effects and

side effects, namely the tendency to induce gastric or intestinal ulcers accompanied by anemia due to blood loss. Side effects arising from the use of NSAIDs can be minimized by looking for alternative treatments to reduce pain and inflammation from natural ingredients so that the side effects will be smaller.

Traditional medicine in Indonesia is a national culture that has been passed down from generation to generation using plants or ingredients found in nature (Samudra, 2017). The Catharanthus roseus plant is one of the plants that are widespread in the tropics and belongs to the Apocynaceae family (Muharram et al., 2018). A study conducted by Hassan et al., (2011) showed results that Catharanthus roseus leaves contain alkaloids, tannins, saponins, flavonoids, and triterpenoids. Catharanthus roseus leaves are one of the natural ingredients that have been widely studied and reported to have many properties in curing various diseases, including anti-cancer (antineoplastic), laxative urine (diuretic), and lowering blood pressure (hypotension), tranquilizers (sedatives), stop bleeding (hemostatic) and remove heat and poison (Verranandа, Febrina & Rijai, 2013).

In previous studies, it was stated that Catharanthus roseus Leaf extract affected the number of fibroblasts in the oral mucosal wounds of Wistar rats (Rattus norvegicus) with the best concentration being 50% on the 7th day (Putri, et al., 2017). The process of wound healing consists of phases that are interconnected with one another, namely hemostasis, inflammation, proliferation, and tissue remodeling. So Catharantus roseus Leaf extract needs to be specifically investigated for its effect as an anti-inflammatory.

Therefore the use of medicinal plants with anti-inflammatory properties needs to be done to find alternative treatments with relatively few and small side effects. This study aims to examine the anti-inflammatory effect of ethanol extract of Catharanthus roseus leaves on leg edema of male white rats induced by carrageenan at the right dose. So that an effective dose will be obtained as an anti-inflammatory. The results of this study are expected to be used by the public as a traditional anti-inflammatory drug and can be followed up by other researchers to make an anti-inflammatory product from Catharanthus roseus leaves.

METHODS

Materials

Catharanthus roseus leaves, Rat probe (Terumo Syringe), 1cc and 5cc injection syringe (Terumo), stamper mortar, analytical balance (Sartorius), plethysmometer, grams and mg scales, stopwatch, measuring cup, tray, sieve, blender, rat cage, water bottle, beaker glass (Pyrex), maceration vessel, rotary evaporator, stir bar, water bath, test tube, measuring cup (Pyrex), CMC Na, ethanol 96%, distilled water, Diclofenac Na (Novell), 1% carrageenan, 0.9% NaCl, concentrated HCl, Mg powder, 2N HCl, 10% FeCl3 solution, 2M HCl, Dragendorf reagent, Mayer reagent, Mayer reagent, Liebermann-Burchard reagent, acetic anhydrite, concentrated acetic acid.

Phytochemical Screening

Alkaloid Test

The extract is evaporated over a porcelain cup. The resulting residue was then dissolved with 5 mL of 2M HCl. The solution obtained was divided into 3 test tubes. The first tube serves as a blank, added with 3 drops of 2M HCl. The second tube added 3 drops of Dragendorf reagent and the third tube added 3 drops of Mayer reagent. A positive test is indicated by the formation of an orange precipitate while the Mayer reagent is indicated by the formation of a yellow precipitate.

Flavonoid Test

The extract is added to hot water, then boiled for 5 minutes then filtered. 5 mL of filtrate was added with 0.05 mg of Mg powder and 1 mL of concentrated HCl, then shaken. A positive test is indicated by the formation of red, yellow, or orange color.

Saponin Test

The extract was put into a test tube, then added with 10 mL of hot water then cooled and shaken vigorously for 10 seconds, then added 1 drop of 2N HCl. Positive results were indicated by the formation of stable froth up to 1-10 cm for 10 minutes.

Tannin Test

The extract was added with a few drops of 10% FeCl3. A positive result is indicated by the formation of a greenish-black color.

Triterpenoid Test

Identification was carried out with Liebermann-Burchard reagent. In this test, 10 drops of acetic anhydride and 2 drops of concentrated sulfuric acid were added sequentially to 1mL of the sample which had been dissolved in ethanol. Then the sample was shaken and left for a few minutes. If the sample turns red and purple, it means that it is positive for triterpenoids.

Dosage

1) Carrageenan Dosage

The dose of carrageenan is 5mg/200gBW of 1% carrageenan or 1g of carrageenan in 100mL of preparation with a maximum administration volume of 0.1mL rat's subplantar for every 200g rat weight.

2) Diclofenac Sodium Dosage

The therapeutic dose of diclofenac sodium in adults is between 50mg. The conversion factor from a 70 kg human to a 200 g rat is 0.018. Then the dosage setting is 50mg x 0.018 = 0.9 mg/200kgBW. The maximum administration volume p.o is 5mL

3) Dosage of 96% Ethanol Extract of Catharanthus roseus Leaf

The doses of Catharanthus roseus Leaf extract used were 100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW.

Edema production

The feet of the rats that had been marked were limited to the ankles, then induced subplantar with 1% carrageenan solution of 0.1mL.

Anti-inflammatory Testing

The anti-inflammatory test procedure is by fasting rats for 18 hours before testing, given drinking water. 25 male white rats were grouped into 5 treatment groups. On the day of testing, the rats were weighed and marked on the right ankle. Each rat from each group was measured the normal volume (Vn) on the right hind leg by immersing it in mercury on a plethysmometer. Then each group was given the following treatment:

- Group I (negative control): given 5 ml of 0.1% Na CMC suspension
- Group II (positive control): given diclofenac Na solution at a dose of 0.9 mg/200 kg BW of 5 ml
- Group III (Test 1): given a suspension of ethanol extract of Catharanthus roseusLeaf at a dose of 100 mg/kg BW of 5 ml
- Group IV (Test 2): given a suspension of ethanol extract of Catharanthus roseus Leaf at a dose of 200 mg/kg BW of 5 ml
- Group V (Test 3): given a suspension of ethanol extract of Catharanthus roseus Leaf at a dose of 400 mg/kg BW

After 30 minutes, the change in fluid volume was recorded as the paw volume of the rat (Vt). Measurements were made every 30 minutes for 180 minutes. Calculate the percentage of edema and DAI (anti-inflammatory power).

From the data obtained, the volume of edema was calculated from the difference in the volume of the rat's feet before and after being induced with 1% carrageenan at a certain time. The formula for the percentage of edema is:

$$Vu = Vt - V_0$$

Description :

Vu: Volume of rat foot edema each time t

Vt: The volume of rat foot edema after being induced by carrageenan at a certain time

V0: Initial volume of rat foot before induction of carrageenan 1%

Quantitative data of the study were the Area Under Curve (AUC) of the average edema volume curve under time and the percentage of anti-inflammatory effect. The AUC value is the average area under the curve which is the relationship between the average volume of edema per unit of time with the formula:

$$AUC = \frac{Vtn-1+Vtn}{2} (t_n - t_{n-1})$$

Description:
Vtn-1: Average volume of edema in tn-1
Vtn: Mean edema volume in tn

The percentage of anti-inflammatory power is calculated based on the percent reduction in edema using the formula:

$$\%DAI = \frac{AUCk-AUCp}{AUCk} x$$

Description:
AUCk: AUC curve of mean edema volume versus time for negative control
AUCp: AUC volume versus time curve for the treatment group in each individual.

Data analysis
The % DAI value obtained was statistically tested using a one-way ANOVA test and then continued with the LSD (Least Significantly Difference) Test with a significance level of 0.05.

RESULTS AND DISCUSSION

Phytochemical Screening
Qualitative tests on Catharantus roseus leaf extract were carried out at the ITEKES Cendekia Utama Kudus Chemical Laboratory. The results of the identification of the compounds contained in Catharantus roseus Leaf extract can be seen in Table 1 below:

Table 1. Phytochemical screening results of Catharanthus roseus Leaf

Content	Reactor	Result
Flavonoid	Mg Powder, HCl, and alcohol 96%	+
Tanin	FeCl3 1%	+
Saponin	HCl 1%	+
Alkaloid	HCl 2M and Dagrendorff	+
Triterpenoid	Glacial acetic acid and H2SO4 concentrated	-

The results of the phytochemical screening showed that Catharantus roseus leaf extract was positive for flavonoids, tannins, saponins, and alkaloids and negative for triterpenoids.

Anti-inflammatory effect
In this study, 5 test groups were used with 25 rats, namely negative control (NaCMC 0.1%), positive control (Diclofenac Sodium 0.9 mg/KgBW), 96% ethanol extract of Catharantus roseus Leaf at a dose of 100 mg/KgBW, 200 mg/KgBW and 400 mg/KgBW. The anti-inflammatory test of Catharantus roseus Leaf extract used the method of making edema on the soles of mice that had been induced with 1% carrageenan and giving a test dose. From the measurement of the volume of edema, the average value of edema, the average AUC value, and the average % DAI value will be obtained. The average volume value of rat paw edema can be seen in the following Table 2:

Table 2. The average volume of rat paw edema

Treatment	The average difference in the decrease in edema volume						
	T0	**T0,5**	**T1**	**T1,5**	**T2**	**T2,5**	**T3**
KN	0,0105	0,0374	0,0372	0,0370	0,0366	0,0360	0,0358
KP	0,0103	0,0330	0,0280	0,0232	0,0212	0,0142	0,0102
D100mg/KgBW	0,0104	0,0332	0,0322	0,0308	0,0292	0,0270	0,0242

D200mg/KgBW	0,0106	0,0360	0,0316	0,0270	0,0238	0,0198	0,0156
D400mg/KgBW	0,0105	0,0320	0,0274	0,0232	0,0202	0,0154	0,0102

Description : KN: Negative Control (Na CMC 0.1%), KP: Positive Control (Diclofenac Sodium 0.09mg/KgBW)

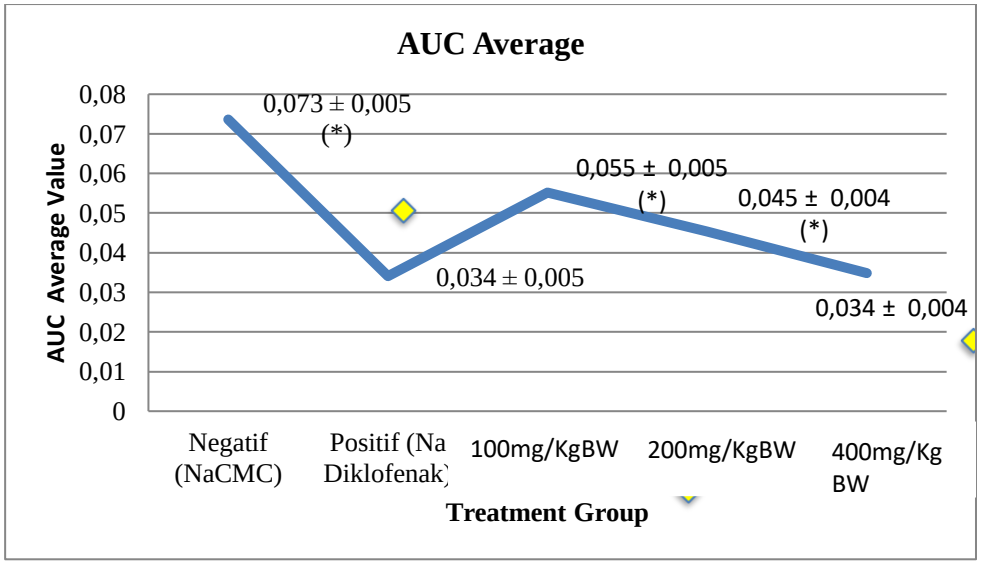
From the data on the average volume of edema on the soles of the rats' feet above, the AUC (Area Under Curve) and %DAI (Anti-Inflammatory Power) values for each test animal in each experimental group are obtained. The results of calculating the average value of AUC and %DAI are as follows in Table 3:

Table 3. Average of AUC ± SD

Treatment	Average of AUC ± SD
Negative Control	0,073 ± 0,005*
Positif Control	0,034 ± 0,005
D100mg/KgBW	0,055 ± 0,005*
D200mg/KgBW	0,045 ± 0,004*
D400mg/KgBW	0,034 ± 0,004

Description: * (significantly different from the positive control)

From the table above, it can be seen that the average AUC is the highest in the negative control. So that a graph of the average AUC relationship on the paws of rats can be made as follows in picture 1:



Picture 1. Graph of Average AUC value

The results of statistical analysis using the One Way ANOVA test with a significant value of 0.000 ($p < 0.05$) indicated that there were significant differences between groups. After that, it was continued with the LSD test with positive control results (Diclofenac Na) which were significantly different from the doses of 100mg, 200mg/KgBW. While the positive control did not have a significant difference with a dose of 400 mg/KgBW. Thus a dose of 400 mg/KgBW is an effective dose to reduce edema volume.

%DAI (Anti-Inflammatory Power)

From the data, the average AUC value was then followed by calculating the % DAI (Anti-Inflammatory Power) value to find out how much the test material could inhibit edema on rat feet induced with 1% carrageenan. The results of the % DAI calculation can be seen in Table 5.

Table 5. Average of %DAI

Treatment	Average of %DAI
Negative Control	-
Positif Control	53,776
D100mg/KgBW	25,112
D200mg/KgBW	38,362
D400mg/KgBW	52,758

From the table above it can be seen that the highest % DAI (Anti-inflammatory Power) calculation results were found in the positive control and the ethanol extract of Catharanthus roseus Leaf extract at a dose of 400 mg/KgBW.

So based on the results of statistical analysis and calculation of the % DAI, it can be concluded that a dose of 400 mg/KgBW is an effective dose because it has an effect equivalent to positive control. This is because Catharantus roseus Leaf extract 400 mg/KgBW contains alkaloid compounds, flavonoids, tannins, and saponins. Flavonoids work as cyclooxygenase (COX) inhibitors. Cyclooxygenase (COX) functions to trigger the formation of prostaglandins where prostaglandins play a role in the inflammatory process and increase body temperature. If prostaglandins are not inhibited, there will be an increase in body temperature which will result in fever (Samudra, 2017). The mechanism of alkaloids as an anti-inflammatory is by suppressing histamine release by mast cells, reducing IL-1 secretion by monocytes and PAF on platelets (Luliana et al., 2017). Tannins work to bind and trigger or precipitate proteins and inhibit protein synthesis and saponins have anti-inflammatory potential by inhibiting protein denaturation, but it is not clear how the mechanism of saponins inhibits protein denaturation. Protein denaturation is one of the causes of inflammation (Erianti et al., 2015).

CONCLUSION

The effective dose of 96% ethanol extract of Catharanthus roseus Leaf extract as an anti-inflammatory in carrageenan-induced rat foot edema is a dose of 400 mg/KgBW.

ACKNOWLEDGEMENT [OPTIONAL]

We thank ITEKES Cendekia Utama Kudus, who has provided support for our research publication

REFERENCES

Khotimah, S. N., & Muhtadi, A. (2014). Article Review: Some Plants That Contain Anti-Inflammatory Compounds. Pharmaca, 14(2), 28–40.

Hasanah, A. N., Nazaruddin, F., Febrina, E., & Zuhrotun, A. (2011). Analysis of Essential Oil Content and Anti-inflammatory Activity Test of Kaempferia galanga L. Extract Journal of Mathematics & Science, 16(3), 147–152.

Rahayu, L., Dewi, R. S., & Ayu, G. (2016). Test of Anti-inflammatory and Analgesic Effects of Senggani Leaf Infusion (Melastoma malabathricum L.). Indonesian Journal of Pharmaceutical Sciences, 14(1), 10–15.

Riskesdas. (2013). BASIC HEALTH RESEARCH. Health Research and Development Agency, Ministry of Health RI.pdf (Vol. 7, Issue 5).

Pramitaningastuti, A. S., & Anggraeny, E. N. (2017). Anti-inflammatory Effectiveness Test of Ethanol Extract Annona Squamosa. Leaf Against Leg Edema of Wistar Male White Rats. Pharmaceutical Scientific Journal, 13(1).

Priamsari, M. R., & Krismonikawati, R. A. (2019). Test of Anti-inflammatory Power of Dutch Eggplant (Solanum betaceum, Cav.) Peel Ethanolic Extract in Carrageenin-Induced Male Mice. Journal of Pharmacy and Clinical Pharmacy (JIFFK), 16(2), 1–7.

Samudra, A. G. (2017). Antipyretic Effectiveness of Catharanthus Roseus Leaf Extract in Mice (Mus musculus). Journal of Borneo Journal of Pharmascientech, 01(01), 28–35.

Muharram, Adnan, Faqih, A., & Jihadi, A. (2018). Conception Power Test of Catharanthus Roseus Leaf Methanol Extract (Catharantus roseus) Against Male Icr Mice (Mus Musculus). Indonesian Journal of Fundamental Sciences, 4(2), 102–109.

Hassan, K. A., Brenda, A. T., Patrick, V., & Patrick, O. E. (2011). In vivo antidiarrheal activity of the ethanolic leaf extract of Catharanthus roseus Linn. (Apocyanaceae) in Wistar rats. African Journal of Pharmacy and Pharmacology, 5(15), 1797–1800.

Verrananda, I., F, V. Y., Febrina, L., & Rijai, L. (2013). Identification of Secondary Metabolites and Antioxidant Activity of Catharanthus Roseus Flower Extract. Journal of Chemical Information and Modeling, 53(9), 1689–1699.

Putri, R. R., Hakim, R. F. and Rezeki, S. 2017. The Effect of Catharanthus Roseus Leaf Extract on the Number of Fibroblasts On the Process of Wound Healing in the Oral Mucosa. Journal of Canine Dentistry. 2(1): 20-30.

Erianti, F., Marisa, D., & Suhartono, E. (2015). Anti-inflammatory Potential of Carambola Fruit Juice (Averrhoa carambola L.) Against Protein Denaturation In Vitro. Periodic Journal of Medicine, 11, 33–40.

Luliana, S., Susanti, R., & Agustina, E. (2017). Antiinflammatory Activity Test of Aqueous Extracts Herb of Physalis angulata L. in Caragenan Inducted Wistar Rat (Rattus norvegicus L.). Traditional Medicine Magazine, 22(3), 199.