Effect of *Muntingia calabura* L. Leaf Extract on Blood Glucose Levels and Body Weight of Alloxan-Induced Diabetic Mice

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ABSTRACT

**Objective:** To evaluate the effect of *Muntingia calabura* L. leaf extract on the blood glucose level and weight of alloxan-induced diabetic mice. **Methods:** The mice were injected using 150mg/kg of alloxan intraperitoneally to induce diabetes. Blood glucose level was tested before alloxan injection and 5 days after injection to confirm diabetes development. *M. calabura* leaf extract with 100 and 300 mg/kg and 600 µg/kg of glibenclamide was given orally for 14 days. **Results:** The statistical results showed a significant decrease in blood glucose level, especially on day-7 and day-14 in the *M. calabura* leaf extract treatment group and glibenclamide treatment group compared to the model control group. There was an increase on weight on day-7 in the glibenclamide group compared to the model control group. **Conclusion:** *M. calabura* leaf extract had a significant antidiabetic effect that can normalize the weight of alloxan-induced diabetic mice.

Key words: Alloxan, Diabetes, *Muntingia calabura* leaf, Mice.

INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disease characterized by hyperglycemia caused by insufficiency in the number and function of insulin which led to an abnormality in the metabolism of carbohydrates, lipid, and protein1,2. DM is a non-contagious disease in Indonesia with increasing prevalence and may lead to fatal complications3. Factors related to the increased prevalence of DM in Indonesia include changes in dietary patterns, obesity, urbanization, and lack of exercise. Several acute and chronic complications can affect DM patients due to oxidative stress caused by hyperglycemia4.

The insulin hormone has the main function of regulating blood glucose levels. Lack of insulin or inadequate activity of the hormone due to reduced sensitivity of the insulin receptor can cause an abnormality in blood glucose homeostasis5. Exposure to the diabetogenic substance of alloxan reduces the level of insulin and disrupts blood glucose homeostasis. Alloxan is a diabetogenic substance that is cytotoxic to the pancreatic islets6.

The current oral medicines for diabetes mellitus are in the sulfonylurea, biguanide, and acarbose groups7. One of the medicines widely used in Indonesia is glibenclamide. Synthetic drugs, aside from expensive, often cause side effects8. On the other hand, natural drugs from selected herbal medicines cost cheaper and have a relatively easy method of application9,10.

One of the herbal medicines in Indonesia that can be used for diabetes mellitus is *M. calabura*, especially its leaves. *M. calabura* is a fast-growing wild plant that is easy to obtain. The leaves of *M. calabura* can be utilized as an antidiabetic drug. *M. calabura* leaf contains high antioxidants. Substance screening from *M. calabura* leaves showed alkaloid, flavonoid, tannin, saponin, triterpenoid, and steroid11,12.

Several previous studies stated that *M. calabura* leaf has potential as an antidiabetic drug, including a study by Zulham, Hendrarti, and Wahyuni (2019)13 who showed that ethanol extract from *M. calabura* leaves with 50, 100, and 150 mg/kg obtained from macemiceion could reduce blood glucose level in alloxan-induced diabetic mice. The results from Herlina, Amriani, Solihah, and Sintya (2018)14 also showed that ethanol extract from *M. calabura* leaves with 65, 130, and 260 mg/kg could reduce blood glucose levels to 28.90%, 32.16%, and 35.66% in male albino mice induced by alloxan.

Based on the statements above, the author is interested in conducting a further study to determine the effect of graded dose of *M. calabura* leaf extract on blood glucose level and body weight of alloxan-induced diabetic mice. This study is expected to be a new source of information for the public regarding the benefit of *M. calabura* leaves as an optimal antidiabetic herbal medicine.

MATERIALS AND METHODS

Prepamiceion of animals

Male mice (*Mus musculus* L.), aged ± 3 months, weighing between 25-35 grams were used in this study. The cage used was a plastic caged covered with wire mesh. Every other day, the cage's bottom was coated in 1 cm high husks. The scientific range for light, moisture, and room temperature were adjusted. Ethical code, institutional, and national regulation on live animals were strictly observed. The Health Research Ethical Clearance Commission, Faculty of Dental Medicine, Airlangga University granted ethical permission for the animals used in this investigation, with the number 507/HREC.

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M. calabura leaves were rinsed from dust and heated in an oven at 60°C. The leaves were then ground with a leaf grinder with a miceio of 1:7 of M. calabura leaf powder immersed in 96% ethanol. They were mixed and let sit still at room temperature for 2 hours. Afterward, the leaves were filtered, and macemices were sepamiced and remacemiced with a miceio of 1:4 with an addition of 96% of ethanol. All macemices were evapomiced at 60°C after filtered to obtain the thick extract. The solvent used was Carboxyl Methyl Cellulose (CMC) with 0.5% concentmiceion to obtain the desired extract.

Flavonoids Test
A solution of hydrochloric alcohol from 2 mL of amyl alcohol was added with Mg powder and 5 mL of M. calabura leaf extract, then vigorously shaken and let to sepamice. The formation of the yellow amyl alcohol layer showed a positive result.

Tannins Test
Steasny reagent was added to the solution of M. calabura leaf extract, then heated in a water bath to obtain a pink deposit. The result was filtered and the filtmicee from the solution was satumiced with NaCl and added with gelatin. The formation of white deposits showed a positive result.

Alkaloids Test
2 grams of M. calabura leaf extract were added to 50 mL of water, heated for 5 minutes, and filtered. Mayer and Dragendorff test was performed by inserting 5 mL of M. calabura leaf extract into a reaction tube. The formation of white deposit showed a positive result in the Mayer test and the formation of orange deposit showed a positive result in the Dragendorff test.

Saponins test
A reaction tube containing 10 mL of M. calabura leaf extract shaken for 10 seconds and let still for 10 minutes. The formation of bubbles or foam showed a positive result.

Polyphenol test
3 drops of FeCl reagent were added to 5 mL of M. calabura leaf extract. The formation of a blue-green color showed a positive result of polyphenol.

Quinone test
NaOH of 1 N was added to 5 mL of M. calabura leaf extract. The formation of a red color showed a positive result of quinone.

Steroids and Terpenoids test
5 mL of ether solution of M. calabura leaf extract was evapomiced in the evapomiceor cup, and 1 drop of thick H2SO4 and 2 drops of anhydrous acetic acid were added to the resulted residue from the extract solution. The formation of a green color showed a positive result of steroids, and the formation of a brown color showed a positive result of terpenoid.

Acute toxicity test
M. calabura leaf extract with 5 mg/kg was given to 6 male mice orally using a probe and was observed every 6 hours for 48 hours. The toxic dose was confirmed if there are 3 or more mice died. However, if only one mice died, then the same dose was given to confirm non-toxicity. Repetition of the procedure with a higher dose of 50, 100, and 2000 mg/kg was performed if there was no death.
Alkaloids can regenemice damaged pancreatic β cells. The antioxidant activity from *M. calabura* leaf is also high and could repair damaged pancreatic β cells. Antioxidant activity can capture free radicals that result in the repair of damaged pancreatic β cells causing DM 1. This leads to increased insulin in the body thus reduced blood glucose in the body 24,25 .

Saponin is a substance containing the isoprene structure of \( \text{CH}_2=\text{C}-(\text{CH}_3)-\text{CH}=\text{CH}_2 \). This saponin can be detected based on the ability to form foam because saponin acts like a soap. Saponin is an active surface substance grouped as triterpene glycosides. Saponin can alleviate oxidative stress by contributing hydrogen atoms from the hydroxyl aromatic group of polyphenol to bind free radicals and release them through the excretion system, preventing a chain reaction from superoxide to hydrogen superoxide. Polyphenol has been shown to protect pancreatic cells against the harmful effects of free radicals generated under chronic hyperglycemia conditions. Antioxidant administration can enhance pancreatic β cell mass and keep insulin levels stable 33,35 . Steroids lowered blood glucose levels through altering insulin activity at the cellular level, distal insulin receptors, and reduced glucose production in the liver 34.

In this study, the mice used were male mice because they have a more stable biological condition compared to female mice, which are not affected by the estrous cycle. Other than that, male mice also have faster drug metabolism. Mice were adapted for 2 weeks to the lifestyle in their new environment and to avoid stress during treatment.

Diabetes mellitus can be caused by several factors. These factors include genetic, nutrition, diabetogenic substance, and free radicals (oxidative stress) 32. Alloxan is a toxic diabetogenic substance, especially on pancreatic β cells. Alloxan given to animal models such as mice can cause diabetes. The entrance of alloxan into pancreatic cells initiates the cytotoxic mechanism of alloxan. The diabetogenic property of alloxan is determined by the speed with which it is collected. Damage to cells is caused by a combination of mechanisms, including the oxidation of the sulphydryl group and the production of free radicals. Alloxan damages pancreatic β cells by destroying biological components that contain the sulphydryl group, cysteine amino acids, and proteins that attach to the SH group (including enzymes containing the SH group) 33.

Alloxan reacts with two SH groups that bind the sides of protein or amino acid and create a disulfide bond, which inactivates the protein and leads to the damaged function of the protein 34,35. Alloxan with 150 mg/kg induced intraperitoneally can increase the level of blood glucose and damage in the pancreatic β cells of mice. The mice were considered hyperglycemic if the blood glucose level reached > 200 mg/dl.

Based on the result of One-Way ANOVA on day 14, *M. calabura* leaf extract with 100 and 300 mg/kg provided a significant effect in reducing blood glucose level compared to the model control group. *M. calabura* leaf was insignificantly different from the positive control. This showed
that *M. calabura* leaf with 100 and 300 mg/kg provided a significant effect in reducing blood glucose levels because they provide similar effects to glibenclamide as a positive control.

The positive control group that was given glibenclamide showed a significant decrease in blood glucose compared to the model control group. Glibenclamide is an oral hypoglycemic drug in the group of sulfonylurea that has a therapeutic effect in reducing blood glucose levels. Therefore, it is chosen as a comparison in this study. This is because glibenclamide works by increasing insulin secretion. Glibenclamide works by inducing the secretion of insulin hormone from the granules of the β cells in pancreatic Langerhans islets. Its interaction with ATP – sensitive K channel in β cells membrane cause membrane depolarization, which will open the Ca channel. After the Ca channel opens, Ca$^{2+}$ ions will go inside β cells and induce granules with insulin that results in insulin secretion$^{14,15}$. The results of this study showed that weight loss occurred on day 7 in the model control group because diabetes mellitus patients often had sudden significant weight loss due to insufficient insulin. Therefore, glucose provides a very low energy source in the body and the body took energy from fat and muscles which causes to weight loss. The administration of *M. calabura* leaf extract in this study helped normalized the weight of mice because the ethanol extract of *M. calabura* leaf contains antioxidant substances such as flavonoid and saponin that can normalize weight.

**CONCLUSION**

The administration of *M. calabura* leaf extract in this study effective in decrease blood glucose level and can normalized the weight of diabetic mice. However, further studies are needed to determine the microscopic effect on pancreatic and liver cells of diabetic mice after administration of *M. calabura* leaf extract.

**COMPETING INTERESTS**

The authors declare that they have no competing interests.

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*Pharmacognosy Journal*, Vol 13, Issue 6, Nov-Dec, 2021

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**GRAPHICAL ABSTRACT**

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**Fasting Blood Glucose Effect**

| Group | Blood glucose (mg/dL) |
|-------|----------------------|
|       | Day 1 | Day 7 | Day 14 |
| Control | 100±6 ± 2.7 | 95±2 ± 4.3 | 92±2 ± 1.3 |
| Model   | 181±6 ± 2.8** | 191±2 ± 1.4** | 191±6 ± 2.5** |
| Glibenclamide | 301±6 ± 2.5** | 290±6 ± 2.9** | 290±6 ± 2.4** |
| M. calabura leaf extract 100 | 103±7 ± 2.7** | 103±7 ± 2.6** | 213±3 ± 2.8** |
| M. calabura leaf extract 500 | 106±6 ± 3.2** | 178±6 ± 2.1** | 106±6 ± 3.6** |

**Body Weight Effect**

| Group | Body weight (g) |
|-------|----------------|
|       | Day 1 | Day 7 | Day 14 |
| Control | 25±3 ± 2.1 | 23±2 ± 2.9 | 26±3 ± 2.9 |
| Model   | 25±3 ± 2.5 | 23±2 ± 2.9 | 26±3 ± 2.9 |
| Glibenclamide | 25±3 ± 2.5 | 23±2 ± 2.9 | 25±3 ± 2.9 |
| M. calabura leaf extract 100 | 23±3 ± 2.5 | 23±3 ± 2.9 | 24±3 ± 2.9 |
| M. calabura leaf extract 500 | 23±3 ± 2.5 | 23±3 ± 2.9 | 24±3 ± 2.9 |
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