

Anti-Inflammatory Effect Of *Carica Papaya* Leaves Extract In Male Wistar Rats Based On Variation Of Concentration

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Abstract.

Treatment of inflammation is currently dominated by using synthetic drugs, which if used in cases of chronic inflammation such as hyperuricemia and rheumatoid arthritis will carry the risk of increasing side effects for the user. Papaya leaves as a potential herb in some communities are also used as a treatment for inflammatory conditions. The aim of this research was to determine the anti-inflammatory effect of papaya leaf extract in vivo based on variations in concentration in Wistar white rats. This research is an in vivo study using 25 male Wistar Rats and divided into 5 groups. Group I was given as a negative control (P0), the group used Diclofenac Sodium as a positive control (P1), the *Carica papaya* leaf extract test group with varying concentrations of 40% (P2), 50% (P3), and 60% concentration (P4). Carrageenan 1% is used as an inflammation inducer. The inflammation observation parameter in this study was the volume of edema in the rats' feet at 30 minute intervals for 180 minutes. The average percentage reduction in edema during 180 minutes of observation in the P2, P3 and P4 extract test groups was 81.00%, 78.33% and 81.00%, respectively. The positive control group (P1) showed a reduction of 81.66%, while the negative control was only 26%. The results of statistical tests show that there is an effect of giving the extract on reducing edema in mice compared to the negative control group. From these results it can be concluded that the ethanol extract of *Carica papaya* leaves with concentrations of 40%, 50% and 60% showed an anti-inflammatory effect on male Wistar rats.

Keywords: *Carica papaya*, anti-inflammatory and wistar rat .

I. INTRODUCTION

Inflammation is a biological problem that results from tissue damage and is exacerbated by pathogenic infections. The appearance of pain, redness, edema and even loss of motor function greatly interferes with daily activities.[1] Inflammatory treatment is currently still dominated by synthetic drugs such as steroids and non-steroids. The use of NSAID drugs can have an impact on the onset of several side effects and complications such as impaired kidney function, edema, hypertension, and bleeding in the gastrointestinal tract especially in long-term use in cases of chronic inflammation.[2] Treatment using corticosteroids is also questionable because of its influence on the decline in joint and tissue function.[3] Currently, the development of research from natural sources of plants and animals is very rapid with the aim of finding alternative medicines that are safe for long-term use, especially in such cases.[4] Treatment using herbal sources is currently believed to be increasing which is influenced by important factors where one of them is low cost.[5] Many plants are known to have strong anti-inflammatory activity. One of the potential herbal plants is *Carica papaya* leaves.

C.papaya leaves are known from various literature sources to contain various compounds such as phenolic acid, namely protocatechin acid, caffeic acid, chlorogenic acid and coumarinic acid, Flavonoids, namely kaemferol, curcetin, and myrisetin. In addition, the types of alkaloids, namely carpain and pseudocarpin, are also reported to be contained by *C.papaya* leaves.[6] Research shows that papaya leaf extract has a role in lowering the amount of interleukin- β that is known to be involved in the inflammatory response process.[7] Other research also revealed that the ability of *C.papaya* leaves to reduce oxidative stress is able to cause tissue damage resulting in inflammation.[8] Research related to the anti-inflammatory activity of *C.papaya* leaves in vitro has also been reported by Kumar, et al, 2021 using the antidenaturation test method where the results of papaya leaf extract showed an IC₅₀ value of 172.3 μ g.[9] *C.papaya* leaf juice with a concentration of 0.72 ml.100 g of body weight is also known based on the results of research can fight the influence of inflammation due to carrageenan induction.[10]

II. METHODS

This study is a laboratory experimental test using maceration containers, wooden stirrers, *vacuum rotary evaporators*, rat cages, plethysmometers, oral sonde, injection syringes, analytical scales, animal scales, and glassware. The ingredients used are *C.papaya* leaves (leaves picked directly from papaya trees that grow in the West Aceh area, Aceh, Indonesia), ethanol 96%, aquadest, carrageenan 1%, Na-CMC (Sodium Carboxymethyle Cellulose) 0.5%, sodium diclophenec tablets 50mg, Animal Testing are used White Rats of the Wistar strain aged 2-3 months, weighing 160-200 grams as many as 25 animals obtained from the Laboratory of Veterinary Medicine, Faculty of Veterinary Medicine, Syiah Kuala University Banda Aceh, Indonesian. Before testing, all mice were acclimatized for 1 week.

Preparation of *Carica papaya* leaf extract

A total of 100 g of dried *C.papaya* leaves powder was soaked using 750 mL of 96% ethanol while stirring for the first 6 hours, then soaked for 18 hours. After that, screening is carried out. The pulp in the first bath is re-soaked with 250 mL of 96% ethanol. The extraction process is repeated up to 2 times. The filtrate obtained is evaporated using a rotary evaporator at a temperature of 50°C so that the filtering solution separates from the extract until a thick extract is obtained.[11]

Test solution preparation

The viscous extract obtained is suspended into CMC sodium solution with a certain concentration so that the concentration of 40%, 50% and 60% of the test solution is obtained. For sodium diclofenac, the same thing was done where 100 mg of sodium diclofenac was dissolved into a solution of Sodium CMC with a volume of 100 mL so that a concentration of 0.1% was obtained.

Carrageenan 1% preparation

A total of 10 grams of carrageenan were weighed and dissolved with 100 mL of physiological NaCl in a glass jar until a concentration of 1% was obtained. The carrageenan solution is stirred until it is soluble and homogeneous.

Anti-inflammatory testing

In this *in vivo* study, anti-inflammatory test uses the Carrageenan-induced paw edema. Before the test, each rat will be fasted for 18 hours. The 25 rats were divided into 5, namely the P0 group as a negative control, P1 positive control of Diclofenac Sodium with a dose of 2.25 mg/kg BW,[12] P2, P3 and P4, namely 40%, 50% and 60% *C.papaya* leaves extract with 5 mice each per group. The left leg of the rat was marked to the ankle with a pointed end marker and the volume of the leg was measured with a plethysmometer as the initial leg volume. Then the rats in the P1 to P4 groups were given an extract test solution and an oral drug. Meanwhile, the P0 group was not given any test runs. After 1 hour all mice in each group were given a 1% carrageenan solution through the intraplantar. 10 minutes later, the volume of the rat's legs was measured after induction (v_0). Measurement of the volume of rat uedema began at 30 minutes after v_0 and was repeated for up to 180 minutes (v_t) using a plethysmometer. To determine the percentage of decrease in uedema in rat legs Data on decrease in uedema volume every 30 minutes was calculated using the formula.[13][14]

$$(v_0 - v_t / v_0) * 100\% \dots\dots\dots(I)$$

III. RESULT AND DISCUSSION

Resulting extract

Through an extraction process that was carried out repeatedly using 96% ethanol solvent, an extract weight of 16.2 g was obtained from 100 g of dry powder of *C.papaya* leaves. The number of extract yields showed a percentage of 16.2%

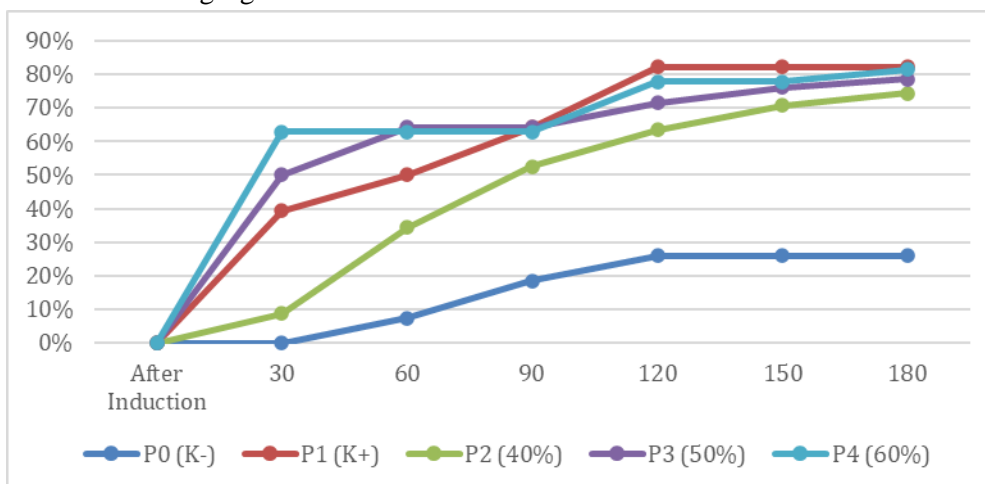
Anti-Inflammatory Test Results

In the anti-inflammatory activity test, the uedema volume measurement data carried out for 3 hours with an interval of 30 minutes after the administration of 1% carrageenan was calculated using equation 1 to determine the percentage of decrease in uedema in rat legs. The data on uedema decrease are presented in the following table 1:

Table 1. Percentage volume decrease in carrageenan - induced paw edema

Group	percentage decrease every minute					
	30	60	90	120	150	180
P0 (K-)	0%	7%	19%	26%	26%	26%
P1 (K+)	39%	50%	64%	82%	82%	82%
P2 (40%)	9%	34%	53%	64%	71%	74%
P2 (50%)	50%	64%	64%	71%	76%	79%
P2 (60%)	63%	63%	63%	78%	78%	81%

For more clarity in seeing the series of increase in the percentage value of the decrease in rat edema, it can be seen in the following figure 1:

**Fig 1.** Increase in activity of decreasing the volume of rat leg edema induced by carrageenan 1%

From this data (Fig.1), we can see that there is a variation in the percentage decline rate between these groups. The P1 group (K+) showed the highest percentage decrease, while the P0 group (K-) showed the lowest percentage decrease. In addition, the test group of *Carica papaya* leaf extract showed the highest decrease at a concentration of 60%, but began to decrease at a concentration of 50% and 40% along with a decrease in concentration. The K+ test group showed activity decreasing edema from the 30th minute and continued to increase until the 120th minute, but the activity began to decrease until the 180th minute. The 40% test group began to show a significant increase in activity at the 60th minute and continued to increase consistently to 74% at the 180th minute. A slightly different thing was shown by the 50% and 60% test groups where both began to show significant activity at the 30th minute but began to stagnate at the 60th and 90th minutes and began to increase again at the 120th minute and again stagnated again in the following minutes. As a group that was not given any test solution, the K- group also showed a decrease in edema until 26%, although statistically showed a significant difference with the test group.

The activity of lowering edema may be influenced by the ability of the rat body to fight inflammation. This is supported by the theory that there are anti-inflammatory agents in mice that are included in Interleukin 10 (IL-10) and TGF- β which are types of cytokines that are able to control the immune response that causes inflammation and maintain tissue homeostasis.[15], [16] The analysis using the statistical test using a significant value of $p < 0.05$ using the percentage of decrease in edema volume data can be explained that *C.papaya* leaf ethanol extract has an effect in reducing edema in rat induced by 1% carrageenan as evidenced by the value of $p = 0.00$. The test continued with the LSD test. The results of the LSD follow-up test showed a significant difference when the significant value of each treatment group was less than 0.05 (< 0.05). The P0 (K-) treatment group significantly showed edema reduction activity compared to P1 P2, P3, and P4 (< 0.05). The 40%, 50% and 60% extract test groups did not show any significant difference between the three marked by a significance value of $p > 0.05$. An interesting thing was seen between the P1 (K+) test group and the extract test group, where there was no significant difference in activity in lowering edema in rat legs. This shows that papaya carica leaves with concentrations of 40%, 50% and 60% have the potential to be an alternative treatment for inflammation.

The ability of *C.papaya* leaf extract as an anti-inflammatory is likely influenced by the content of the active ingredients it contains. Nandini, *et al.* (2020), reported that the ethanol extract of *C.papaya* leaves contains secondary metabolite compounds such as alkaloids, tannins, saponins, quinine, containing 0.196% total flavonoids and 0.06% total phenols.[17] The papain enzyme content in *C.papaya* leaves is known to contribute significantly to significantly lowering TGF- β which contributes to the occurrence of inflammation. Phenols are known to have been proven to have the activity of suppressing the action of cytokines, and activating enzymes that work as cellular antioxidants.[10] The effect is amplified when combined with other enzymes such as trypsin and chemotrypsin. The type of alkaloid in *C.papaya*, namely carpine, which has never been reported to contribute to anti-inflammatory activities, can further enrich the literature on the anti-inflammatory activity of *C.papaya* leaves.

IV. CONCLUSION

The conclusion of this study is that *C.papaya* leaves extract with concentration variations of 40%, 50% and 60% is able to provide anti-inflammatory effects in vivo on the Wistar white rats carrageenan-induced-paw edema

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