

Decreasing Blood Glucose Levels Using *Muntingia calabura* L. Leaf Extract in Rats with Diabetes Mellitus

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ABSTRACT

The aim of this study was to determine the decrease in blood glucose levels using *Muntingia calabura* L. leaf extract in hyperglycemic rats. This study used a completely randomized design with six treatments and four replications. The experimental animal used 24 male rats (*Rattus norvegicus*) aged 3 months, body weight 200-250 grams. Group P1 as a negative control was only given distilled water. All rats in the P2, P3, P4, P5, P6 groups were induced with a single dose of alloxan 75 mg/kg body weight. Groups P3, P4, P5 were given 150, 300 and 450 mg/kg body weight of *Muntingia calabura* L. leaf extract and P6 were given metformin (positive control) for 28 days. The results showed that the mean glucose levels at P1, P2, P3, P4, P5, P6 were 59.75 ± 7.67 , respectively; 151.75 ± 17.03 ; 83.25 ± 26.46 ; 74.5 ± 25.27 ; 72.5 ± 31.77 ; 69.5 ± 7.04 . Based on Anova, *Muntingia calabura* L. leaf extract had a very significant effect on the blood glucose levels of rats ($P < 0.01$). It can be concluded that *Muntingia calabura* L. leaf extract 450 mg/kg BW can reduce blood glucose levels in hyperglycemic rats.

Keywords: blood glucose level, hyperglycemic, *Muntingia calabura* leaf extract, rat

1. INTRODUCTION

The using plants is believed by humans to prevent, reduce and cure certain diseases [1]. In Indonesia there are various types of plants are used as traditional medicine for generations to treat diabetes. One of them is the Kersen plant. The Kersen plant is a plant that is widely consumed by the community but has not been fully researched [2]. Kersen plant contains several chemical compounds, such as Kersen leaf contains tripenoid, carbohydrates, proteins, polyphenols, flavonoids, ascorbic acid, α -tocopherol, and chlorophyll [3]. Kersen leaves contain a group of compounds that exhibit antioxidative activity [4,5]. Antioxidants are thought to be able to protect liver cells from damage caused by free radicals. Qualitatively known that the dominant compound in Kersen leaves are flavonoids [5]. The flavonoid content in Kersen fruit is also thought to inhibit the growth of cancer cells in the laboratory [6].

According to WHO, Indonesia ranks 4th in the number of people with Diabetes Mellitus in the world. In 1995, the number of diabetes mellitus sufferers in Indonesia reached 5 million, in 2000 the number of sufferers was 8,400,000, in 2003 the number of sufferers was 13,797 million, in 2005 around 24 million people. This number is expected to increase in the coming year [7].

Diabetes is a disease in which the patient's body is unable to control blood sugar levels. Patients experience metabolic disorders in the process of absorption of sugar by the body, because the body cannot release or use insulin normally. Insulin is a hormone released by the pancreas, which is the main substance responsible for maintaining blood sugar levels [8].

Research carried out regarding the benefits of leaves of Kersen, among others, is antibacterial, anti-

inflammatory, anti-cancer and tumor [9] reported that Kersen leaf decoction was proven to be effective in killing *C. dipteri*, *S. aureus*, *P. vulgaris*, *S. epidermidis*, and *K. rhizophila* bacteria. [5] reported that the Kersen plant (*Muntingia calabura* L.) utilized the antinociceptive activity of the water (*Muntingia calabura* L.) extract and the involvement of L-arginine/nitratoxide /cyclic guanosine monophosphate pathway in the activity observed in mice, repairing liver damage, as an anti-cancer [9], as a gastroprotective [5], as an antiproliferative [5], antitoxic [9], as well as an antioxidant and anti-inflammatory.

2. MATERIALS AND METHODS

2.1 Research Design

Methods this study used quantitative methods. This study used a completely randomized design (CRD) consisting of six treatments and each treatment was repeated four times. The treatments consisted of P1= negative control (given distilled water), P2= positive control (75 mg/kg BW of alloxan and incubated for 28 days), P3 (75 mg/kg BW of alloxan and 150 mg/kg BW of Kersen leaf extract for 28 days), P4 (75 mg/kg BW of alloxan and 300 mg/kg BW of Kersen leaf extract for 28 days), P5 (75 mg/kg BW of alloxan and 450 mg/kg BW of Kersen leaf extract for 28 days), and P6 (75 mg/kg BW alloxan + commercial products - Metformin 20 mg).

2.2 Materials

The tools used in this study were OHAUS scales, Sartorius analytical scales, erlenmeyer flasks, mouse cage, rotary mikrotom, oven, light microscope, glass objects, cover glasses, scissors, pinset, *hot plate*, *staining jar*, *Block holder*, fotomikroskop, Nesco Multi Check, Nesco DrTM Test Strip, gavage, and stationery.

Materials used in this study were 24 male mice (*Ratus norvegicus*) three-month-old male, in which mice (*Ratus norvegicus*) easy to handle and has a relatively short growth period and has a blood component that can represent other mammals, particularly humans. Mice that were selected are male, because the immune system in male rats tend to be influenced by reproductive hormones, weighing 200-250 g are from the Faculty of Veterinary Pathology Laboratory Unsyiah, pellet types 789-S production PT. Charoen Phokpahan Medan-Indonesia, alloxan monohydrate comes from the Unsyiah Microtechnic Laboratory of Faculty of Mathematics and Natural Sciences, kersen leaves (*Muntingia calabura* L) from Ule lheu Banda Aceh, ethanol, aquades.

2.3 Research Objects

The objects in this study were 24 male rats (*Ratus norvegicus*) aged three months with a body weight of 200-250 g from the Pathology Laboratory of the Faculty of Veterinary Medicine, Universitas Syiah Kuala.

2.4 Preparation of Experimental Animals

This study used 24 male rats (*Ratus norvegicus*) three-month old male weighing 200-250 g. Mice derived from the stable maintenance of the Department of Clinical Veterinary Pathology Laboratory of the Faculty of Veterinary Medicine University of Syiah Kuala. These mice were acclimatized for seven days in the cage. Rat cage made of plastic tubs measuring 70 cm x 44 cm x 20 cm at the top of the closed wire mesh and covered with a hull bottom with a thickness of 3 cm. The experimental animals were given feed in the form of feed pellets in the form of 789-S pellets. Food and drink for rats were given ad libitum.

2.5 Extraction of *Muntingia calabura*

Making the Kersen leaf extract based on the Leaf *Muntingia calabura*. L cleaned, dried without being exposed to direct sunlight, and cut into small pieces and then blend. Furthermore, leaf *Muntingia calabura*. L was extracted using maceration method that is soaked with 96% ethanol. All extracts were filtered separately, then evaporated with a rotary evaporator at low pressure.

2.6 In Vivo Assay

Before the treatment was carried out, all rats were weighed for alloxan dose determination using the OHAUS scale with a weighing power of 2610 g. Giving alloxan done once on the first day of treatment intraperitoneally with a dose of 75 mg/kg for four days and continued provision leaf extract Kersen with different doses of 150 mg/kg, 300 mg / kg and 450 mg / kg refers in Manohar *et al.* [10]. According to Santos *et al.* [11] conducted cherry leaf extract administered orally (intubation of the esophagus) for 30 days for all treatments. Control animals were only given alloxan and water. The treatment was carried out at 16.00 WIB before the experimental animals were fed. The rats were fasted for six hours before and after being treated.

2.7 Examination of Blood Glucose Levels

Examination of blood glucose levels was carried out on the first day before treatment and on the seventh day after alloxan injection by taking blood from the tail

in a rat tail flick. Examination of blood glucose levels was also carried out one day after the treatment ended with necropsy. Blood tests were performed using the Nesco Test Meter. The blood obtained is dripped on the Nesco™ test strip, then after 11 per second the blood glucose level is displayed on the Nesco™ test meter screen and after that reading the data. The observed blood glucose levels were in mg/dL units.

2.8 Data Analysis

Data obtained from observations of blood glucose levels were carried out by analysis of variance. If the variance results show a significant difference, a further test is carried out to determine the difference between one treatment and another, determined based on the coefficient of diversity (KK), namely

1. If the KK is large (at least 10% in homogeneous conditions or at least 20% in heterogeneous conditions), the next test should be used is Duncan
2. If the KK is moderate (between 5-10% in homogeneous conditions or between 10-20% in heterogeneous conditions), the next test that should be used is the Honestly Real Difference test.

3. RESULTS AND DISCUSSION

The results showed that the administration of *Muntingia calabura* L leaf extract on blood glucose levels in mice at the start of the treatment were all the same, that is in the range of 85-108.5 mg/dL, on the seventh day it was seen that alloxan administration caused hyperglycemic rats ranging from between 179-324 mg/dL, in P2, P3, P4, P5, and P6 treatments, while the control group was not given alloxan glucose levels were not high, that is normal. After being given the extract treatment on the 28th day, it can be seen that giving Kersen leaf extract as much as 450 mg/kg BW can reduce blood glucose levels, namely between 69.5-83.25 mg/dL, this decrease in blood glucose levels is the same as the controls, namely P1 and P6 namely the commercial drug administration of metformin. However, when compared with P2, P3, and P4, the glucose levels were different from P5.

Results the mean blood glucose levels in various treatments can be seen in the following of Table 1.

Table 1. Mean blood glucose levels of mice in various treatments

Treatment		Mean blood glucose levels mg/dL X ± SD		
		Day 1	Day 7	Day 28
P1	Aquadest	85 ± 20.19 ^C	88±12.24 ^C	59.75 ± 7.67 ^A
P2	75 mg/kg BW of alloxan and incubated for 28 days	87.75 ± 20.15 ^C	324 ± 40,54 ^D	151.75±17.03 ^C
P3	75 mg/kg BW of alloxan and 150 mg / kg of <i>Muntingia calabura</i> L leaf extract	85.25 ± 6.99 ^C	213.25±37.33 ^D	83.25 ± 26.46 ^C
P4	75 mg/kg BW of alloxan and 300 mg / kg of <i>Muntingia calabura</i> L leaf extract	108.5±11.35 ^C	179.75±17.85 ^D	74.5 ± 25.27 ^{BC}
P5	75 mg/kg BW of alloxan and 450 mg / kg of <i>Muntingia calabura</i> L leaf extract	102.5 ± 46.07 ^C	192.75±52.16 ^D	72.5 ± 31.77 ^{AB}
P6	75 mg/kg BW of alloxan and commercial drug giving	102.75±24.7 ^C	191.75 ±86.6 ^D	69.5 ± 7.04 ^{AB}

Note: Superscript table of different capital letters (A, B, C) shows significant differences (P < 0.01)

In the table above shows the blood glucose levels of mice over the observation vary greatly. One of the factors is the different resistance of individual mice to alloxan which causes the initial conditions of diabetes to be not uniform. Normal blood glucose levels range from 50- 135 mg/dL [11]. For more details on the differences between the treatments, it can be seen in Figure 1.

From the results of statistical analysis showed that the average blood glucose level at P2 (positive control) before alloxan injection was around 112 mg/dL, and after alloxan mg/kg BW was induced there was an increase on the seventh day of 292 mg/dL. The mean blood glucose level of P2 (positive control) mice injected on the twenty-eighth day was 151.75 mg/dL higher than the P1 mice (negative control) on the twenty-eighth day, namely

59.75 mg/dL. Normal blood glucose levels in mice range from 50- 135 mg/dL [11].

Based on the range of normal glucose levels, P2 rats experienced blood glucose levels (hyperglycemia) after being injected with alloxan, as well as P3, P4, P5 and P6. Alloxan compound is one of the diabetogenic substances which is toxic, especially to pancreatic beta cells, and when given to experimental animals such as mice, it can cause mice to become diabetic. Damage to pancreatic beta cells causes the body to be unable to produce insulin, causing blood glucose levels to increase (hyperglycemia occurs). Alloxan induction in the peritoneum of experimental animals caused selective damage to pancreatic β cells. Alloxan is an agent that causes diabetes mellitus. *In vitro* alloxan causes necrosis of pancreatic β cells by stimulating intracellular H_2O_2 . Alloxan causes permanent hyperglycemia within 2-3 days. Alloxan also interferes with homeostasis in cells, this is the beginning of cell death due to disruption of the cell oxidation process. Increasing the concentration of calcium ions, accelerates the destruction of pancreatic β cells. When β cells are damaged by alloxan, insulin secretion is disrupted resulting in a reduced amount of insulin. The decrease in insulin secretion results in the body unable to use glucose as an energy source [12].

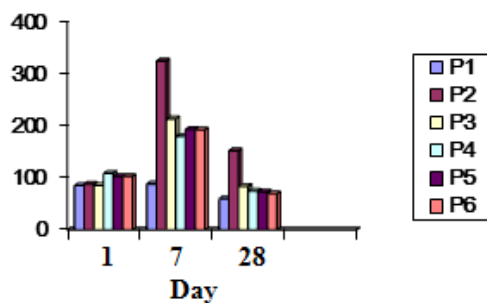


Figure 1 Diagram of average blood glucose levels in rats

In P3, P4, and P5 alloxan 75 mg/kg BW were injected and added with *Muntingia calabura*. L leaf extract 150 mg/kg BW, 300 mg/kg BW, and 450 mg/kg BW mean blood glucose levels namely 50.67 mg/dL, 37 mg/dL, 24.33 mg/dL. Based on the results obtained in the group giving Kersen leaf extract (*Muntingia calabura*. L) with different doses, it showed that the P5 treatment was giving Kersen leaf extract (*Muntingia calabura*. L) (300 g/kg BW) in the test animals experiencing a decrease in glucose levels, which is very significant when compared with other doses as well as metformin. This is because in Kersen leaf extract (*Muntingia calabura*. L) there are several metabolic compounds that can reduce blood glucose levels, including flavonoids which are useful for lowering blood glucose levels [13].

Muntingia calabura L. is one of the plants that thought to have active antidiabetic substances, namely ascorbic acid, fiber, niacin and β -carotene. In his

research on the effectiveness test of kersen fruit juice (*Muntingia calabura* l.) on decreasing blood glucose levels in white rats (*Ratus norvegicus*) showed that *Muntingia calabura* L. fruit juice had an effect in lowering blood glucose. The best test that can lower blood glucose is the kersen fruit juice with 4 ml doses [14].

On day 28, there was a decrease in blood glucose levels of male white rats (*Ratus norvegicus*) in *Muntingia calabura*. L leaf extract treatment at a dose of 450 mg/kg BW. This is because *Muntingia calabura*. L leaf extract can repair pancreatic beta cells, so that pancreatic beta cells produce insulin, and insulin regulates glucose levels to normal [12].

The administration of *Muntingia calabura*. L leaf extract has the same effect as the commercial drug administration in the form of metformin (P6). Metformin is an oral hypoglycemic antidiabetic drug, sulfonyl urea derivative, which works actively to reduce blood sugar levels. The use of antidiabetic drugs is able to stimulate insulin secretion from the pancreas. Giving a single dose of metformin will lower blood glucose and excreted with feces and as metabolites with urine [12]. Metformin stimulates the β cells of the pancreatic islets of Langerhans so that insulin secretion is increased. In addition, the sensitivity of β cells to blood glucose levels is enhanced by their influence on glucose transport proteins. There are indications that the drug also improves the sensitivity of the target organ for insulin and reduces insulin absorption by the liver. It was observed that the extracts from Kersen leaf at day 28 decreased levels of blood glucose male rats were used as an experiment at a dose of 450 mg/kg bb. At this dose it can improve pancreatic beta cells so that pancreatic beta cells produce insulin and increase sugar levels to normal again.

4. CONCLUSION

The *Muntingia calabura* L. leaf extract of 450 mg/kg BW can be develop as antidiabetic drug because of its ability to reduce blood glucose levels in hyperglycemic rats.

AUTHORS' CONTRIBUTIONS

All authors equally contributed to this study in Laboratory and also review the result.

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