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Research Article

### Activity of *Muntingia calabura* Leaves Ethanolic Extract on Glucose and Insulin Blood Levels in Streptozotocin-induced Rat

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#### ABSTRACT

**Objectives:** The research aims to determine the effect of *Muntingia calabura* leaves extract on improving diabetes conditions, by analyzing blood glucose and insulin levels of male *Rattus norvegicus* which is given high fat diet and induce streptozotocin 30 mg/kg BB with a two-week interval.

**Design:** This research was a purely experimental with pretest and post test control group design. This research was divided into 6 groups. Three treatment groups were treated by *Muntingia calabura* leaves extract with the doses of 125, 250, and 500 mg/kg. Other groups are negative control (CMC-Na), positive control (glibenklamid) and normal control.

**Interventions:** The variables specified in this study are variations in the concentration of extract used. variations in extract concentration are expected to affect the results of glucose concentration and blood insulin in rats.

**Main outcome measure:** The main parameters measured in this study were rat blood glucose and insulin levels.

**Results:** The result of this research indicate that the ethanol extract of *Muntingia calabura* leaves able to reduce blood glucose levels effectively at dose 500 mg/kg (97, 17 mg/dL) so that insulin levels increase well (0,839 µg/mL).

**Conclusion:** *Muntingia calabura* leaves ethanol extract can reduce glucose levels and increase blood insulin levels in rats.

**Keywords:** *Muntingia calabura*, anti-hyperglycemia, insulin levels

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#### INTRODUCTION

The kerson (*Muntingia calabura* L.) is a neotropic plant originating from the Philippines that entered Indonesia in the 19th century. *Muntingia calabura* leaves contain groups of compounds including alkaloids, flavonoids, triterpenoids, steroids, tannins, anthraquinones and saponins<sup>1,2</sup>.

Based on various literature, *Muntingia calabura* leaves have many benefits. Based on research conducted by various researchers of *Muntingia calabura*, it is known to have antioxidant, antimicrobial, antiproliferative, hypotensive, antinociceptive, cardioprotective, platelet anti-aggregation, anti-inflammatory and antidiabetic activities<sup>2-6</sup>.

Research on the antidiabetic activity of *Muntingia calabura* leaves was carried out using alloxan as an induction of pancreatic beta cell damage in mice. Based on the results of these studies, kersen leaf extract was able to reduce blood sugar levels in rats induced by alloxan. *Muntingia calabura* leaves extract doses of 260 mg / kg BB can reduce blood sugar levels by 35.66%<sup>7</sup>.

Based on this background, a study was conducted to look at blood glucose and insulin levels in streptozotocin-induced rats. The results of these studies are discussed in this article.

## MATERIALS AND METHODS

### Plant and Chemicals Materials

*Muntingia calabura* harvested came from Lamgugob Village, Syiah Kuala District, Banda Aceh City, Aceh Province, Indonesia. The leaves taken are whole green leaves with varying sizes. The chemicals used in this study were ethanol 96%, streptozotocin, physiological NaCl, Na CMC, glibenklamide tablets, and Elisa Insulin Kit.

### Preparation of Extract

10 kg of *Muntingia calabura* leaves cleaned using running water then drained and dried. Dry ingredients are called simplicia. simplicia is then pollinated using a machine blender. Simplicia is then put into a container and stored in a place protected from sunlight. Simplicia powder was extracted by maceration method by soaking ethanol 96% closed and left for 5 days protected from light, stirring once in a while. After 5 days the filter is done and the pulp is squeezed until sufficient maserate is obtained. macerate is then evaporated with vacuum rotary evaporator until thick extract is obtained <sup>8</sup>.

### Antihyperglycemia Test in Streptozotocin-induced Rats

Test animals were divided into 6 groups, then measured each blood sugar level normally after fasting for 8 hours. Rats were given a high-fat diet for four weeks and then induced streptozotocin intraperitoneally twice as much injection at a dose of 30 mg / kg body weight at intervals of 2 weeks. rats that have blood sugar levels 200 mg / dl and above are considered to have diabetes and enter into the object to be studied. 6 groups of experimental animals were divided into

normal (not induced) groups, negative controls (without treatment), positive controls (glibenclamide), extracts 125, 250 and 500 mg/kg. In each group the test material was given for 15 consecutive days orally. Then measured the blood sugar levels of mice on days 3, 6, 9, 12 and 15. Serum test animals were taken to measure insulin levels <sup>9</sup>.

### Insulin Levels Test

The microplate is coated with antibodies and the sample is inserted into the column. Samples were inserted and incubated for 2 hours at 4°C and washed using buffer 5 times. Incubation again for 30 minutes at room temperature and washed using buffer 7 times. Enter the substrate enzyme solution and re-incubate for 40 minutes at room temperature. The enzyme reaction is stopped by adding stop solution. Measured insulin concentration using a spectrophotometer at absorbance 450-630 nM. This test is carried out in accordance with the user guide <sup>10,11</sup>.

### Data Analysis

The data obtained were analyzed to see the significance of the difference in the control group with the treatment group using the One Way Anova test and Paired t-test.

## RESULTS AND DISCUSSION

### Antihyperglycemic Aactivity in Streptozotocin-induced Rats

The activity of ethanol extract of *Muntingia calabura* leaves in reducing blood sugar levels in streptozotocin-induced rats can be seen in table 1.

Table 1: The results of rat blood glucose level measurements

No	Groups	Blood Sugar Level Value (dl ± Deviation)						
		Initial Value	Post Induction	3rd Day	6th Day	9th Day	12th Day	14th Day
1	Normal	86.67± 4.08	86.67± 4.08*	87.50 ± 4.04*	86.33 ± 3.93*	86.50 ± 1.87*	87 ± 3.10*	87 ± 2.83*
2	Positive Control	76.50 ± 11.17	440.17± 53.82*	368 ± 44.86*	285 ± 45.29	207.67 ± 18.92	142.83 ± 15.94*	83.17 ± 8.84*
3	Negative Control	89.83 ± 9.30	297.50± 22.92	279.17 ± 27.87	263 ± 30.57	249.17 ± 36.56	236.67 ± 32.10	220.67 ± 33.10
4	Extract 125 mg/Kg	89.67 ± 3.56	343.83± 72.85	306.50 ± 58.93	254.83 ± 55.17	198.1 ± 34.03*	142.83 ± 14.8*	103.33 ± 10.86*
5	Extract 250 mg/Kg	85.50 ± 5.65	329.17± 46.5	272.67 ± 46.43	240.17 ± 51.98	193.50 ± 32.22*	135.17 ± 28.44*	99.17 ± 6.37*
6	Extract 500 mg/Kg	94.17 ± 6.18	391.50± 48.06*	317.83 ± 27.86	249.83 ± 35.51	191.33 ± 32.8*	140.50 ± 30.34*	97.17 ± 7.14*

\* Significantly different from the negative control group  $p \leq 0.05$

Based on the table above it can be seen that the administration of glibenclamide decreases blood sugar levels in mice. This also occurs in rats given a suspension of ethanol extract of kersen leaves. Whereas in the negative control group did not experience a significant decrease in blood sugar levels.

Blood sugar levels were measured on days 3, 6, 9, 12, and 14 days in each group. The doses of extracts given were 125, 250 and 500 mg/kg BW. Decreasing blood sugar levels for

all doses decreases with time. Based on the data in the table it can be concluded that the greater the extract dose given, the greater the decrease in blood sugar levels in experimental animals. Decreased blood sugar levels are caused by the content of the leaves of ginger, namely flavonoids. Compounds of flavonols that are thought to have activity in reducing glucose levels in the blood are quercetin. The mechanism of action of quercetin in reducing blood glucose levels is reducing oxidative stress and will cause protective

effects of pancreatic  $\beta$  cells and can increase insulin sensitivity. Flavonoids can also inhibit GLUT 2 which can reduce the absorption of glucose and fructose from the intestine so that blood glucose levels drop. Flavonoids also have a phosphodiesterase inhibition mechanism so that the level of cAMP in pancreatic  $\beta$  cells increases so that stimulation of insulin secretion occurs through the Ca pathway<sup>12</sup>.

## Results Measurements of insulin levels

The value of rat insulin levels after streptozotocin was induced and the active ingredient was extracted and compared with negative and positive controls can be seen in table 2.

**Table 2;** The value of rat insulin levels

No	Groups	Insulin Levels ( $\mu\text{g/mL}$ ) $\pm$ SD
1	Normal	1.126 $\pm$ 0.87*
2	Glibenklamid	0.902 $\pm$ 0.56*
3	CMC Na	0.249 $\pm$ 0.05
4	EEDK 125 mg/Kg BB	0.683 $\pm$ 0.72
5	EEDK 250 mg/Kg BB	0.749 $\pm$ 0.28*
6	EEDK 500 mg/Kg BB	0.839 $\pm$ 0.70*

\* Significantly different from the negative control group  $p \leq 0.05$

Measurement of insulin is very important to do in this study, because the value of insulin describes the condition of pancreatic  $\beta$  cells. Insulin functions as a medium that converts glucose into energy. When the amount of insulin is not normal for example it decreases, it will disrupt this system so that the glucose will increase in number in the blood.

The highest results of insulin values in this study were at an extract dose of 500 mg/kg BW, which was 0.839  $\mu\text{g/mL}$ . In this study the greater the dose given, the higher the insulin level. This is related to the mechanism of action of Flavonoids namely inhibition of phosphodiesterase so that the level of cAMP in pancreatic  $\beta$  cells increases which will stimulate insulin secretion through the Ca pathway. Insulin levels obtained at a dose of 500 mg / kg BB are not too far from the value of glibenclamide, which is 0.902  $\mu\text{g/mL}$ . But even so in this study the insulin level of the glibenclamide group was higher when compared to other groups. The mechanism of action of glibenclamide is to stimulate insulin secretion from the pancreatic Langerhans  $\beta$  cell granules. Its stimulation is through its interaction with ATP-sensitive K channel on the membrane of  $\beta$  cells which results in Ca depolarization. With the formation of a Ca channel,  $\text{Ca}^{++}$  ions will enter  $\beta$  cells, stimulating insulin-containing granules and insulin secretion will occur with an amount equivalent to the C-peptide<sup>12-14</sup>.

Streptozotocin administration to rats increased blood glucose and decreased insulin and C- peptide levels. Rutin- treated streptozotocin- diabetic rats exhibited a decrease in plasma glucose and an increase in insulin and C- peptide levels. Rutin by its ability to scavenge free radicals and to inhibit lipid peroxidation, prevents streptozotocin- induced oxidative stress and protects  $\beta$ - cells resulting in increased insulin secretion and decreased blood glucose levels. In this context, previous research have shown that quercetin, the aglycone of rutin decreased blood glucose concentration and increased insulin release in streptozotocin- induced diabetic rats. In streptozotocin- induced diabetic rats, quercetin protected pancreatic  $\beta$ - cells by decreasing oxidative stress and preserving pancreatic  $\beta$ - cell integrity. Increased insulin levels could also be due to the stimulatory effect of rutin, thereby potentiating the existing  $\beta$ - cells of the islets of Langerhans in diabetic rats<sup>15-18</sup>.

## CONCLUSION

Based on the results obtained in this study it can be concluded that the ethanol extract of the leaves of *Muntingia calabura* can reduce blood sugar levels in rats induced by streptozotocin and can increase the value of insulin.

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