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# Antihyperglycemic Potential of 70% Ethanol Extract of Kersen (*Muntingia calabura* L.) Fruit in Mice: An In Vivo Study

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## ABSTRACT

**Background:** Diabetes mellitus requires affordable therapeutic alternatives. Kersen (*Muntingia calabura* L.) fruit contains secondary metabolites with antioxidant and potential antihyperglycemic properties. **Objective:** To evaluate the antihyperglycemic activity of ethanol extract of *M. calabura* fruit in alloxan-induced hyperglycemic mice. **Method:** An in vivo experimental study used 30 mice divided into normal control, diabetic control, and extract treatment groups receiving 10%, 15%, or 20% extract orally for 14 days. Hyperglycemia was induced using alloxan at 150 mg/kg body weight. Blood glucose levels were measured before induction and on days 7 and 14. Phytochemical screening was performed qualitatively. Data were assessed using *Shapiro-Wilk* and *Levene's tests*, followed by *one-way ANOVA* and *Tukey's HSD* test. **Results:** The extract yield was 24.25%, and phytochemical screening identified flavonoids, alkaloids, tannins, and saponins. Alloxan successfully induced hyperglycemia, with diabetic-control blood glucose levels reaching approximately 299 mg/dL on day 14. Extract treatment produced a dose-dependent reduction in blood glucose. The 20% extract group showed the greatest effect, with a mean final blood glucose level of approximately 116 mg/dL and no significant difference from the normal control group ($p=0.209$). *One-way ANOVA* showed significant differences among groups ($p<0.001$). **Conclusion:** Ethanol extract of *M. calabura* fruit demonstrated significant antihyperglycemic activity in alloxan-induced mice, with the 20% concentration providing the most effective glucose-lowering response.

**Keywords**: *Muntingia calabura*, antihyperglycemic activity, ethanol extract, blood glucose, in vivo, diabetes mellitus

## INTRODUCTION

Indonesia is a country rich in natural resources, including a wide variety of plants with potential applications as traditional medicines. In recent years, the use of herbal remedies has increased significantly due to their natural origin, accessibility, and relatively lower side effects compared to synthetic drugs. One plant that has attracted attention for its antihyperglycemic potential is *Muntingia calabura* L. (kersen), particularly its fruits and leaves, which have been widely utilized in traditional medicine (Pratiwi & Santika, 2023).

Previous studies have demonstrated that kersen exhibits significant activity in reducing blood glucose levels. Extracts from both leaves and fruits have been shown to lower glucose levels in experimental animals and improve pancreatic β-cell function (Pratama et al., 2024). In addition, other studies reported that kersen extract enhances glucose transporter (GLUT-4) activity and significantly decreases blood glucose levels (Ramadhiani et al., 2025).

Kersen contains various bioactive compounds, including flavonoids, tannins, saponins, and terpenoids, which act as antioxidants and help reduce oxidative stress while improving insulin sensitivity (Telaumbanua et al., 2025). Flavonoids, particularly quercetin, are known to inhibit α-glucosidase activity and enhance glucose metabolism, contributing to antidiabetic effects (Pratiwi & Santika, 2023). Furthermore, kersen fruit also contains essential nutrients such as vitamin C and other antioxidant compounds that protect cells from oxidative damage (Khotimah & Chatri, 2024).

Based on these findings, this study aims to investigate whether kersen fruit extract (*Muntingia calabura* L.) exhibits effective antihyperglycemic activity in reducing blood glucose levels in mice. This research is particularly important considering the increasing prevalence of diabetes mellitus globally and nationally. Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting from impaired insulin secretion, insulin action, or both (Hasan et al., 2024). The rising incidence is closely associated with lifestyle changes, unhealthy dietary patterns, obesity, and lack of physical activity.

In addition, plant-based therapies remain widely used by the community, including for diabetes management, indicating a strong potential for natural products such as kersen fruit to be developed as supportive treatments. Therefore, further scientific investigation is necessary to validate their efficacy and safety, along with proper education to ensure rational and optimal use.

Separately, bioactive compounds such as flavonoids also play a role in reducing lipid peroxidation (Isyraqi et al., 2020). In other medicinal plants, such as *Moringa oleifera* L., flavonoids have been reported to exhibit analgesic activity by inhibiting cyclooxygenase enzymes, thereby reducing prostaglandin synthesis and pain levels. Although Moringa leaves are commonly consumed in traditional forms such as decoctions, infusions, juices, or teas, these methods are often considered impractical. Therefore, the development of more convenient dosage forms, such as effervescent powder preparations derived from plant extracts, is highly desirable to improve usability and patient compliance.

## MATERIALS AND METHODS

### Study Design and Experimental Animals

This in vivo experimental study evaluated the antihyperglycemic activity of ethanol extract of kersen (*Muntingia calabura* L.) fruit in alloxan-induced hyperglycemic mice. The experiment was conducted in the Pharmacology Laboratory of STIKes Rajekwesi Bojonegoro. A total of 30 healthy male and female mice, aged 2-3 months and weighing 20-30 g, were acclimatized for 7 days with free access to standard feed and drinking water. Each mouse was weighed before induction and treatment to determine the required alloxan dose and treatment volume.

The mice were randomly allocated into five groups, with six mice in each group: K0, normal control; K1, diabetic control; P1, 10% extract; P2, 15% extract; and P3, 20% extract. The K0 group was not induced with alloxan. The K1 group was induced with alloxan and received no kersen extract. The P1, P2, and P3 groups were induced with alloxan and received the designated extract concentration. No pharmacological positive-control group was included in the final experimental dataset.

### Preparation of Kersen Fruit Extract

Fresh kersen fruit (7 kg) was processed into dried simplicia, yielding 500 g of powdered material with a moisture content of 6.91%. The powdered simplicia was macerated using 96% ethanol at a ratio of 500 g to 5 L solvent for 7 × 24 hours. The combined filtrate was concentrated under reduced pressure using a rotary evaporator at approximately 50°C. This procedure produced 121.25 g of a thick extract with a dark reddish-black color and characteristic kersen odor. The extract yield was calculated as 24.25% of the dried simplicia weight.

### Phytochemical Screening

Qualitative phytochemical screening was conducted to identify alkaloids, flavonoids, tannins, and saponins. Alkaloids were tested using Bouchardat, Wagner, and Mayer reagents. Flavonoids were screened using concentrated hydrochloric acid and magnesium powder, sodium hydroxide, and ammonia. Tannins were tested with ferric chloride, gelatin, and hot distilled water followed by ferric chloride. Saponins were evaluated using hot distilled water, hydrochloric acid, and Bouchardat reagent. A positive result was indicated by the characteristic color change, precipitate, or stable foam described in Table 1.

### Alloxan Induction, Treatment, and Blood Glucose Measurement

Hyperglycemia was induced in groups K1, P1, P2, and P3 by intraperitoneal injection of alloxan monohydrate at 150 mg/kg body weight. The normal-control group was not induced. Blood glucose was measured from tail-tip capillary blood using a OneMed glucometer at baseline, 72 hours

after alloxan induction, and on treatment days 7 and 14. Hyperglycemia was considered established when the post-induction blood glucose level was at least 200 mg/dL.

The extract was administered orally by gavage once daily for 14 consecutive days. The P1, P2, and P3 groups received 10%, 15%, and 20% extract, respectively. The administration volume was adjusted to the individual animal and ranged from 0.2 to 0.4 mL/mouse/day. The experimental regimen was documented as extract concentrations rather than standardized milligram-per-kilogram doses. Therefore, all treatment effects are interpreted as concentration-based responses.

**Statistical Analysis**

Blood glucose data are presented as mean ± standard deviation (SD). Normality of day-14 blood glucose values was assessed using the Shapiro–Wilk test, and equality of variances was assessed using Levene's test. Differences in day-14 blood glucose among groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test for pairwise comparisons. Statistical significance was set at $p < 0.05$. The inferential analysis focused on the day-14 endpoint, while changes across the observation period were interpreted descriptively.

## RESULT AND DISCUSSION

### ReESULT

### Extract Characteristics and Phytochemical Profile

Maceration of 500 g dried kersen fruit simplicia with 96% ethanol yielded 121.25 g of thick extract, corresponding to a 24.25% yield. The extract was dark reddish-black and had the characteristic odor of kersen fruit. Qualitative screening showed positive reactions for alkaloids, flavonoids, tannins, and saponins (Table 1).

**Table 1. Phytochemical screening of *Muntingia calabura* L. fruit extract**

| Compound Class | Reagents | Color Change | Result |
|---|---|---|---|
| Alkaloids | • Burchard | Orange | + |
| | • Wagner | Orange | + |
| | • Mayer | White precipitate | + |
| Flavonoids | • Conc. HCl + Mg powder | Orange | + |
| | • NAOH | Orange | + |
| | • Ammonia | Orange | + |
| Tannins | • $FeCl_3$ | Greenish-black | + |
| | • Gelatin | White precipitate | + |
| | • Hot aquadest + $FeCl_3$ | Brown precipitate | + |
| Saponins | • HCl + Hot aquadest | Foam | + |
| | • Burchard | Green | + |
| | • Hot aquadest | Foam | + |

*Note: (+) presence of compound, (-) absence of compound*

### Blood Glucose Response Following Alloxan Induction and Treatment

All alloxan-induced groups fulfilled the predefined hyperglycemia criterion at 72 hours, with mean glucose values ranging from 215.00 ± 6.78 to 224.00 ± 4.43 mg/dL. The diabetic-control group then showed a continued increase in glucose, reaching 299.00 ± 3.41 mg/dL on day 14. In contrast, the treatment groups showed concentration-related reductions in blood glucose. The day-14 means were 181.00 ± 6.69 mg/dL in P1, 142.17 ± 5.81 mg/dL in P2, and 115.50 ± 6.28 mg/dL in P3. Relative to the 72-hour post-alloxan value, glucose changed by +33.48% in K1 and declined by 15.81%, 34.69%, and 46.77% in P1, P2, and P3, respectively.

**Table 2. Mean blood glucose levels during the experiment (mg/dL, mean ± SD; n = 6/group)**

| Group | Treatment | Baseline | 72 h after alloxan | Day 7 | Day 14 | Change from 72 h to day 14 (%) |
|---|---|---|---|---|---|---|
| K0 | Normal control | 116.50 ± 28.05 | – | 116.00 ± 6.78 | 108.83 ± 2.64 | – |
| K1 | Diabetic control | 120.83 ± 36.36 | 224.00 ± 4.43 | 290.50 ± 3.02 | 299.00 ± 3.41 | +33.48 |
| P1 | Extract 10% | 112.00 ± 17.45 | 215.00 ± 6.78 | 223.33 ± 10.80 | 181.00 ± 6.69 | −15.81 |
| P2 | Extract 15% | 108.17 ± 15.25 | 217.67 ± 8.29 | 201.67 ± 5.32 | 142.17 ± 5.81 | −34.69 |
| P3 | Extract 20% | 103.67 ± 21.37 | 217.00 ± 9.78 | 169.17 ± 29.73 | 115.50 ± 6.28 | −46.77 |

*Note : K0: normal control; K1: diabetic control; P1: 10% extract; P2: 15% extract; P3: 20% extract. The normal-control group was not induced with alloxan. Negative values indicate a reduction from the post-induction mean.*

**Table 3. Results of alloxan induction (Blood glucose levels, mg/dL)**

| Group | Treatment | Mouse | Baseline | 72 h after alloxan | Day 7 | Day 14 |
|---|---|---|---|---|---|---|
| K0 | Normal control | 1 | 160 | - | 112 | 108 |
| | | 2 | 75 | - | 114 | 112 |
| | | 3 | 104 | - | 110 | 111 |
| | | 4 | 126 | - | 126 | 105 |
| | | 5 | 111 | - | 111 | 107 |
| | | 6 | 123 | - | 123 | 110 |
| K1 | Diabetic control | 1 | 147 | 224 | 290 | 298 |
| | | 2 | 124 | 220 | 288 | 295 |
| | | 3 | 142 | 227 | 295 | 300 |
| | | 4 | 88 | 230 | 293 | 305 |
| | | 5 | 66 | 218 | 287 | 299 |
| | | 6 | 158 | 225 | 290 | 297 |
| P1 | Extract 10% | 1 | 104 | 210 | 240 | 190 |
| | | 2 | 123 | 212 | 230 | 182 |
| | | 3 | 118 | 220 | 225 | 179 |
| | | 4 | 97 | 226 | 215 | 170 |
| | | 5 | 138 | 208 | 210 | 185 |
| | | 6 | 92 | 214 | 220 | 180 |
| P2 | Extract 15% | 1 | 118 | 230 | 210 | 150 |
| | | 2 | 85 | 219 | 205 | 140 |
| | | 3 | 113 | 223 | 200 | 142 |
| | | 4 | 127 | 216 | 198 | 135 |
| | | 5 | 96 | 207 | 202 | 148 |
| | | 6 | 110 | 211 | 195 | 138 |
| P3 | Extract 20% | 1 | 90 | 229 | 190 | 125 |
| | | 2 | 111 | 225 | 180 | 115 |
| | | 3 | 143 | 221 | 185 | 120 |
| | | 4 | 102 | 208 | 110 | 110 |
| | | 5 | 87 | 204 | 170 | 108 |
| | | 6 | 89 | 215 | 180 | 115 |

**Assumption Testing and Endpoint Analysis**

The day-14 glucose data met the assumptions for parametric analysis. Shapiro-Wilk p-values ranged from 0.611 to 0.874 across groups, indicating no evidence of a departure from normality.

Levene's test showed homogeneous variances (F=1.081, p=0.387). One-way ANOVA demonstrated a significant difference in day-14 blood glucose among groups, F (4, 25) = 1330.890, p < 0.001.

**Table 4. Assumption tests for day-14 blood glucose values**

| Test | Group or basis | p-value |
|---|---|---|
| Shapiro–Wilk | K0 | 0.863 |
| Shapiro–Wilk | K1 | 0.611 |
| Shapiro–Wilk | P1 | 0.874 |
| Shapiro–Wilk | P2 | 0.696 |
| Shapiro–Wilk | P3 | 0.785 |
| Levene's test (F = 1.081) | Based on mean | 0.387 |

**Table 5. One-way ANOVA for day-14 blood glucose values**

| Source | Sum of squares | df | Mean square | F | p-value |
|---|---|---|---|---|---|
| Between groups | 145475.133 | 4 | 36368.783 | 1330.890 | <0.001 |
| Within groups | 683.167 | 25 | 27.327 | – | – |
| Total | 146158.300 | 29 | – | – | – |

**Table 6. Tukey HSD pairwise comparisons for day-14 blood glucose**

| Comparison | Mean difference (mg/dL) | 95% CI lower | 95% CI upper | Adjusted p-value | Significant |
|---|---|---|---|---|---|
| K0 vs K1 | 190.167 | 181.303 | 199.030 | <0.001 | Yes |
| K0 vs P1 | 72.167 | 63.303 | 81.030 | <0.001 | Yes |
| K0 vs P2 | 33.333 | 24.470 | 42.197 | <0.001 | Yes |
| K0 vs P3 | 6.667 | −2.197 | 15.530 | 0.209 | No |
| K1 vs P1 | −118.000 | −126.864 | −109.136 | <0.001 | Yes |
| K1 vs P2 | −156.833 | −165.697 | −147.970 | <0.001 | Yes |
| K1 vs P3 | −183.500 | −192.364 | −174.636 | <0.001 | Yes |
| P1 vs P2 | −38.833 | −47.697 | −29.970 | <0.001 | Yes |
| P1 vs P3 | −65.500 | −74.364 | −56.636 | <0.001 | Yes |
| P2 vs P3 | −26.667 | −35.530 | −17.803 | <0.001 | Yes |

*Note : Mean difference is calculated as the first-named group minus the second-named group. Tukey HSD was calculated from the individual day-14 blood glucose values in Table 3.*

**Table 7. Homogeneous subsets based on Tukey HSD for day-14 blood glucose**

| Subset | Groups | Interpretation |
|---|---|---|
| 1 | K0, P3 | No significant difference between K0 and P3 |
| 2 | P2 | Distinct from K0, P3, P1, and K1 |
| 3 | P1 | Distinct from K0, P3, P2, and K1 |
| 4 | K1 | Highest blood glucose level |

## DISCUSSION

The experimental model successfully generated persistent hyperglycemia in the alloxan-induced groups. At 72 hours, all induced groups had mean blood glucose values above 200 mg/dL, while the non-induced normal-control group remained within its baseline range. The diabetic-control group did not show spontaneous improvement; instead, its mean glucose increased from 224.00 mg/dL after induction to 299.00 mg/dL on day 14. This pattern supports the internal validity of the model because the observed glucose reductions in the extract-treated groups occurred against a background of persistent hyperglycemia in K1. Alloxan is known to accumulate preferentially in pancreatic beta cells and generate reactive oxygen species through redox cycling, thereby producing beta-cell injury and impaired insulin secretion (Lenzen, 2008).

The extract-treated groups showed a graded response that strengthened with increasing concentration. P1, P2, and P3 decreased by 15.81%, 34.69%, and 46.77%, respectively, from their 72-hour post-alloxan glucose levels to day 14. The separation was not merely descriptive. Tukey HSD

analysis showed that each extract group differed significantly from K1, and that each concentration differed significantly from the others. Therefore, the data support a concentration-related antihyperglycemic effect within the tested range. The 20% extract produced the lowest day-14 glucose value, 115.50 ± 6.28 mg/dL, whereas the 10% and 15% extracts produced 181.00 ± 6.69 mg/dL and 142.17 ± 5.81 mg/dL, respectively.

The lack of a significant difference between P3 and K0 ($p=0.209$) requires careful interpretation. It indicates that this study did not detect a difference between the day-14 glucose levels of P3 and the normal-control group. It does not prove that the two groups are biologically or therapeutically equivalent. Equivalence requires a predefined margin and an equivalence or non-inferiority design. The appropriate conclusion is that the 20% extract brought blood glucose near the normal-control range in this model, while still recognizing the limited sample size and the endpoint-based design.

The qualitative phytochemical profile offers a biologically plausible explanation for the observed response, but it does not establish the active constituent or mechanism. The extract tested positive for flavonoids, alkaloids, tannins, and saponins. Previous work on *M. calabura* has identified a broad range of phytoconstituents and pharmacological activities across plant parts (Mahmood et al., 2014). Flavonoid- and phenolic-rich extracts may reduce oxidative stress, while some plant constituents may influence intestinal carbohydrate digestion, peripheral glucose utilization, or insulin-related signaling. However, this experiment did not quantify total phenolics or flavonoids, measure alpha-glucosidase activity, assess plasma insulin, or examine pancreatic tissue. Thus, any mechanism beyond the blood-glucose outcome remains a hypothesis rather than a demonstrated pathway.

The extraction yield of 24.25% confirms that the maceration procedure recovered a substantial amount of ethanol-soluble material from the dried fruit. Nevertheless, a high yield should not be interpreted as evidence of high pharmacological potency. Yield reflects total soluble extractives, not the concentration of the specific compounds responsible for glucose lowering. The current result is therefore more informative when interpreted together with the concentration-response pattern and the phytochemical screen. Future work should standardize the extract by at least one measurable marker, such as total phenolic content, total flavonoid content, or a chromatographic fingerprint, to permit comparison among batches and studies.

The alloxan model is useful for preliminary screening, yet it has a narrow translational scope. Alloxan primarily models beta-cell toxicity and insulin deficiency. It does not reproduce the full metabolic context of common type 2 diabetes, including obesity, insulin resistance, dyslipidemia, and chronic low-grade inflammation. Pancreatic beta cells are highly sensitive to oxidative imbalance, but diabetes pathophysiology involves multiple interacting mechanisms beyond oxidative injury alone (Mukai et al., 2022). Consequently, these findings support preclinical antihyperglycemic potential, not a clinical recommendation for diabetes treatment.

Several methodological issues should guide interpretation. First, the study used concentration-based preparations rather than standardized mg/kg doses, which limits dose extrapolation. Second, no pharmacological positive control was represented in the final raw dataset, so the extract cannot be compared directly with an established antidiabetic medicine. Third, male and female mice were included without sex-stratified analysis. Fourth, the repeated measurements were summarized descriptively, while the statistical comparison focused on the day-14 endpoint. A future study should use a predefined mg/kg dosing scheme, a vehicle control and a pharmacological comparator, sex-balanced groups, blinded outcome assessment, and repeated-measures or mixed-effects analysis. It should also assess insulin, glycated hemoglobin, lipid profile, liver and kidney function, oxidative-stress markers, and pancreatic histopathology.

A recent study reported antidiabetic activity in a combined ethanolic extract containing *M. calabura* leaves, but that evidence should not be treated as directly interchangeable with the present fruit extract. Plant part, extraction profile, co-administered species, dosage, and model characteristics may change the observed effect (Widodo et al., 2024). The present study nevertheless provides an internally consistent signal that the ethanol extract of kersen fruit warrants further standardized pharmacological and toxicological evaluation.

## CONCLUSION

The ethanol extract of kersen (*Muntingia calabura* L.) fruit contains bioactive compounds such as flavonoids, alkaloids, tannins, saponins, and phenolic compounds, which are potentially responsible for its antihyperglycemic activity. The extraction process using maceration with 96% ethanol resulted in a high yield, indicating good extraction efficiency. Administration of the kersen fruit extract to alloxan-induced mice significantly reduced blood glucose levels compared to the positive control group. Among the tested concentrations, the 20% dose was found to be the most optimal, as it effectively reduced blood glucose levels close to normal conditions, highlighting its potential as an alternative antidiabetic therapy.

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