Research article

Anti-inflammatory Activity of Andong Leaf Extract (Cordyline Terminalis Kunth) Against Edema in the Soles of Wistar Rats

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Abstract.
The Balinese plant andong (*Cordyline terminalis* Kunth) has anti-inflammatory properties. The focus of this research was to determine whether andong leaf extract has anti-inflammatory properties and is effective against carrageenan-induced edema in rat soles. In this study, carrageenan was used to induce inflammation in the soles of Wistar rats. 25 rats were divided into five treatment groups. Group I was given aquadest as the control; group II was given diclofenac sodium at a dose of 4.5 mg/kgbw as a drug control; and groups III, IV and V were given ethanol extract of andong leaves at doses of 150 mg/kgbw, 300 mg/kgbw, and 600 mg/kgbw orally as test groups. For six hours, the inflammation volume was measured using a plethymometer. One-way ANOVA was used to test differences between the treatment groups. The results showed that the ethanol extract of andong leaves had the highest anti-inflammatory activity in the six-hour period when given at doses of 150 mg/kgbw (36.35% 0.02), 300 mg/kgbw (26.30% 0.20), and 600 mg/kgbw (20.67% 0.16) with a significant difference ($p < 0.05$) compared to the negative control (86.84% 0.092), but this was not significantly different from the positive control (22.21% 0.01). Based on these findings, it can be concluded that a dose of 600 mg/kgbw is the most effective in reducing inflammation.

Keywords: Anti-inflammatory, Red andong leaf, Carrageenan, Wistar rat, Edema

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1. Introduction

Skin inflammation is a protective response in the skin caused by injury or tissue damage and infection. inflammation is caused by a complex reaction in connective tissue that has vascularity due to exogenous and endogenous stimuli [1]-[2]. The inflammatory process is a protective mechanism by which the body attempts to neutralize and kill harmful agents at the site of injury and to prepare the tissues. Five characteristics of inflammation, known as the main signs of inflammation, are redness (rubor), heat (calor), swelling (edema), pain (dolor) and loss of function (functiolaesa) [3].

Inflammation treatment can use non-steroidal anti-inflammatory drugs (NSAIDs) and steroid class drugs that can relieve inflammatory reactions well but in long-term use will

have side effects in the form of gastrointestinal disorders such as gastric ulcers, impaired kidney function and pregnancy induction. Long-term use of steroid drugs will reduce the body's immune response to infections, osteoporosis, moonface, and hypertension [1]-[2].

Currently, treatment using natural ingredients, namely plants, is increasing. The community's need to treat diseases by using plants is one of the current phenomena. Medicinal plants contain many components of active compounds and have various pharmacological effects that need to be scientifically proven.

One of the medicinal plants that can be used for anti-inflammatory treatment is andong leaf (*Cordyline terminalis*), which is traditionally used as a swelling medicine in Bali. The chemical components contained in the leaves of Andong are saponins, tannins, flavonoids, polyphenols, polysaccharides [4]-[5]. One of the content of Andong leaf extract that has the potential as an anti-inflammatory drug is flavonoids. Siriwardana *et al*. (2013) reported that flavonoids have the potential to inhibit the cyclooxygenase enzyme so that the formation of prostaglandins is inhibited in the inflammatory process. flavonoids are able to protect lipid membranes against damaging reductions[6]. Flavonoids can also inhibit the release of inflammatory mediators such as histamine and prostaglandins [7].

Based on the description above, the research objective was to evaluate the anti-inflammatory activity and effectiveness of the ethanol extract of andong leaves on the soles of rats induced by 1% carrageenan.

2. Methodology

2.1. Material and Tools

In this study, the test material used was andong leaf [5], the chemicals used were lambda (λ) type carrageenan (Sigma Chemical Co), Sodium Chloride 0.9% w/v (Otsuka, Indonesia), diclofenac sodium (Pharos), 96% ethanol (IkapharmindoPutramas), Carboxymethyl Cellulose Sodium (CMC), and distilled water.

The equipment used in this study included: beaker (Pyrex), measuring cup (Pyrex), oral injection syringe (Terumo), blender, Rotary Vacuum Evaporator (Heidolph), analytical balance (Mettler toledo), 60 mesh sieve, rat cage , stirring rod, ad libittum mouse drinking bottle, plesthymometer PANLAB LE 7500 model/series, and a set of maceration tools.

2.2. Andong leaves extraction

The powder 450 g of andong leaves was extracted by maceration technique using 96% ethanol, according to Bogoriani *et al*., 2021[5].

2.3. Activity test of andong leaf extract on test animals

The test animals used were white rats with the Wistar strain weighing 116-160 grams and 2-3 months old. The condition of the animal is healthy. The number of rats used were 25 which were divided into 5 groups and each group consisted of 5 rats, namely the normal control rat group which was given distilled water (K1), the rats group were given a suspension of 4.5 mg/kgbw sodium diclofenac (K2), and The rats were given red andong leaf extract with successive doses of 150 mg/kgbw (K3), 300 mg/kgbw (K4), 600 mg/kgbw (K5). The rats were adapted in cages for approximately 1 week for the acclimatization process. During the process, it is ensured that the needs for food and drink are met. Rats were fasted for 18 hours before treatment, but drinking water was still given (ad libitium) [8]. The rats were then weighed and marked on the joint of the left hind leg using a marker [9]. This mark is used as the limit in the immersion of the foot on the plesthymometer to measure the volume of edema. After that, the rat's left leg was inserted into the plesthymometer to the mark that had been made previously, and the initial volume (V_0) of the rat's foot was recorded each group, namely the volume of the foot before being given the drug and induced with a carrageenan solution. Each rat was given a suspension of the test material orally according to its group. One hour later the treatment group was induced subplantarly with 0.1 ml solution of 1% carrageenan, after 1 hour the measurements were taken with a plesthymometer. The volume of the rat's feet (Vt) was recorded asvolume and diameter of the feet after being given the drug and induced with carrageenan solution. Measurements were taken every 60 minutes for 360 minutes.

The volume of inflammation is the difference in the volume of the rat's paws after and before the carrageenan injection.

2.4. Calculation of inflammation percentage

$$\% \ Inflammation = \frac{Vt - Vo}{Vo} \times 100\%$$

Information:

Vt = Volume of rat paws in each group at time t after treatment

Vo = Volume of rat paws in each group before treatment[10].

1. (a) Analysis of statistic

Data analysis was carried out statistically using one way ANOVA and determined according to Bogoriani *et al*., 2020[11]

3. Result and Discussion

The percentage of anti-inflammatory activity of the ethanol extract of red andong leaves can be seen in Table 1.The increase in the average percentage of anti-inflammatory in all test groups showed that the negative control group who was given orally aquadest had the largest percentage of inflammation compared to the other test groups because the negative control group did not contain active substances that could inhibit the formation of edema. In the negative control, maximal edema was formed at the fifth hourand the sixth.

TABLE 1: Average percentage of foot edema of Wistar rats at each observation time (%).

Observati Time	Treatment				
	Average percentage of edema (%) + SD every 1 hour for 6 hours				
hours	K_1	K_2	K_3	K_4	K_5
1	34.01 + 0.07	7.40 + 0.10	18.17 + 0,014^{a,b}	13.79 + 0.13^{a,b}	10.51 + 0.21^{a,b}
2	44.73 + 0.06	22.12 + 0.06	36.35 + 0.02^{a,b}	31.03 + 0.16^{a,b}	15.77 + 0.22^{a,b}
3	60.52 + 0.07	33.32 + 0.36	45.44 + 0.18^{a,b}	41.37 + 0.39^{a,b}	21.04 +0.23^{a,b}
4	65.77 + 0.08	33.32 + 0.05	45.44 + 0.03^{a,b}	44.83 + 0.16^{a,b}	31.56 + 0.24^{a,b}
5	84.20 + 0.10	40.73 + 0.04	58.61 +0.17^{a,b}	54.53 +0.02^{a,b}	52.62 + 0.29^{a,b}
6	86.83 + 0.09	22.21 + 0.01	36.35 + 0.03^{a,b}	26.30 +0.20^{a,b}	20.67 + 0.16^{a}

Description: K1 (normal control group given aquadest); K2 (drug control group given diclofenac sodium at a dose of 4.5 mg/kgbw), and K3,K4,K5 (test group given ethanol extract of andong leaves at a dose of 150 mg/kgbw, 300 mg/kgbw, and 600 mg/kgbw)

^{a} Shows difference of significantly from K1 $p < 0.05$

^{b} Shows difference of significantly from K2 $p < 0.05$

This anti-inflammatory test aimed to evaluate the anti-inflammatory effectiveness of andong leaf extract against male white rats. Carrageenan-induced rat paw edema is the

standard experimental model of acute inflammation [12]. Carrageenan is a mucopolysaccharide obtained from Irish red seaweed (*Chondruscrispus*). Carrageenan plays a role in the formation of edema in a model of acute inflammation [13]. Carrageenan is a foreign substance (antigen) which when it enters the body will stimulate the release of inflammatory mediators such as histamine, causing inflammation due to the body's antibodies reacting to these antigens to counter their effects [14].

The results of phytochemical screening of andong leaves were reported to contain flavonoid, alkaloids, saponins, tannins, and phenols compounds [5].

Anti-inflammatory activity testing was carried out using a plesthymometer with the principle of measurement based on Archimedes' law. In this study, carrageenan injection was carried out 60 minutes after treatment, the formation of inflammation by carrageenan did not cause tissue damage even though the inflammation could last for 360 minutes and gradually decrease over one day[15].

Based on the calculation results, it was found that the K3 group was 150mg/kgbw; K4300 mg/kgbw; K5 600 mg/kgbw has had an inhibitory effect of edema on the soles of rats with inflammation percentage of 36.35 ± 0.03%; 26.30±0.20%; 20.67±0.16% respectively;

The greatest anti-inflammatory effect was found in the 600 mg/kgbw group. The anti-inflammatory effect was shown by the smallest percentage of inflammation during the 6-hour observation. Based on the LSD test results showed that the K3 group was 150 mg/kgbw; K4300 mg/kgbw; K5600 mg/kgbw hadanti-inflammatory effect, from the three doses, the dose that showed the lowest percent inflammation during 6 hours of observation was the K5 dose of 600 mg/kgbw. It is suspected that this dose is the optimal dose that can inhibit the formation of edema on the soles of the rat's feet.

The existence of an anti-inflammatory effect is thought to be due to the activity of secondary metabolites contained in the ethanol extract of andong leaves, namely flavonoids, alkaloids, saponins, tannins, steroid and phenols. One of the secondary metabolites thought to have anti-inflammatory activity is flavonoids, the mechanism of action of flavonoids as anti-inflammatory can be through several pathways by inhibiting cyclooxygenase (COX) and lipoxygenase activities, inhibiting leukocyte accumulation, inhibiting neutrophil degranulation, inhibiting histamine [16]. In addition, the mechanism of flavonoids in inhibiting inflammation is through two ways, namely by inhibiting arachidonic acid and the secretion of lysosomal and endothelial enzymes so that proliferation and exudation of the inflammatory process occur. The inhibition of the release of arachidonic acid from inflammatory cells will lead to less availability of

arachidonic substrate for the cyclooxygenase pathway and the lipoxygenase pathway[1]-[2]. In addition to flavonoids, other bioactive compounds that have the potential as anti-inflammatory are saponins. Saponins have an anti-inflammatory mechanism by inhibiting the formation of exudate and inhibiting vascular permeability[17].

4. Conclusion

The results showed that the ethanol extract of Andong leaves at doses of 150 mg/kgbw, 300 mg/kgbw, and 600 mg/kgbw had anti-inflammatory activity against carrageenan-induced Wistar rat edema with a significant difference compared to negative controls and not significantly different from positive control.

Andong leaf ethanol extract which gave the highest anti-inflammatory activity for rat foot edema was at a dose of 600 mg/kgbw in terms of volume measurement of edema and the percentage of inflammation produced was close to the value of the positive control, which was 20.67± 0.16%.

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