



Anti Inflammation Activity Of Duku Seed Extract Gel In Carrageenan Induced Wistar White Rats

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Abstract

Introduction: Inflammation is the body's initial response to tissue damage caused by trauma, exposure to toxins, or infection. Hyaluronic acid was one of the drugs of choice to reduce inflammation due to trauma, but it has side effects. One of the herbal plants that had anti-inflammatory potential was duku seeds. **Purpose:** The purpose of this study was to determine the anti-inflammatory activity of the duku seed extract gel on male white rats wistar were induced by carrageenan. **Method:** In vivo study with a pre and post-test control group design was conducted at the Biochemistry Laboratory and Animal House of the Faculty of Medicine Sriwijaya University and Palembang Clinical Laboratory Center. Thirty rats were divided into five groups. Group I, II, and III were treated with 5%, 10%, and 20% duku seed extract gel. Groups IV and V were treated with 0.2% hyaluronic acid gel and a placebo gel, respectively. Rats were induced by 0.1 ml carrageenan solution on the labial gingival of the rat's lower lip and treated with duku seed extract gel twice a day for four days and taking 2 ml of blood from the orbital sinus. The number of neutrophil cells was counted using a hematology analyzer and statistically analyzed. **Results:** There was no difference in the number of neutrophil cells before and after treatment in all groups. There was no difference in the number of neutrophil cells after treatment between the extract gel groups and the control. The least increase in neutrophil cells was found in the 20% extract group, but it was not significant. **Conclusion:** Duku seed extract gel at concentrations of 5%, 10%, and 20% did not have anti-inflammatory activity in male wistar strain rats.

Keywords: Anti-inflammatory; duku seed extract gel; neutrophil

Introduction

Inflammation is the body's initial response to tissue damage caused by trauma, exposure to toxins, or infection.¹ One of the causes of inflammation in dentistry is trauma. Trauma that occurs due to accidental or dental care mistakes, or perhaps due to negligence of the dentist, is called iatrogenic trauma.^{2,3} Hyaluronic acid was one of the drugs of choice to reduce inflammation due to trauma, but it has side effects. The disadvantage of this topical hyaluronic acid is contraindicated in a patient with a history of allergies or hypersensitivity reactions. Therefore, a safer substitute derived from herbal plants was needed.⁴

Lansium domesticum Corr. or better known as duku fruit was very easy to find in Indonesia, one of which was the Komerling duku originating from South Sumatra.⁵ Duku had several active compounds, including flavonoids, triterpenoids, alkaloids, lancioside acids, and



dukunolid.^{6,7} Research conducted by Klungsunya P. (2015) and Subandrate (2016) showed that flavonoids contained in duku seeds had antioxidant effects that can reduce free radical.^{7,8}

Flavonoids had anti-inflammatory effects, which are: reducing the amount of Interleukin 6 (IL-6), Tumor Necrosis Factor Alpha (TNF- α); inhibiting the secretion of Interleukins 1 and 8 (IL-1 and IL-8), and inhibiting the production of Nitric Oxide (NO).⁹ Triterpenoids had an anti-inflammatory effect with a mechanism that significantly suppresses the expression of Cyclooxygenase 2 (COX-2) and inducible Nitric Oxide Synthase (iNOS).¹⁰ Research conducted by De-Almeida showed that the triterpenoids contained in *Himatanthus drasticus* also inhibit the secretion of TNF- α and Interleukin 1 Beta (IL-1 β), which are cytokines during inflammation.¹¹

The purpose of this study was to determine the anti-inflammatory activity of the duku seed extract gel on male white rats Wistar strain induced by carrageenan.

Methods

This was in vivo study with a pretest-posttest control group design. The study was carried out in the Biochemistry Laboratory Faculty of Medicine Sriwijaya University Palembang for the making of duku seed extract, Animal house Faculty of Medicine Sriwijaya University Palembang for acclimatization, wound induction on the lower lip of rats and giving treatment to rats, and Health Laboratory Center Palembang for counting the neutrophil cells count. This study used white male Wistar rats (*Rattus norvegicus*) obtained from the Palembang Tikus Center. The inclusion criteria used in this study was Wistar strain white rat (*Rattus norvegicus*) male with 200-250 grams weight, aged 2-3 months, in good health with no defect. The exclusion criteria used in this study was Wistar strain white rats that were sick and stressed.

The samples in this study were divided into 5 groups. The number of samples was calculated using the Federer formula. Based on the calculation, the minimum number of rat samples for each group was 5 rats. The total number of rat samples required is as many as 25 rats. During the study, the probability of the rats dying was 5 rats for each group.

Each sample in each treatment group was determined by simple random sampling. The sampling of population members was carried out randomly, or in other words, it was carried out with equal opportunities for each member of the population to become a sample member. The independent variable in this study was duku seed extract with concentrations of 5%, 10%, and 20%. The dependent variable in this study was the number of neutrophil cells in the blood



of wound-induced Wistar rats and 1% carrageenan to see the anti-inflammatory activity of the duku seed extract.

Prior to conducting research on experimental animals, an ethical feasibility test was carried out by the Research Ethics Commission of the Rumah Sakit Umum Pusat Mohammad Hoesin (RSMH) Palembang and the Faculty of Medicine, Sriwijaya University with an ethics certificate. The preparation of experimental animals begins with acclimatization. Rats were acclimatized in the study room at a temperature of 20-25°C for seven days. At the time of acclimatization, rats were fed in the form of pellets and water ad libitum (given unlimited).

Duku seeds were used to make extracts by maceration. Fifty kg duku was prepared, then the seeds were separated from the skin and flesh, cleaned with running water, drained, then naturally dried in the sun. Furthermore, the dried seeds are mashed using a blender to make powder (simplicia). The 750 grams of simplicia were macerated using 96% ethanol solvent for 48 hours, then filtered using filter paper. The macerate was collected, and the solvent was evaporated using a rotary evaporator to obtain a thick duku seed extract.

The 3% Na CMC was mixed with heated distilled water until homogeneous and allowed to expand. The 15 ml of duku seed extract dissolved with propylene glycol was added and stirred until homogeneous. Then 0.2% metal paraben dissolved in distilled water was added to the mixture and stirred until homogeneous. The gel was then weighed using an analytical balance to obtain 10 grams of gel which would then be packed in a container and labeled.

A total of 0.05 grams of carrageenan was weighed and mixed in 5 ml of 0.9% NaCl solution in a volumetric flask. Carrageenan was induced in the labial mucosa of the mandible of rats to induce acute inflammation. Previously, the labial mucosa of the rats was sterilized with 10% povidone-iodine solution and using a cotton swab. Tweezers were used to pull the lower lip of the rat, then 1% carrageenan 0,1 ml was induced in the labial mucosa of the lower jaw of the rat using a syringe.

The treatment groups were rats applied with duku seed extract gel with concentrations of 5%, 10%, and 20%; hyaluronic acid 0.2% as the positive group; and placebo gel as the negative group. Before being treated, Rats were anesthetized with ketamine 0.2 ml/head intramuscularly on the thighs of the rats' hind legs. Afterward, the lower lip of the rat was gently pulled using tweezers and then wound with a cylinder diamond bur to a depth of 1 mm or according to the diameter of the bur used. Sterile cotton moistened with distilled water was used to clean the blood and then dried. Their blood was taken to count the number of neutrophil cells before



treatment. On the next day, the lower lip of the rats was induced with 1% carrageenan suspension near the previously induced wound. Duku seed extract gel with various concentrations, 0.2% hyaluronic acid gel, and placebo gel were administered using a cotton bud by slightly pulling the rat's lower lip and then smearing evenly on the wound. On the 4th day, the rat blood was taken again to count the number of neutrophil cells after treatment.

The number of segmented neutrophil cells in rat blood was calculated using a hematology analyzer at the Central Health Laboratory (BBLK) Palembang. The data were analyzed statistically.

Results

To test the anti-inflammatory activity of the duku seed extract gel, the number of neutrophil cells was calculated before and after treatment using a hematology analyzer. The calculation of the number of neutrophil cells can be seen in Table 1.

Table 1. The average and difference in the number of neutrophil cells before and after being given duku seed extract gel.

Groups	Average Number of Neutrophil Cells ($10^3/\mu\text{L}$)		
	Before treatment	After treatment	Average Difference
Duku seeds extract 5%	1,65	3,69	2,04
Duku seeds extract 10%	1,86	4,12	2,26
Duku seeds extract 20%	1,43	2,40	0,97
Positive control (0.2% hyaluronic acid gel)	3,15	4,31	1,16
Negative control (pacebo)	2,46	3,32	0,86

The normality and homogeneity test was performed using Kolmogorov-Smirnov and Levene's test, respectively. The results showed $p > 0.05$, which means that the distribution of data was normal and homogeneous. Then, the data were analyzed using paired T-test to determine the ratio of the average number of neutrophil cells before and after being given treatment. The results are in Table 2.

Table 2. Paired T-test results regarding the number of neutrophil cells before and after being given duku seed extract gel

Groups	Average Number of Neutrophil Cells ($10^3/\mu\text{L}$)		Sig.
	Mean \pm Standard Deviation		
	Before	After	
G. I	1,65 \pm 1,035	3,69 \pm 2,567	0,051
G. II	1,86 \pm 1,239	4,12 \pm 2,677	0,113
G. III	1,43 \pm 0,744	2,40 \pm 1,645	0,076
G. IV	3,15 \pm 1,017	4,31 \pm 2,841	0,404
G. V	2,46 \pm 1,561	3,32 \pm 2,076	0,479



Description: Paired T-test, G.I; duku seed extract gel group 5%, G.II; 10% duku seed extract gel group, G.III; Duku seed extract gel group 20%, G.IV; positive control group (0.2% hyaluronic acid gel), G.V; negative control group (placebo gel)

Table 2 showed that there was an increase in the number of neutrophil cells after treatment in all groups, but the difference was not significant. Followed by a one-way ANOVA test, the results of p-value = 0.670, which indicates that there is no significant difference (Table 3).

Table 3. One Way ANOVA test results mean the number of neutrophil cells.

	P Value
Between groups	0,670

Significant at level $p \leq 0.050$

Discussion

Based on the results of the current study, there was an increase in the average number of neutrophil cells in all groups before and after being injured and induced by carrageenan. The greatest increase in the average number of neutrophil cells was in the group given 10% duku seed extract gel, this is maybe due to the active ingredients possessed by the duku seed extract gel, such as flavonoids and triterpenoids.¹²⁻¹⁶ Flavonoids can act as anti-inflammatory and immunomodulatory. It could increase the adhesion of neutrophils to the endothelial cell wall so that it would increase the number of neutrophil cells in the blood.¹⁷ Neutrophils play a role in phagocytosis of microbes or other irritants that cause inflammation.¹⁸ In contrast the research conducted by T.P. Umar (2020) showed that duku seed extract ointment with a concentration of 10% had wound healing and antiseptic effects.¹²

The results also showed an increase in the average number of neutrophil cells in each group after treatment. In the group of 20% duku seed extract gel, the average increase in the number of neutrophil cells was the least compared to the other treatment groups. This is because the active ingredient in the duku seed extract gel is 20% more than the duku seed extract gel at 5% and 10%, so the anti-inflammatory activity is greater. Likewise, G. Susanti (2017) showed that 40% binahong leaf extract caused a greater decrease in the number of neutrophil cells compared to 10% and 20% concentrations.¹⁹

The flavonoid compounds contained in duku seeds have anti-inflammatory activity through cyclooxygenase inhibition so that they can limit the number of inflammatory cells, such as neutrophil cells in inflamed tissues. This causes the inflammatory process to last shorter



and immediately progress to the proliferative stage.²⁰ The role of flavonoid compounds as anti-inflammatory works by reducing the number of pro-inflammatory cytokines such as IL-6 and TNF- α ; inhibiting the secretion of IL-1 and IL-8; and inhibiting the production of Nitric Oxide (NO).⁹ Flavonoids also have the potential to inhibit the biosynthesis of prostaglandins by inhibiting the COX enzyme.²¹ Research by Almeida (2019) on the anti-inflammatory effect of the triterpenoid-rich fraction of *Himatanthus drasticus* showed that there was an inhibition of edema in carrageenan-induced rat paws. This study also stated that there was an inhibition of neutrophil migration to the region of edema. This shows that the triterpenoid compounds present in *Himatanthus drasticus* can reduce the inflammatory response by inhibiting the migration of neutrophils to the inflammatory area. This study also states that triterpenoids can inhibit the secretion of inflammatory mediators such as TNF- α and IL-1 β . During inflammation, there is an increase in the production of secretions by inflammatory cells. Triterpenoids can inhibit the production of Nitric Oxide (NO) by macrophages which is an inflammatory cell.²²

The results of the study in the positive control group using 0.2% hyaluronic acid gel showed a greater increase in the average number of neutrophil cells compared to the group of 20% duku seed extract gel. This may be attributed to many chemical compounds in duku seeds that are more complex than 0.2% hyaluronic acid. It is in line with A. Pramono (2017) showed that 0.2% hyaluronic acid was less effective than sweet starfruit extract gel in the healing process of traumatic ulcers.²³

In the negative control group treated with a placebo gel, there was the least increase in the average number of neutrophil cells compared to the whole group. This could be because the placebo gel applied to the wounds on the rat's lips also played a role in covering the wound from the entry of pathogens into the body. It is in line with research by G. Susanti (2017) showed that even ointment bases without any active ingredients could help reduce the average number of neutrophil cells in injured rats.¹⁹

The data from the paired T-test and the one-way ANOVA test showed that there was no significant difference in the mean number of neutrophil cells ($p > 0.05$). The results of these tests suggest that the duku seed extract gel in all concentrations did not have anti-inflammatory activity compared to positive and negative controls. This could be due to the research being carried out on the application extract topically, but the neutrophil count parameters in rat blood



were examined without histopathological examination of the inflamed tissue. In a study conducted by A. Tamara (2019), it was shown that there was a significant decrease in the average number of neutrophil cells on the topical application of kelulut propolis flavonoid extract and seen histopathologically.²⁴ On the other hand, H. Ervando (2019) examined the number of neutrophils in the blood of rats induced by carrageenan treated with kesum leaf extract intraorally. It showed a significant decrease in the number of neutrophils in each group.²⁵ This indicates that the intraoral application of the extract will show more significant results if the examination of the neutrophil cell count through blood samples, as well as the topical application of the extract, will be more significant if the neutrophil cell count is seen histopathologically.

Conclusions

It can be concluded that duku seed extract gel with concentrations of 5%, 10%, 20% have not been proven to have anti-inflammatory activity. Duku seed extract gel 20% was more effective than the positive control group in influencing the number of neutrophil cells because the increase in the average number of neutrophil cells in the 20% duku seed extract gel was less than the positive control group.

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