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Research Article

Anti-Inflammatory Effects of Rutin, Quercetin, and Catechin on Leukocyte Count and Edema in Carrageenan-Induced Male Sprague Dawley Rats

Indah Kartika Sari[1], Hotlina Nainggolan[1,2]

[1]Department of Pharmacy, Gunadarma University, Depok, Indonesia
[2]Department of Medicine, Gunadarma University, Depok, Indonesia

nainggolanhotlyna@gmail.com

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ABSTRACT

Inflammation is the response of the body to infection or tissue injury. This reaction triggers the release of inflammatory mediators and increases leukocyte count. NSAIDs and corticosteroids are the most commonly used drugs to reduce inflammation; however, long-term use can cause side effects. Therefore, medicinal plants with anti-inflammatory properties have been explored, as their activity is largely attributed to flavonoid content. Flavonoids possess various pharmacological activities, such as antioxidant, anti-inflammatory, anticancer, and antibacterial effects. This study aimed to assess the anti-inflammatory effect of flavonoid on leukocyte counts in carrageenan-induced male Sprague Dawley rats. The study involved 24 rats divided into six groups: normal control, negative control (1% Na-CMC), positive control (diclofenac sodium, 4.5 mg/kg BW), and treatment groups (rutin, quercetin, and catechin at 50 mg/kg BW). The evaluated parameters included the percentage of inhibition of inflammation and the leukocyte count. Flavonoid reduced inflammation, with quercetin showing the most similar effect to the positive control, followed by rutin and catechin. One-way ANOVA indicated no significant difference in the percentage of inflammation inhibition between the 1st and 2nd hours ($p = 0.087$), but a significant difference was observed between the 2nd and 3rd hours ($p = 0.042$). Leukocyte counts also varied significantly among the groups ($p = 0.004$). Flavonoid reduces number of leukocyte and potential as a antiinflammation agents.

Keywords: anti-inflammatory; carrageenan; flavonoid; leukocyte count; rats

INTRODUCTION

Inflammation is the body's natural response to tissue injury, typically identified by five main signs: rubor (redness), calor (heat), tumor (swelling), dolor (pain), and *functio laesa* (loss of function) (Buana & Mauludin, 2024; Karin & Shalapour, 2022). It can result from various conditions, such as microbiological infections, physical trauma, chemical exposure, allergic and hypersensitivity reactions, and autoimmune diseases. These conditions cause the release of proinflammatory mediators, including an increase in leukocyte count (Akrom, 2021). Leukocytes, or white blood cells, are vital components of the immune system that play a key role in the inflammatory response. They fight infections by engulfing pathogenic microorganisms and aiding in the production or transport of antibodies (Akrom, 2021; Tigner et al., 2022).

Most drugs used to reduce inflammation-related symptoms work by inhibiting cyclooxygenase activity or blocking cytokine receptors. However, long-term use of these medications can cause adverse side effects. The clinical use of nonsteroidal anti-inflammatory drugs (NSAIDs) is continually being reassessed due to evidence of potential risks, which encourages the search for natural plant-based compounds as safer options for treating inflammatory conditions (Nunes et al., 2020).

Several studies have reported that various medicinal plants, including *Cordyline fruticosa*

(andong), *Annona squamosa* (sugar apple), *Carica papaya*, Musa spp., *Ocimum basilicum*, *Curcuma longa*, *Moringa oleifera*, *Eurycoma longifolia* (tongkat ali), and *Areca catechu*, exhibit anti-inflammatory properties. The anti-inflammatory activity of these plants is largely attributed to their flavonoid content, as approximately 5%-10% of their secondary metabolites are flavonoids (Ningsih et al., 2023). Flavonoids are phenolic compounds abundantly found in plants and have diverse pharmacological activities, including anti-allergic, cardioprotective, antidiabetic, antioxidant, and radical scavenging effects (Sari et al., 2023).

Previous studies have shown that leaf extracts from medicinal plants, such as Carica pubescens and Clerodendrum squamatum, exhibit significant anti-inflammatory activity. This is demonstrated by a reduction in leukocyte count in azoxymethane (AOM)-induced rats. The extracts achieved up to 95.04% inhibition of inflammation, comparable to sodium diclofenac. This effect is believed to be linked to the presence of bioactive compounds, particularly flavonoids, which play a key role in modulating inflammatory responses. Therefore, this study aims to evaluate the anti-inflammatory effects of flavonoid such as rutin, quercetin, and catechin on leukocyte counts in rats with carrageenan-induced inflammation.

METHODS

Equipment

The equipment used in this study included oral gavage needles, syringes, an analytical balance (Ohaus®, USA), an animal weighing scale, beakers (Duran®, Germany), test tubes (Iwaki®, Japan), spatulas, glass stirring rods, parchment paper, drop pipettes, forceps (OneMed®, Indonesia), a vernier caliper, EDTA vacutainers (Vaculab®, Indonesia), and a hematology analyzer.

Materials

The materials used in this study included male Sprague Dawley rats, flavonoid (Sigma-Aldrich, USA), sodium diclofenac (Tokyo Chemical Industry, Japan), carrageenan (Sigma-Aldrich, USA), NaCl, and *sodium carboxymethyl cellulose* (Sigma-Aldrich, USA).

Preparation of Flavonoid Solution

Flavonoid (50 mg) were dissolved in 10 mL of distilled water-soluble and belong to the polyphenol family (Idos *et al*., 2023).

Preparation of Carrageenan Induction Solution

Carrageenan (0.1 g) was dissolved in 0.9% NaCl solution to a final volume of 10 mL.

Preparation of 1% Na-CMC Solution

Na-CMC (0.1 g) was dissolved in 100 mL of warm water (70°C) and continuously stirred until a homogeneous solution was obtained (Suryandari *et al*., 2021).

Preparation of Sodium Diclofenac Suspension

Sodium diclofenac was administered at a dose of 4.5 mg/kg BW. For preparation, 10 mg of sodium diclofenac was weighed and dissolved in 10 mL of Na-CMC solution, then stirred until a uniform suspension was achieved (Sari & Sulistiany, 2021).

Carrageenan-Induced Inflammation Procedure

Twenty rats were induced with 1% carrageenan at a dose of 0.1 mL/kg BW via subplantar injection. Each test preparation was administered once to each group. Before carrageenan induction, the thickness of each rat's left hind paw was measured using a Vernier caliper. Paw thickness was remeasured after carrageenan injection to assess the inflammatory response (Sari et al., 2023).

Anti-Inflammatory Activity Test of Flavonoid

The anti-inflammatory test was conducted by first weighing each rat and marking the left hind paw. The initial left paw diameter was measured using a Vernier caliper and recorded as the baseline. In groups 2–6, inflammation was induced by subplantar injection of 1% carrageenan at 0.1 mL/kg body weight. After paw edema appeared, the paw diameter was remeasured.

Each group then received different treatments: flavonoid at 50 mg/kg body weight for the test groups, 1% Na-CMC as the negative control, and sodium diclofenac at 4.5 mg/kg body weight as the positive control. All treatments were administered orally. Paw edema was observed every hour for 3 h before blood sample collection (Fratiwi et al., 2022). The percentage of inflammation inhibition was calculated using the equation 1 (Ulasi et al., 2025).

Percentage of inflammation inhibition= $\frac{a-b}{a}$ .....(1)

Where, a = percentage of inflammation in the negative control group

b = percentage of inflammation in the treatment or reference group

Blood Sample Collection

Blood samples (approximately 2 mL) were collected via intracardiac puncture under anesthesia with ketamine (360 mg/kg body weight) and xylazine (40 mg/kg body weight) administered intramuscularly until the animal was unconscious. Blood was collected into EDTA Vacutainer tubes.

Leukocyte Count Analysis

Blood analysis was performed using a hematology analyzer for complete blood count (CBC), including leukocyte count, platelet count, and hemoglobin level measurements. This analyzer offers high throughput, time efficiency, minimal labor, and rapid results (Brahmananda *et al*., 2023). The working principle involves mixing blood samples with a dilution reagent at a 1:200 ratio during hemolysis to quantify leukocyte levels (Dabukke et al., 2023).

Data Analysis

Data on the percentage of inflammation inhibition and leukocyte count were statistically analyzed using the Statistical Package for the Social Sciences (SPSS). Data were tested for homogeneity and normality to determine whether parametric or non-parametric tests were appropriate. If data were homogeneous and normally distributed, one-way ANOVA was performed. If not, the Kruskal–Wallis test was conducted.

RESULTS AND DISCUSSION

The inhibition of inflammation reflects the suppression of inflammatory symptoms, where the percentage of anti-inflammatory activity indicates a compound's ability to exert anti-inflammatory effects (Nessa et al., 2024). In this study, 24 rats were used, 20 induced with inflammation induction and 4 served as the normal group. Immediately after induction (0 h), paw edema diameter increased by approximately 58.85%. Test compounds were administered at 1 h, and the inhibition percentage was calculated from 1 to 3 h.

Table 1. Average Percentage of Inflammation Inhibition

Group	Percentage of inflammation inhibition				P Value		
	0 h	**1 h**	**2 h**	**3 h**	**From the 1nd hour to 2rd hour**	**From the 2nd hour to 3rd hour**	**Post hoc**
N	0±0.000	0±0.000	0±0.000	0±0.000			
KN	0±0.000	0±0.000	0±0.000	0±0.000			
KP	0±0.000	14.01±5.27	25.38±6.95	32.35±6.67			[c]0.027
P1	0±0.000	11.15±8.52	16.15±13.13	24.26±14.16	[a]0.087	[b]0.042	[c]1.000
P2	0±0.000	12.02±7.59	21.53±5.24	27.94±4.83			[c]1.000
P3	0±0.000	6.37±7.75	16.15±3.6	14.71±3.4			[c]0.030

Notes:
N : Normal group (no treatment)
KN : Negative control (Na-CMC 1%)
KP : Positive control (sodium diclofenac, 4.5 mg/kg BW)
P1 : Rutin (50 mg/kg BW)
P2 : Quercetin (50 mg/kg BW)
P3 : Catechin (50 mg/kg BW)
Data are expressed as mean ± SD.
(a) : p-value from one-way ANOVA comparing mean percentage inhibition between hours 1 and 2.
(b) ; p-value from Kruskal–Wallis test comparing hours 2 and 3.
(c) : p-value from post hoc analysis following label (b).

The results showed that carrageenan successfully induced inflammation compared to the normal group as shown in **Table 1**. As a polysaccharide commonly used for inflammation

induction, carrageenan acts as a foreign substance that triggers the release of inflammatory mediators such as histamine, serotonin, and bradykinin. These mediators increase capillary permeability, allowing fluid and cells to move from the bloodstream into interstitial tissues, which leads to paw swelling in rats (Santoso, 2021). One-way ANOVA showed no significant difference in the mean percentage of inflammation inhibition between hours 1 and 2 ($p = 0.087$, $p > 0.05$). However, between hours 2 and 3, a significant reduction in edema was observed ($p = 0.042$, $p < 0.05$) as shown in **Table 1**.

These results indicate that flavonoid can inhibit edema formation. The mechanism of flavonoids involves multiple pathways, including the inhibition of cyclooxygenase (COX) enzyme activity, which blocks prostaglandin biosynthesis (Yuniza & Ginanjar, 2021). This finding is consistent with that of Saputri *et al*. (2020), who reported that A. conyzoides leaf extract containing flavonoids significantly reduced edema formation. Likewise, Deniyanti (2024) showed that the ethanolic extract of yellow root stem, which contains flavonoids, exhibits anti-inflammatory effects by decreasing edema, with the 3% b/v concentration being the most effective.

Table 2. Average Leukocyte Counts

Group	Mean Leukocyte Count (µL)	P Value	
N	8.75 ± 1.58		0.001[b]
KN	12.58 ± 1.68		
KP	9.43 ± 0.59	0.004[a]	0.005[b]
P1	9.33 ± 1.29		0.921[b]
P2	8.10 ± 1.91		0.198[b]
P3	10.55 ± 0.89		0.271[b]

Notes:
N : Normal group (no treatment)
KN : Negative control (Na-CMC 1%)
KP : Positive control (sodium diclofenac, 4.5 mg/kg BW)
P1 : Rutin (50 mg/kg BW)
P2 : Quercetin (50 mg/kg BW)
P3 : Catechin (50 mg/kg BW)
Data are expressed as mean ± SD. Label (a) represents one-way ANOVA, and label (b) represents post hoc analysis.

Based on the calculation of the number of leukocytes as shown in **Table 2,** the group treated with flavonoid compounds rutin, quercetin, cathecin and the positive control (diclofenac sodium) showed significant differences compared to the negative control group. The negative control group had significantly higher leukocyte counts (>10,000/µL), exceeding the normal range of 2,000–10,000/µL for rats (Usman *et al*., 2021). Meanwhile, there was no significant difference between the positive control and treatment groups, indicating that the flavonoid tested were able to reduce number of leucocyte to a level similar to that of the reference drug as in **table 2**. Among the tested flavonoids, quercetin showed the greatest effectiveness in inhibiting inflammation with reduce leucocyte.

Quercetin, a flavonol-type flavonoid, has been widely reported to possess potent antioxidant and anti-inflammatory properties (Baqer et al., 2024). Quercetin, a flavonoid compound found in red onion peel extract, has been reported to exhibit various pharmacological activities, Juliadi et al. (2019) demonstrated that quercetin inhibits the activity of the cyclooxygenase (COX) enzyme, which plays a crucial role in the arachidonic acid pathway associated with inflammation. In vitro studies have also shown that quercetin effectively reduces oxidative stress and displays strong anti-inflammatory properties. Furthermore, quercetin has been reported to decrease blood glucose levels in diabetic rats, suggesting its potential benefits in metabolic disorders (Ansari et al., 2022; Xia et al., 2025).

The anti-inflammatory mechanism of quercetin involves the suppression of edema formation through several pathways: binding to the active site of COX-2, inhibiting the transcription of pro-inflammatory genes, and reducing the production of pro-inflammatory mediators, such as prostaglandins and leukotrienes, by inhibiting lipoxygenase and cyclooxygenase enzymes (Aggarwal et al., 2025).

The rutin group also exhibited inhibitory activity, with leukocyte counts similar to the positive control group. Rutin, a glycosylated form of quercetin, possesses antioxidant, anti-inflammatory, and antibacterial activities (Choi et al., 2021). Ardhana and Rahman (2024) reported that ethanol extract of fig leaves, containing secondary metabolites such as quercetin and rutin, effectively reduced leukocyte counts in rats with carrageenan-induced inflammation.

The catechin group showed a non-significant reduction in leukocyte count. However, a previous

study by Pitriyah (2016) indicated that catechin isolated from gambier at a dose of 10 mg/kg BW could reduce paw edema volume, confirming its potential anti-inflammatory activity. The lack of significant results in this study may be attributed to the instability of catechins under physiological conditions and their relatively low bioavailability. Catechins are poorly absorbed in the gastrointestinal tract following oral administration and are rapidly eliminated from the bloodstream after intravenous dosing, resulting in low systemic and tissue concentrations (Ferenczyová *et al*., 2021).

CONCLUSION

Flavonoid such as quercetin, rutin, and catechin exhibit anti-inflammatory activity, as evidenced by the reduction in paw edema diameter and leukocyte count in carrageenan-induced rats. Administration of flavonoids at a dose of 50 mg/kg BW demonstrated that quercetin had the highest efficacy, with results comparable to the positive control. The anti-inflammatory mechanism is likely mediated through the inhibition of cyclooxygenase (COX) enzyme activity, leading to reduced synthesis of prostaglandins and other inflammatory mediators.

SUGGESTIONS

Future studies should explore varying dose levels of individual flavonoid to determine the most effective dosage range and clarify the dose–response relationship. Additionally, leukocyte count measurements should be performed both before and after treatment to obtain a more accurate assessment of immune response changes.

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