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Leukocyte Responses to Karika (*Vasconcellea pubescens*) Leaf Ethanol Extract in Carrageenan-Induced Mice

Respons Leukosit terhadap Ekstrak Etanol Daun Karika (*Vasconcellea pubescens*) pada Mencit yang Diinduksi Karagenan

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ABSTRACT

Inflammation involves systemic changes in leukocyte counts. This study characterized the phytochemical constituents of ethanol extract of karika (*Vasconcellea pubescens*) leaves and evaluated leukocyte responses in carrageenan-induced mice. The extract was prepared by maceration with 70% ethanol and analyzed by qualitative phytochemical screening and thin-layer chromatography. Twenty-four male Swiss Webster mice were randomized to six groups: negative control, vehicle control, diclofenac, and extract at 100, 200, or 400 mg/kg. Leukocyte counts were measured at baseline and 3 and 6 h after carrageenan induction. Friedman tests showed significant within-group changes over time in all groups ($p < 0.05$). Mean leukocyte counts at 3 and 6 h were descriptively lower in extract-treated groups than in negative and vehicle controls, with the 400 mg/kg group approaching diclofenac values. Alkaloids, flavonoids, and tannins were detected. These findings are consistent with potential anti-inflammatory activity, although between-group efficacy was not statistically tested.

Kata kunci:

Aktivitas antiinflamasi;
Jumlah leukosit;
Karagenan;
Skrining fitokimia;
Vasconcellea pubescens

ABSTRAK

Inflamasi dapat disertai perubahan sistemik jumlah leukosit. Penelitian ini mengkarakterisasi kandungan fitokimia ekstrak etanol daun karika (*Vasconcellea pubescens*) dan mengevaluasi respons leukosit pada mencit yang diinduksi karagenan. Ekstrak dibuat dengan maserasi etanol 70% dan dianalisis melalui skrining fitokimia kualitatif serta kromatografi lapis tipis. Dua puluh empat mencit jantan Swiss Webster diacak ke enam kelompok: kontrol negatif, kontrol vehikulum, diklofenak, serta ekstrak dosis 100, 200, atau 400 mg/kg. Jumlah leukosit diukur pada awal pengamatan serta 3 dan 6 jam setelah induksi karagenan. Uji Friedman menunjukkan perubahan bermakna dalam kelompok selama waktu pengamatan pada semua kelompok ($p < 0,05$). Secara deskriptif, rerata leukosit pada jam ke-3 dan ke-6 lebih rendah pada kelompok ekstrak dibanding kontrol negatif dan vehikulum, dengan dosis 400 mg/kg mendekati nilai diklofenak. Alkaloid, flavonoid, dan tanin terdeteksi. Temuan ini konsisten dengan potensi aktivitas antiinflamasi, meskipun efektivitas antar kelompok belum diuji secara statistik.

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1. INTRODUCTION

Inflammation is a coordinated biological response to tissue injury, infection, or other harmful stimuli and involves vascular, cellular, and molecular processes (Oronsky et al., 2022). When excessive or prolonged, inflammation can contribute to tissue damage and chronic disease. Nonsteroidal anti-inflammatory drugs (NSAIDs), including diclofenac and ibuprofen, are widely used to control inflammatory symptoms, but prolonged use may cause gastrointestinal and renal adverse effects (Radiah et al., 2023). These limitations support continued investigation of plant-derived compounds as potential sources of anti-inflammatory agents.

Vasconcellea pubescens, locally known as karika, is cultivated in the Dieng Highlands and belongs to the family Caricaceae. Plants in this family contain diverse secondary metabolites, including flavonoids, alkaloids, tannins, saponins, and terpenoids, several of which have recognized anti-inflammatory properties (Choy et al., 2019). Previous work on karika seeds has reported immunomodulatory activity and changes in leukocyte-related outcomes (Luhurningtyas et al., 2020, 2024). In contrast, evidence on the anti-inflammatory potential of karika leaves remains limited.

Carrageenan-induced inflammation is a widely used acute experimental model because it produces a reproducible biphasic response. The early phase is driven mainly by histamine and serotonin, whereas the later phase involves prostaglandins, neutrophil recruitment, and other pro-inflammatory mediators (Myers et al., 2019). These processes are accompanied by leukocyte mobilization and activation, making circulating leukocyte counts a useful systemic indicator of the inflammatory response (Ansari et al., 2021). Total leukocyte count was therefore selected as the primary hematological outcome in this study.

Although paw edema is commonly used to evaluate carrageenan-induced inflammation, the systemic leukocyte response to *Vasconcellea pubescens* leaf extract has not been adequately characterized. Phytochemical characterization of the leaf ethanol extract by qualitative screening and thin-layer chromatography (TLC) has also rarely been evaluated alongside leukocyte responses. Therefore, this study aimed to characterize the phytochemical constituents of the ethanol extract of *Vasconcellea pubescens* leaves and to evaluate changes in total leukocyte counts in carrageenan-induced mice treated with different extract doses.

2. METHODS

2.1. Research Sites and Study Activities

The study was conducted at two research sites. The animal experiment and evaluation of total leukocyte counts in carrageenan-induced mice were conducted at the Biology Laboratory, Universitas Ngudi Waluyo, Indonesia. Additional characterization of the ethanol extract, including qualitative phytochemical screening by tube tests and thin-layer chromatography (TLC) profiling using reference standards, was conducted at the Pharmacy Laboratory, Universitas Tidar, Indonesia.

2.2. Equipment and Materials

Equipment included an oven (Memmert GmbH + Co. KG, Schwabach, Germany), laboratory blender (Philips, Amsterdam, The Netherlands), 40-mesh sieve, maceration jar, glassware (Pyrex, Corning Inc., Corning, NY, USA), filter paper, porcelain dish, RE-100-Pro rotary evaporator (DLAB Scientific Co., Ltd., Beijing, China), funnel (Pyrex, Corning Inc., Corning, NY, USA), vernier caliper (Mitutoyo Corporation, Kawasaki, Japan), oral gavage needle, RT-7800 Vet hematology analyzer (Rayto Life and Analytical Sciences Co., Ltd., Shenzhen, China), EDTA microtubes (Monotes, Medan, Indonesia), mortar and pestle, and analytical balance (OHAUS Corporation, Parsippany, NJ, USA).

Materials included karika leaves (*V. pubescens*), 70% ethanol, sodium carboxymethyl cellulose (Na-CMC), n-butanol, glacial acetic acid, ethyl acetate, and n-hexane (Merck KGaA, Darmstadt, Germany); 1% carrageenan, quercetin, rutin, Dragendorff reagent, Mayer reagent, concentrated hydrochloric acid (HCl), and magnesium powder (Sigma-Aldrich, St. Louis, MO, USA); physiological saline (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan); sodium diclofenac (PT First Medipharma, Bekasi, Indonesia); and distilled water (Brataco, Bandung, Indonesia).

2.3. Plant Authentication

Karika leaves were collected from Dieng, Wonosobo, Central Java, Indonesia, in 2022. Taxonomic authentication of the plant material was performed at the Ecology and Biosystematics Laboratory, Department of Biology, Faculty of Science and Mathematics, Diponegoro University (Semarang, Indonesia). The specimen was formally identified and certified as *Vasconcellea pubescens* A.DC. (family Caricaceae, genus *Vasconcellea*) based on the established identification key: 1b-2b-3b-4b-6b-7b-9b-10b-11b-12b-13b-14a-15a-109b-119b-120b-121b-124b-125a (**Figure 1**).

2.4. Sample Preparation

Approximately 5 kg of fresh karika leaves were wet sorted, washed under running water, and cut into small pieces. The leaves were dried under indirect sunlight for approximately 7 days while covered with black cloth. The dried material was ground with a blender, passed through a 40-mesh sieve, and stored in a tightly closed container at room temperature.

2.5. Preparation of Karika Leaf Ethanol Extract

Extraction was performed via maceration using 70% ethanol. Briefly, 250 g of *karika* leaf powder was placed in a dark container with 1,750 mL of 70% ethanol for 3 days with occasional stirring, followed by remaceration with 750 mL of 70% ethanol for 2 days.



Figure 1. Morphological characteristics of *Vasconcellea pubescens*: (A) Intact whole fruit; (B) Fruit cross-sections displaying the internal arrangement of seeds and pulp; (C) Adaxial surface of a representative lobed leaf. Scale bars in (A) and (C) indicate metric units in centimeters (cm).

The combined filtrates were concentrated using a rotary evaporator and subsequently on a water bath at approximately 60 ˚C until a viscous extract was obtained (Luhurningtyas et al., 2021). The extraction yield was calculated, and the dried leaf powder and ethanol extract were characterized for moisture content, total ash content, and organoleptic properties (color, odor, and taste). Moisture content was determined using the gravimetric method with a moisture balance; approximately 2–3 g of the samples were weighed and dried at 105 ˚C to a constant weight, with moisture content calculated based on the weight loss. Total ash content was determined via the gravimetric incineration method, where approximately 2 g of each sample was incinerated in a muffle furnace at 600 ˚C for approximately 6 h until the carbonaceous material was completely removed. The determinations of moisture and total ash contents were performed in reference to the *Parameter Standar Umum Ekstrak Tumbuhan Obat* (General Standard Parameters of Medicinal Plant Extracts).

2.6. Phytochemical Characterization

Qualitative phytochemical screening was performed to detect alkaloids, flavonoids, and tannins. Alkaloids were tested with Mayer and Dragendorff reagents, flavonoids with the Mg-HCl reaction, and tannins with 1% ferric chloride ($FeCl_3$). Precipitate formation or characteristic color changes were used as qualitative indicators of the corresponding metabolite classes.

The extract was further characterized by TLC. Briefly, 10 mg of ethanol extract was dissolved in 1 mL of 70% ethanol and applied to silica gel 60 F254 plates. Chromatographic separation used n-butanol:acetic acid:water (4:1:5, v/v/v) as the mobile phase. Developed plates were observed under UV light at 254 and 366 nm and then derivatized with citroborate reagent. Retention factor (Rf) values and fluorescence patterns of extract spots were compared with quercetin and rutin reference standards (Luhurningtyas et al., 2021).

2.7. Animal Experiment and Leukocyte Assessment

This study evaluated the leukocyte response to the ethanol extract of *V. pubescens* leaves (EEDK) in carrageenan-induced male Swiss Webster mice. The animal protocol was approved by the Ethics Committee of Universitas Ngudi Waluyo (EC No. 26/KEP/EC/UNW2022). Twenty-four mice (~3 months old, 20–30 g), sourced from a local breeder (Salatiga, Indonesia), were acclimatized for 7 days in polypropylene cages at 24–25 ˚C with standard pellet feed and water *ad libitum*. Following an 18-h fast to minimize dietary influences on pharmacokinetics, the animals were randomly assigned to six groups (n = 4 per group, determined via the Federer formula). The specific treatment groups, doses, and administration routes are summarized in **Table 1**. Oral treatments were administered by gavage 1 h prior to inflammation induction, which was achieved by a subplantar injection of 1% carrageenan (0.1 mL) into the left hind paw of all groups. Blood samples (50–100 µL) were collected from the tail vein into EDTA microtubes at 0, 3, and 6 h post-induction, and total leukocyte counts were quantified using an RT-7800 Vet automated hematology analyzer (Rayto Life and Analytical Sciences Co., Ltd., Shenzhen, China).

2.8. Statistical Analysis

Leukocyte counts are presented as mean ± standard deviation (SD) for each treatment group and observation time. Statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA).

Table 1. Treatment Groups and Administration Schedule

Group	Treatment	Dose	Route	Vehicle
Negative control	Carrageenan only	-	-	None
Vehicle control	0.5% Na-CMC	-	Oral gavage	0.5% Na-CMC
Positive control	Sodium diclofenac	6.5 mg/kg BW	Oral gavage	0.5% Na-CMC
EEDK-100	Ethanol extract	100 mg/kg BW	Oral gavage	0.5% Na-CMC
EEDK-200	Ethanol extract	200 mg/kg BW	Oral gavage	0.5% Na-CMC
EEDK-400	Ethanol extract	400 mg/kg BW	Oral gavage	0.5% Na-CMC

Note. EEDK = ethanol extract of *V. pubescens* leaves. All groups received a subplantar injection of 0.1 mL of 1% carrageenan into the left hind paw. Oral treatments were administered 1 h before carrageenan induction. The negative control received carrageenan without oral treatment.

Because leukocyte counts were repeatedly measured in the same animals and the data did not meet the assumptions for parametric analysis, the Friedman test was used to assess within-group changes across 0, 3, and 6 h. A p-value < 0.05 was considered statistically significant. The reported inferential analysis did not test differences between treatment groups; comparisons among group means are therefore descriptive. The experimental workflow is shown in **Figure 2** and includes plant authentication, sample preparation, extraction, phytochemical characterization, animal treatment, blood collection, and statistical analysis.

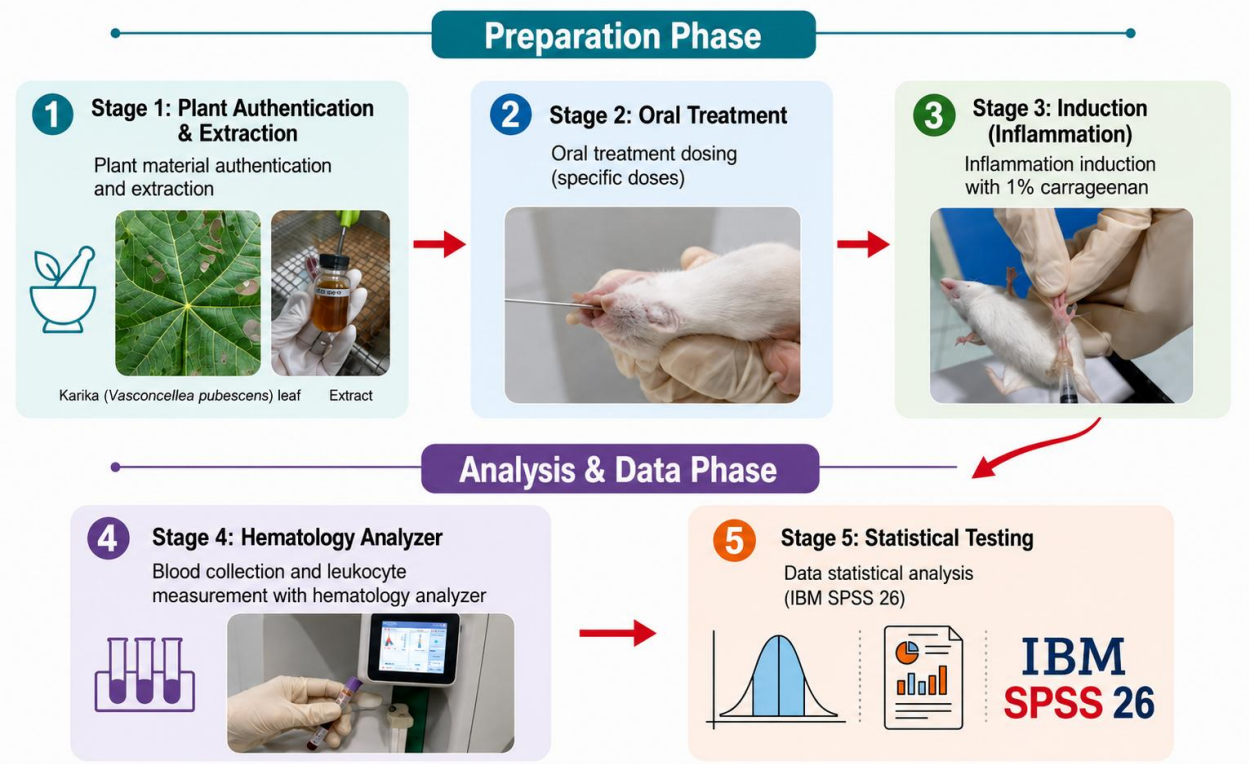


Figure 2. Experimental workflow for evaluating leukocyte responses to the ethanol extract of *Vasconcellea pubescens* leaves in carrageenan-induced mice.

3. RESULTS AND DISCUSSION

3.1. Extract Characterization

The leaves were dried for seven days under indirect sunlight while covered with black cloth; the covering was intended to limit direct ultraviolet exposure (Mahmudah et al., 2024). Maceration avoided direct heating during extraction (Putri et al., 2021), and 70% ethanol provided a hydroalcoholic solvent suitable for extracting compounds across a broad polarity range (Maskura et al., 2023). The extraction yield was 22.0%. Moisture content was 8.87% in the dried leaf powder and 4.14% in the ethanol extract, whereas total ash content was 11.00% and 16.00%, respectively (**Table 2**). The extract was dark green, had a characteristic odor, and tasted bitter. These findings describe the physical and basic quality characteristics of the prepared extract; interpretation of moisture and ash values should follow the analytical standard used for their determination.

3.2. Phytochemical Screening and TLC Profile

Qualitative screening detected alkaloids, flavonoids, and tannins in the ethanol extract (**Table 3**). These tests establish the presence of broad metabolite classes rather than their concentrations (Maheshwaran et al., 2024). TLC produced three major extract spots using n-butanol: acetic acid: water (4:1:5, v/v/v) as the mobile phase (**Table 4**; **Figure 3**). Extract spot 1 had the same Rf value as quercetin (Rf = 1.00), but its fluorescence profile under UV 366 nm differed from the quercetin standard; therefore, quercetin could not be confirmed from the chromatogram. The rutin standard had an Rf of 0.70 and did not match the extract spots. Spot 2 (Rf = 0.65) showed blue fluorescence and was tentatively assigned as tannin-like, whereas spots 1 and 3 were tentatively classified as flavonoid-like. These assignments remain presumptive because TLC migration and fluorescence patterns alone do not establish compound identity.

Table 2. Characterization of karika dried leaf powder and ethanol extract

Parameter	Dried leaf powder	Ethanol extract
Extraction yield (%)	-	22.0
Moisture content (%)	8.87	4.14
Total ash content (%)	11.00	16.00
Color	Green	Dark green
Odor	Characteristic	Characteristic
Taste	Bitter	Bitter

Table 3. Qualitative phytochemical screening of the ethanol extract of karika leaves

Secondary metabolite	Test	Reagent/procedure	Observation	Result
Flavonoid	Willstatter	2-3 drops concentrated HCl + 2-3 small pieces Mg	Dark yellow	+
Alkaloid	Mayer	5 mL HCl + 3 drops Mayer reagent	Orange-brown precipitate	+
	Dragendorff	5 mL HCl + 3 drops Dragendorff reagent	Yellow precipitate	+
Tannin	$FeCl_3$ test	2-3 drops of 1% $FeCl_3$	Bluish-green to black	+

Table 4. TLC profile of the ethanol extract of *Vasconcellea pubescens* leaves

Sample	No.	Rf	UV 254 nm	UV 366 nm	After citroborate (366 nm)	Tentative assignment
Extract	1	1.00	Yellowish-green	Red	Blue	Flavonoid-like
Extract	2	0.65	Yellowish-green	Fluorescent blue	Fluorescent blue	Tannin-like
Extract	3	0.50	Yellow	Blue	–	Flavonoid-like
Quercetin (standard)	4	1.00	Yellow	Blue	Blue	Quercetin
Rutin (standard)	5	0.70	Green	Pale green	Pale green	Rutin

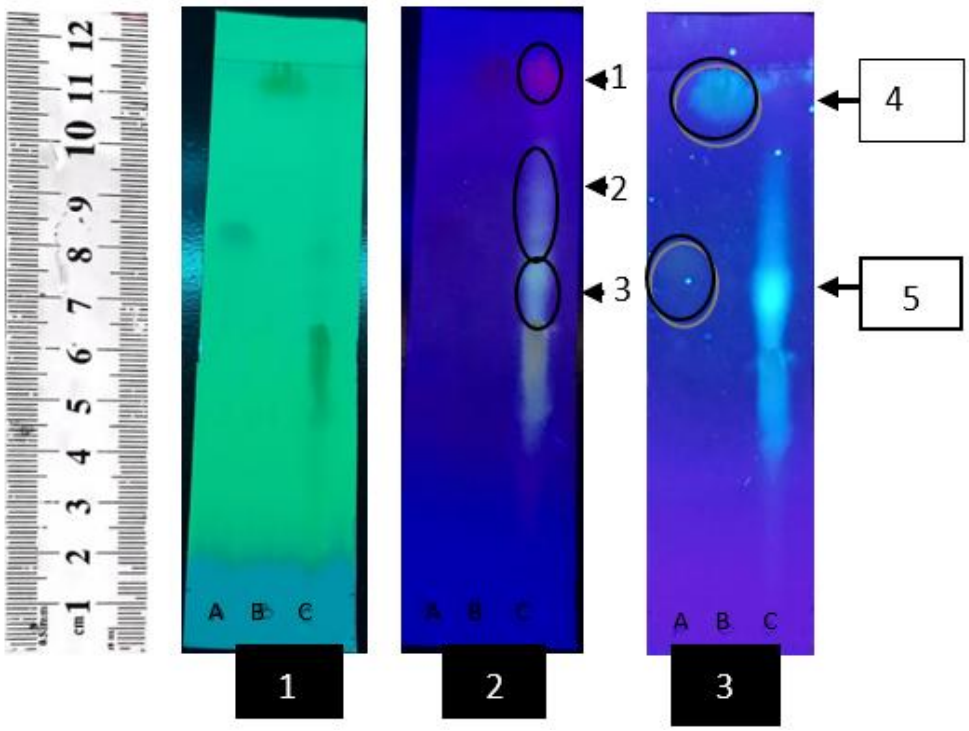


Figure 3. Thin-layer chromatogram of the ethanol extract of karika leaves using n-butanol:acetic acid:water (4:1:5, v/v/v) as the mobile phase. Chromatograms were visualized under UV 254 nm (1), UV 366 nm (2), and after derivatization with citroborate reagent (3). A, rutin standard; B, quercetin standard; C, ethanol extract.

3.3. Leukocyte Responses and Anti-Inflammatory Interpretation

All groups showed an increase in mean leukocyte counts at 3 h relative to baseline, consistent with an acute inflammatory response after carrageenan administration (**Figure 4**). Carrageenan-induced inflammation involves histamine, serotonin, bradykinin, prostaglandins, and leukocyte recruitment during its early and later phases (Abdulkhaleq et al., 2018; Myers et al., 2019). Mean leukocyte counts subsequently decreased by 6 h in every group, including the negative and vehicle controls, indicating that part of the temporal change reflects the natural evolution of the carrageenan response.

Across the extract groups, mean leukocyte counts at 3 h were 12.78, 11.76, and 9.86 × 10^9/L for 100, 200, and 400 mg/kg BW, respectively; at 6 h, they were 10.34, 8.62, and 6.87 × 10^9/L (**Figure 4**). These descriptive values decreased as the extract dose increased. The 400 mg/kg BW group approached the diclofenac group at 3 h (9.86 vs. 9.32 × 10^9/L) and had a slightly lower mean at 6 h (6.87 vs. 7.75 × 10^9/L). Because no between-group inferential test was available, these comparisons should not be interpreted as statistical equivalence or proof of a dose-response effect.

The Friedman test identified significant changes across 0, 3, and 6 h within every group, with p values ranging from 0.018 to 0.039. Significant temporal changes were observed in the negative and vehicle controls as well as in the treated groups, confirming that the test detects change over time but does not isolate a treatment effect. Figure 4 therefore presents descriptive group trajectories based on means and SDs. Taken together, the pattern is consistent with a potential treatment-related reduction in leukocyte counts, particularly at 400 mg/kg BW, but comparative efficacy remains unconfirmed.

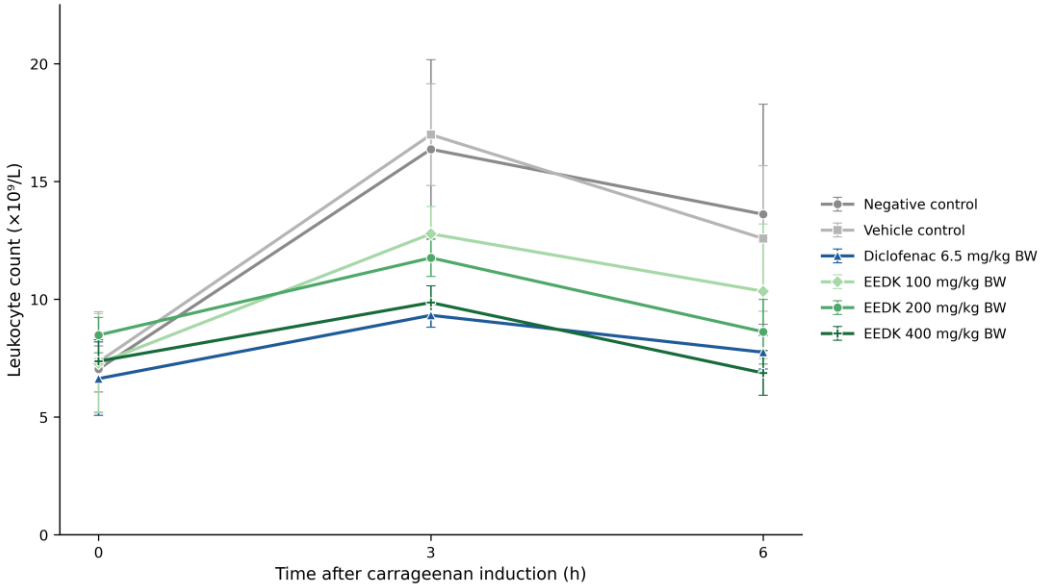


Figure 4. Temporal profiles of total leukocyte count across treatment groups following carrageenan induction. Values are mean ± SD (n = 4). Error bars represent SD. EEDK = ethanol extract of *Vasconcellea pubescens* leaves. Between-group differences were not tested inferentially.

The biological plausibility of the observed pattern may relate to alkaloids, flavonoids, and tannins detected in the extract. Flavonoids have reported immunomodulatory and anti-inflammatory effects (Hosseinzade et al., 2019; Sabini et al., 2025), and experimental evidence indicates that some alkaloids can modulate inflammatory signaling (Guo et al., 2020). Plant-derived preparations containing related constituents have also been associated with modulation of neutrophil and inflammatory responses (Tavasoli et al., 2019; El Faqer et al., 2022). However, the present study did not identify individual active compounds or measure mechanistic outcomes, so these pathways should be regarded as plausible explanations rather than demonstrated mechanisms. The study was limited to total leukocyte counts and did not assess paw edema, differential leukocyte counts, inflammatory cytokines, or histopathology. Further studies should include appropriate between-group statistical testing and complementary inflammatory outcomes.

4. CONCLUSION

The ethanol extract of *Vasconcellea pubescens* leaves was associated with descriptively lower leukocyte counts at 3 and 6 h than the negative and vehicle controls, with larger reductions observed at higher extract doses. Alkaloids, flavonoids, and tannins were detected in the extract. Because the reported statistical analysis assessed only within-group changes over time, comparative anti-inflammatory efficacy cannot be confirmed. These findings provide preliminary evidence consistent with anti-inflammatory potential and support further studies using between-group statistical analysis and complementary inflammatory outcomes.

AUTHOR CONTRIBUTIONS

Conceptualization, F.P.L.; Methodology, F.P.L. and D.C.K.; Investigation, F.P.L. and D.C.K.; Data curation, F.P.L. and D.C.K.; Formal analysis, F.P.L.; Writing - original draft, F.P.L. and D.C.K.; Writing - review and editing, F.P.L. and D.C.K.; Supervision, F.P.L. and D.C.K. All authors have read and agreed to the published version of the manuscript.

INSTITUTIONAL REVIEW BOARD STATEMENT

The animal study protocol was reviewed and approved by the Ethics Committee of Universitas Ngudi Waluyo (protocol code: 26/KEP/EC/UNW2022).

INFORMED CONSENT STATEMENT

Not applicable. This study did not involve human participants.

DATA AVAILABILITY STATEMENT

The raw leukocyte count data, phytochemical screening observations, and statistical outputs supporting the findings of this study are available from the corresponding author upon reasonable request.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ROLE OF FUNDERS

Not applicable. This research received no external funding.

DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) USE

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) solely for language editing, improvement of clarity, and

organization of the manuscript. The authors subsequently reviewed and revised the generated output and take full responsibility for the accuracy, integrity, and final content of the manuscript.

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