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# In Vivo Anti-Inflammatory Activity of Durio zibethinus Murray Leaf Ethanol Extract in Male Wistar Rats

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***Abstrakc***

*Inflammation is a physiological response that can become a serious health problem if excessive. This study aims to evaluate the anti-inflammatory effectiveness of durian leaf ethanol extract (Durio zibethinus Murray) on male white rats. The study used a post-test only control group design with male Wistar rats aged 2–3 months, weighing 180–210 g. The test animals were divided into five groups: negative control (CMC Na), positive control (diclofenac sodium), and three treatment groups with doses of 200, 400, and 800 mg/kg BW of durian leaf ethanol extract. Inflammation was induced by egg white injection into the sole of the foot. Edema volume was measured using a mercury plethysmometer. Data analysis used one-way ANOVA and Tukey's post-hoc test. The results showed that the extract contains flavonoids, saponins, and steroids. A dose of 400 mg/kg BW provided the second highest edema inhibition (34.34% at 120 minutes) after the standard drug sodium diclofenac (37.83% at 360 minutes), and the results of the ANOVA test showed a p-value <0.05 there was a significant difference compared to the negative control. Ethanol extract of durian leaves has anti-inflammatory potential, although its effectiveness has not yet matched standard drugs, so it has the potential to be developed as a safer natural-based therapy.*

***Keywords:*** *Anti-inflammatory, Durian leaf, Edema, Flavonoids, Rattus norvegicus*

## INTRODUCTION

Inflammation is the body's natural physiological response to tissue injury, infection, or harmful stimuli, characterized by classic symptoms such as redness, swelling, heat, pain, and loss of function (Buana & Mauludin, 2024); (Buana et al., 2020). Although inherently protective, excessive or chronic inflammation can trigger various degenerative and chronic diseases (Buana & Mauludin, 2024).

The use of conventional anti-inflammatory drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs) and steroids has become the mainstay of therapy. However, long-term side effects such as gastrointestinal irritation, cardiovascular disorders, and the risk of dependence present challenges in their use (Khotimah & Muhtadi, 2016); (Emelda, Nugraeni, & Damayanti, 2023). This situation has prompted the search for safer and more natural alternatives, particularly from traditional Indonesian medicinal plants rich in secondary metabolites such as alkaloids, flavonoids, tannins, and phenolic compounds, which are known to possess anti-inflammatory activity (Buana & Mauludin, 2024).

One local plant that is beginning to attract attention in pharmacological research is durian (Durio zibethinus). Although better known as a fruit with a distinctive aroma, various parts of the durian plant—including the leaves, skin, seeds, and fruit flesh—have been reported

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to contain bioactive compounds such as flavonoids, phenols, tannins, and terpenoids that have pharmacological potential, including antioxidant and anti-inflammatory activity (Alkandahri et al., 2021); (Yen Yee, 2020).

Previous research has explored the pharmacological potential of durian plant parts. (Charoenphun & Klangbud, 2022) reported that flour from various parts of the durian plant exhibited antioxidant and anti-inflammatory activity. (Tran et al., 2024) found that durian peel extract contains flavonoids with the highest antioxidant, antidiabetic, and anticancer activity. Furthermore, (Syafira, Mambang, Dalimunthe, & Nasution, 2023) demonstrated that durian leaf ethanol extract has antipyretic effects in test animals. However, these studies have not specifically evaluated the anti-inflammatory effects of durian leaf ethanol extract, particularly in relation to its dose-response and mechanism of action.

To date, no studies have focused on the dosimetry and anti-inflammatory effectiveness of durian leaf ethanol extract in test animals. Therefore, there is a research gap that needs to be filled to understand the potential of durian leaf parts as natural anti-inflammatory agents. This study also used male white rats as test animals because their hormonal profiles are more stable than female rats, minimizing biological variability that could influence the results (e.g., the estrous cycle in female rats).

Based on this background, this study aimed to evaluate the effectiveness and dose-response of durian leaf ethanol extract on induced inflammation in male white rats. It is hoped that the results of this study can provide a scientific contribution in developing alternative anti-inflammatory therapies based on natural ingredients that are safer and have the potential to be followed up in pre-clinical and clinical studies.

## METHOD

This study used a laboratory experimental design with *a post-test only control group design* to evaluate the anti-inflammatory effect of durian leaf ethanol extract (*Durio zibenthinus* Murray) in male white rats. The research was carried out in August-September 2024 at the Batam University Laboratory.

The tools used included Erlenmeyer flasks, measuring cylinders, rat cages, filter paper, spirit lamps, mortars and pestles, analytical balances, test tube clamps, dropper pipettes, mercury plexiometers, dropper plates, rotary evaporators, 1 ml probes, 1 ml injection syringes, stopwatches, test tubes, and SPSS software.

The test materials used included distilled water, acetic acid, sulfuric acid, durian leaves, 70% ethanol, $FeCl_3$, 2 N HCl, chloroform, sodium diclofenac, NaOH, Dragendorf reagent, Mayer reagent, Wagner reagent, and egg white. The test animals used were rats weighing between 180–210 grams and aged 2–3 months,

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which were used for the egg white-induced anti-inflammatory study. Fifteen rats were divided into five groups, each consisting of three male rats.

The extracts are tested for the content of secondary compounds (flavonoids, tannins, saponins, alkaloids, triterpenoids and steroids) according to the standard procedures of the Indonesian Herbal Pharmacopoeia.

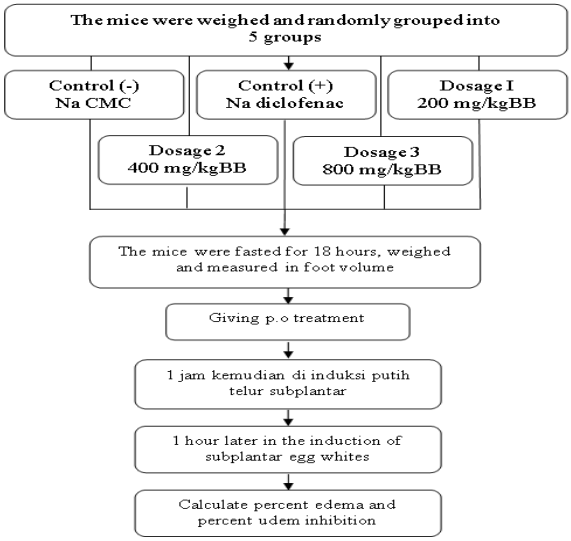


**Figure 1**. Working Scheme of Anti-Inflammatory Test

Edema volume data were analyzed using a one-way ANOVA test, followed by the *Tukey post-hoc* test ($\alpha = 0.05$) to determine significant differences between groups.

## RESULTS AND DISCUSSION

**Table 1**. Results of Durian Leaf Extract Acquisition

| | |
|---|---|
| Wet Leaves | 4 kg |
| Simplisia | 1.1 kg |
| Volume Pelarut | 4 L |
| Liquid Extract | 3.7 L |
| Thick Extract | 27.2 grams |
| Rendamen | 6.8 % |
| Shape | Pasta |
| Color | Dark Green |
| Smell | Distinctive |

**Table 2**. Phytochemical Screening Results of Durian Leaf Extract

| No | Senyawa | Observation Results | |
|---|---|---|---|
| | | Color | Ket |
| | | Clear | (-) |
| 1 | Alkaloid | Red | (-) |
| | | Red | (-) |
| 2 | Flavonoid | Yellow | (+) |
| 3 | Tanin | Green | (-) |
| 4 | Saponin | Busa | (+) |
| 5 | Steroid | Blue-green | (+) |
| 6 | Triterpenoid | Bluish-green | (-) |

Based on the results of phytochemical tests, durian leaf extract showed the presence of flavonoids, saponins, and steroids, as indicated by the appearance of yellow, foamy, and bluish-green colors in each test. Meanwhile, alkaloids, tannins, and triterpenoids were not detected, indicated by negative results in the corresponding color tests. The presence of active compounds such as flavonoids, saponins, and steroids indicates the pharmacological potential, particularly the anti-inflammatory, antioxidant, and immunomodulatory activities of the tested extract.

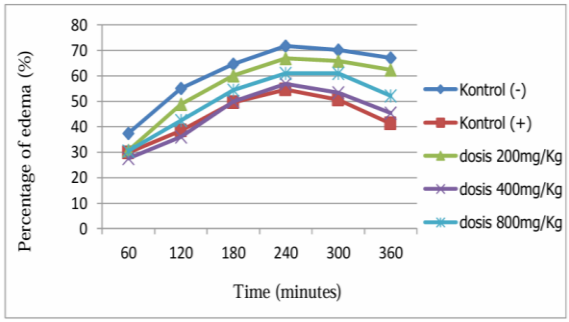


**Figure 2**. An Average Line Diagram of The Percentage Of Leg Edema In Rats At Each Observation Time

**Figure 2,** above shows that the percentage of edema increased in all groups, namely control (-), control (+), and treatment doses of 200 mg/kg, 400 mg/kg, and 800 mg/kg, with a decrease starting to

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be seen at the 300 to 360 minutes. The highest increase in the 60 to 240 minutes occurred in the CMC group, followed by dose I, dose III, control (+), and dose II. At 180 to 360 minutes, the sequence remains the same, i.e. CMC, dose I, dose III, dose II, and control (+).

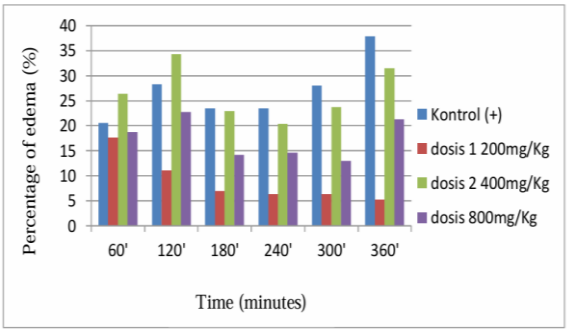


**Figure 3**. Bar Diagram of The Average Percentage of Rat Leg Edema Resistance At Each Observation Time.

**Figure 3,** above shows that the highest edema resistance at the 360th minute was found in the (+) control group of 37.83%. In the first dose group, the greatest resistance occurred at 11.04% at the 120th minute and the lowest at the 180th minute at 3.39%. The second dose group had the highest resistance at the 120th minute at 34.34%, while the third dose group reached the largest resistance at the 120th minute at 22.76%.

**Table 3**. Average Percentage Of Edema Inhibition In The Feet Of Mice At Each Observation Time

| Waktu Pengamata (menit) | Sig | | | | |
|---|---|---|---|---|---|
| | CMC | Diklofenak | Ekstrak Daun Durian | | |
| | | | Dosis I | Dosis II | Dosis III |
| 60 | ,517* | ,236* | ,314* | ,057* | ,856* |
| 120 | ,990* | ,602* | ,811* | ,174* | ,210* |
| 180 | ,211* | ,387* | ,313* | ,763* | ,113* |
| 240 | ,909* | ,229* | ,730* | ,202* | ,201* |
| 300 | ,050* | ,628* | ,703* | ,378* | ,201* |
| 360 | ,308* | ,080* | ,270* | ,057* | ,085* |

(*) menunjukan terdistribusi normal, dengan sig > 0,05.

**Table 4**. Levene Test Results of Rat Edema Percentage At Each Observation Time.

| | Levene Statistics | DFL | df2 | Significance |
|---|---|---|---|---|
| Change 60' | 1,461 | 4 | 20 | ,251* |
| Change 120' | ,028 | 4 | 20 | ,998* |
| Change 180' | ,732 | 4 | 20 | ,581* |
| Change 240' | ,589 | 4 | 20 | ,675* |
| Change 300 | 1,434 | 4 | 20 | ,259* |
| 360' Change | 1,578 | 4 | 20 | ,219* |

(*) shows normal data, with a sig >0.05.

**Table 5**. Results of the One-Way ANOVA Test Change in the Percentage of Leg Edema in Rats at Each Observation Time.

| Perubahan Edema | Sig |
|---|---|
| Edema changes 60 minutes | ,039* |
| Edema changes 120 minutes | ,000* |
| Edema changes 180 minutes | ,000* |
| Edema changes 240 minutes | ,000* |
| Edema changes 300 minutes | ,000* |
| 360-minute edema changes | ,000* |

(*) shows that there is a significant difference, with a significance of <0.05.

**Table 6**. LSD Test Results Change In Percentage Of Leg Edema In Rats At Each Observation Time

| Wkt | Perubahan 60' | | | | | Perubahan 120' | | | | | Perubahan 180' | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Kel | - | + | D1 | D2 | D3 | - | + | D1 | D2 | D3 | - | + | D1 | D2 | D3 |
| - | - | ,034* | ,088 | ,002* | ,072 | - | ,000* | ,011* | ,000* | ,000* | - | ,000* | ,427 | ,000* | ,013* |
| + | ,034* | - | ,632 | ,252 | ,708 | ,000* | - | ,000* | ,145 | ,192 | ,000* | - | ,001* | ,907 | ,041* |
| D1 | ,088 | ,632 | - | ,111 | ,916 | ,011* | ,000* | - | ,000* | ,008* | ,427 | ,001* | - | ,001* | ,068 |
| D2 | ,002* | ,252 | ,111 | - | ,135 | ,000* | ,145 | ,000* | - | ,010* | ,000* | ,907 | ,001* | - | ,052 |
| D3 | ,072 | ,708 | ,916 | ,135 | - | ,000* | ,192 | ,008* | ,010* | - | ,013* | ,041* | ,068 | ,052 | - |

| Wkt | Perubahan 240' | | | | | Perubahan 300' | | | | | Perubahan 360' | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Kel | - | + | D1 | D2 | D3 | - | + | D1 | D2 | D3 | - | + | D1 | D2 | D3 |
| - | - | ,000* | ,109 | ,000* | ,001* | - | ,000* | ,098 | ,000* | ,002* | - | ,000* | ,143 | ,000* | ,000* |
| + | ,000* | - | ,000* | ,430 | ,040* | ,000* | - | ,000* | ,272 | ,001* | ,000* | - | ,000* | ,197 | ,002* |
| D1 | ,109 | ,000* | - | ,003* | ,052 | ,098 | ,000* | - | ,000 | ,072 | ,143 | ,000* | - | ,000* | ,004* |
| D2 | ,000* | ,430 | ,003* | - | ,179 | ,000* | ,272 | ,000* | - | ,008* | ,000* | ,197 | ,000* | - | ,040* |
| D3 | ,001* | ,040* | ,052 | ,179 | - | ,002* | ,001* | ,072 | ,008* | - | ,000* | ,002* | ,004* | ,040* | - |

Description:
(-) : CMC Control (-)
(+) : control (+) na diclofenac
D1 : Dosage 200mg/kgbb
D2 : Dosage 400mg/kgbb
D3 : Dosage 800mg/kgbb
(*) : Shows Significant Differences

This study demonstrates that ethanol extract of durian leaves (Durio zibethinus Murray) has significant anti-inflammatory activity in an animal model of male white rats induced by egg white. Key findings indicate that a dose of 400 mg/kg BW resulted in greater edema inhibition than a dose of 800 mg/kg BW, although both were

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more effective than the negative control. The best activity approached the effectiveness of the positive control (sodium diclofenac), particularly at the 120-minute time point.

This anti-inflammatory effect is supported by the presence of active compounds such as flavonoids, saponins, and steroids identified in phytochemical tests. Flavonoids, for example, are known to inhibit the enzymes cyclooxygenase (COX) and lipoxygenase (LOX), thereby suppressing the production of prostaglandins and leukotrienes, which play a role in the inflammatory process (Buana & Mauludin, 2024); (Emelda et al., 2023). According to (Tran et al., (2024), flavonoids from durian peel exhibit strong antioxidant activity, which can indirectly modulate the inflammatory response by reducing oxidative stress. Furthermore, the similarity of pharmacological effects to research by (Syafira et al., 2023), which demonstrated the antipyretic effect of durian leaf extract, indicates that the pathway of action of this active compound likely involves inhibition of inflammatory mediators such as prostaglandin E2.

Interestingly, a dose of 800 mg/kg BW actually showed lower activity than 400 mg/kg BW. This phenomenon can be explained by the possibility of a saturation effect, where increasing the dose no longer directly correlates with an increase in pharmacological effects due to limited absorption of the active compound by the body. Furthermore, antagonistic effects between compounds in the extract at high doses may also cause decreased effectiveness (Khotimah & Muhtadi, 2016). This emphasizes the importance of determining the optimal dose when using natural ingredients, especially those in the form of crude extracts.

Although the effectiveness of durian leaf extract has not yet fully matched diclofenac, these results remain promising considering The potential side effects of NSAIDs with long-term use (Buana et al., (2020). Compared with synthetic agents, natural ingredients tend to have a better safety profile. This potential safety profile is one of the main contributions of this study in the context of developing natural-based anti-inflammatory agents (Mollie, 2023).

However, this study has several limitations that should be noted. This study did not include long-term toxicity testing, so it cannot yet determine the safety limits for using the extract for longer durations or at high doses. Furthermore, the active compounds have not been specifically separated, so it is not yet clear which compounds are most responsible for the anti-inflammatory activity.

Overall, this study contributes to strengthening the scientific evidence that durian leaf ethanol extract has potential as a natural anti-inflammatory candidate. The findings regarding the non-linear dose response provide important information for the development of more effective formulations and dosages. Further studies including purification of the active compounds, analysis of molecular

mechanisms, and long-term toxicity testing are highly recommended to support the potential of this extract in the development of safe and effective herbal medicines.

## CONCLUSION

Ethanol extract of durian leaves (Durio zibethinus Murray) exhibited significant anti-inflammatory activity, particularly at a dose of 400 mg/kg body weight, thought to be due to its flavonoid, saponin, and steroid content. Although not yet comparable to standard drugs, its potential safety makes it a promising alternative candidate for development in natural-based anti-inflammatory therapy.

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