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ANTIDIABETIC EFFECT OF COMBINED *Muntingia calabura* L. LEAF EXTRACT AND METFORMIN ON RATS

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ABSTRACT

Background: Diabetes mellitus is a disease that causes blood glucose levels to increase. There are several therapies that can be done to reduce blood glucose levels in diabetes such as metformin, biguanides medicine and kersen (*Muntingia calabura* L.) leaves as an alternative. Treatment with combination of both is expected to further reduce blood glucose levels. This study aims to measure the effectiveness of the combination of metformin and *Muntingia calabura* L. leaf extract on blood glucose levels.

Methods: This was an experimental research with pretest-posttest control group. The samples were 24 Sprague-Dawley rats divided into 4 groups, first group (K1) negative control was given only aquadest, second group (K2) positive control (+) was given 45 mg/kgBW metformin, third group (K3) was treated with 300 mg/kgBW *Muntingia calabura* leaf extract, and the fourth group (K4) was given the combination of 300 mg/kgBW *Muntingia calabura* leaf extract and 45 mg/kgBW metformin. Blood glucose levels were measured and analyzed using paired t-test and one-way ANOVA.

Results: The combination of 300 mg/kgBW of *Muntingia calabura* extract and 45 mg/kgBB of metformin reduces 131.77 ± 3.57 mg/dl of blood glucose levels ($p < 0.05$). This result is better than only metformin (92.68 ± 3.10 mg/dl) or only *Muntingia calabura* leaf extract (91.70 ± 4.40 mg/dl). There is a possibility that the synergistic effect of *Muntingia calabura* leaf extract and metformin caused the increased effectivity in reducing blood sugar level.

Conclusion: The combination of *Muntingia calabura* and metformin is more effective in reducing blood glucose levels compared to a single dose of metformin or *Muntingia calabura* L. extract alone.

Keywords: Diabetes mellitus, *M. calabura* leaf extract, blood sugar levels.

INTRODUCTION

Diabetes mellitus is one of the non-communicable diseases that can occur due to damage the pancreatic beta cells, insensitive insulin receptors or both. Diabetes mellitus is one of the non-communicable diseases that can occur due to damage the pancreatic beta cells, insensitive insulin receptors or both. This disease is characterized by increased blood sugar which can cause various kinds of complications such as diabetic ulcer, diabetic ketoacidosis, impaired renal function, diabetic retinopathy, heart disorders, and even death [1]. Data obtained by IDF (International Diabetes Federation) in 2017 show that there are 425 million people in the world suffering diabetes and is expected to increase up to 48% or 629 million people who will suffer from diabetes 2045 [2]. While, Indonesia was ranked 7th in the world in 2015 along with China, India, the United States, Brazil, Russia and Mexico. The results of Basic Health Research (RISKESDAS)

in 2018 found that the prevalence of diabetes in Indonesia tended to increase from 6.9% in 2013 to 8.5% in 2018 [3,4].

Diabetes generally occurs because of an unhealthy lifestyle. The case commonly found is type 2 diabetes or also called NIDDM (Non-Insulin Dependent Diabetes Mellitus). This disease requires special treatment to control blood sugar levels through diet, exercise, and medication. Treatment of diabetes is an oral antidiabetic medicine, one of which is metformin which belongs to the biguanid group [5]. This medicine is used as the first line in the treatment of diabetes and can reduce blood glucose levels. The use of metformin aims to improve impaired glucose uptake of peripheral tissue. Treatment using metformin has a deficiency in type 2 diabetes in the presence of insulin deficiency. Insulin deficiency is caused by damage of the pancreatic beta cells, so that the produced insulin is not adequate to transport sugar from the blood to the tissues. Therefore, metformin is not effective for the treatment of type 2 diabetes with insulin deficiency [6]. In addition to using medicine, people generally believe alternative/traditional medicine, one of which is Kersen leaf (*Muntingia calabura* L) which is believed that it has the effect of reducing hyperglycemia [7,8]. *Muntingia calabura* leaves contain groups of compounds or lignans including flavonoids, tannins, triterpene, saponins, and polyphenols [9]. Flavonoid compounds consist of flavones and flavan which show antioxidative activity. The examples of flavonol compounds are camphor, quercetin and myricetin. The flavonol compound that is thought to have activity in reducing blood glucose levels is quercetin [10]. In reducing blood glucose levels, quercetin keeps pancreatic β cells working normally by preventing oxidative injury and cell death [10,11].

Previous research shows that *Muntingia calabura* L extract is able to reduce blood glucose levels since the extract has an effect on pancreatic β cell regeneration [8]. Therefore, this study aims to compare the effectiveness of antidiabetic in metformin, extract of *Muntingia calabura* leaf and its combinations.

METHODS

This study uses experimental research design with pretest and posttest control group. It was conducted at the Food and Nutrition Laboratory of Gajah Mada University, Yogyakarta, especially to make *Muntingia calabura* extract and measure blood sugar levels. This research has obtained Ethical clearance with No.41/EC/FK/XI/2018 on 15 November 2018 by the Ethics Commission of the Faculty of Medicine, Universitas Swadaya Gunung Jati.

Animal protocol

24 Sprague Dawley rats (aged 3 months) with a weight of 200-250 grams which looked healthy and actively moved were randomly divided into 4 groups. The control group (-) was given aquades, the control group K2 (+) was given metformin 45 mg/kgBB, the K3 group treatment 1 was given 300 mg/kgBB *Muntingia calabura* leaf extract, and K4 treatment group 2 was given *Muntingia calabura* extract of 300 mg/kg combined with metformin of 45 mg/kgBB. Rats were adapted for 7 days (grounded separately, given standard feed and drinks in ad libitum). Afterwards, the rats were induced with alloxan 150 mg/KgBB, then the treatments were conducted. All treatments were given once a day in the morning.

Extraction of *Muntingia Calabura*

One kilogram of *Muntingia calabura* leaves were sorted out washed thoroughly from soil and dirt, and dried in an oven at 40°C for one hour. The dried leaves were mashed using a grinder and the leaves were ready to be extracted. Extracted leaves using the maceration method was then put into a tightly closed container and protected from light and added with 70% ethanol until it was perfectly submerged. The marinade was

then filtered and evaporated with a rotary evaporator so that the thick extract of *Muntingia calabura* was obtained.

Data Measurement

Blood sugar levels were measured in the morning before feeding and carried out 3 times before alloxan was induced. After 5 days of alloxan induction (pretest), hyperglycemia mice were found. After 15 days of treatment (posttest), mice were first anesthetized with ketamine 0.25 mg intramuscularly. Afterwards, rat blood was taken through the orbital sinus for 1-2 ml using a hematocrit micropipette. The blood that comes out was then accommodated in the Eppendorf given EDTA. The collected blood was then centrifuged for 15 minutes until blood plasma was separated and blood sugar levels were measured using a sp-300 spectrophotometer.

The collected data obtained were then processed using a computer program. The statistical test used was Shapiro-Wilk because the number of subjects was less than 50. The test was to see whether the data distribution was normal and homogeneous. The result shows that the data distribution was normal and homogeneous. After that, it was followed by a paired T-test to see the difference in the pretest and posttest. Finally, Anova with Post-Hoc Bonferroni test was conducted to see the difference in average glucose change for each group.

RESULTS

Blood sugar levels in the sample show changes at the pretest and posttest. The results of blood glucose examination can be seen in the following table

Table 1. Mean decrease of blood glucose levels

Group	N	Pretest ± SD (mg/dl)	Posttest ± SD (mg/dl)	Delta ± SD (mg/dl)	p-value
K1	6	214.48 ± 3.65	215.97 ± 3.06	-1.50 ± 1.24	0.032 ^a
K2	6	208.04 ± 4.55	115.36 ± 3.57	92.68 ± 3.10	0.000 ^b
K3	6	216.47 ± 4.09	124.78 ± 3.07	91.70 ± 4.40	0.000 ^b
K4	6	218.85 ± 2.57	87.08 ± 1.56	131.77 ± 3.57	0.000 ^b

^a means significant increase

^b means significant decrease

The highest decrease in blood glucose levels was found in K4 i.e. 131.77 ± 3.57 mg/dl, followed by K2 at 92.68 ± 3.10 mg/dl and K3 at 91.70 ± 4.40 mg/dl. Decreases in blood glucose levels can be found in all groups except K1.

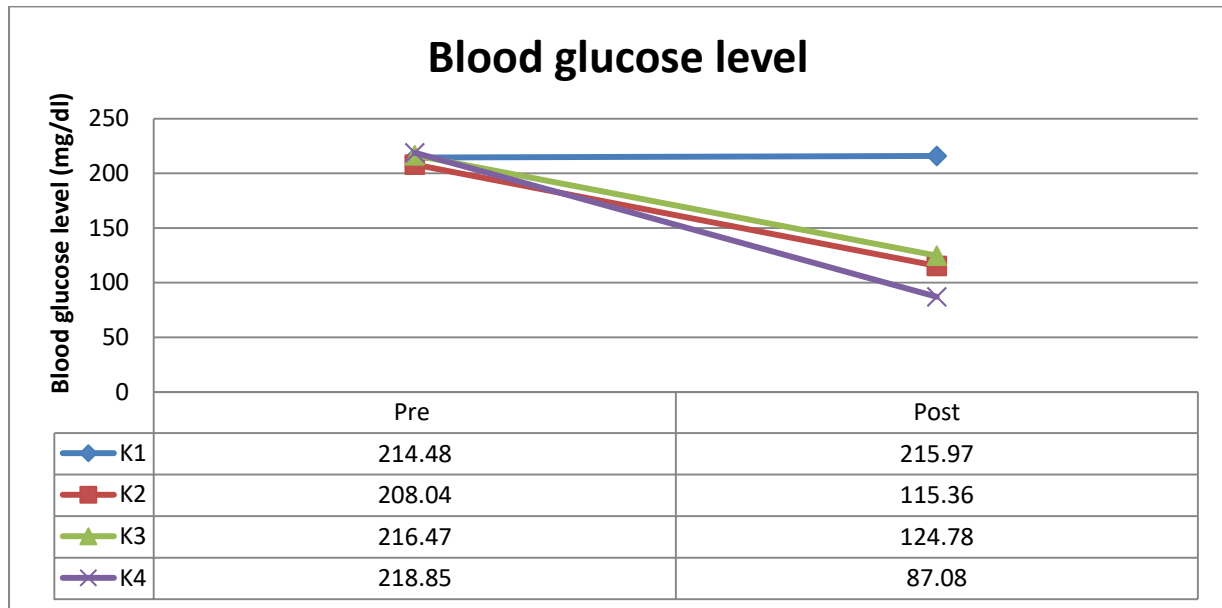


Fig 1. Mean decrease glucose level. A. graphic showing the mean of blood glucose levels in all groups (pretest and posttest) B. mean decrease of blood glucose level of each group. K1 = negative control, K2 = positive control (metformin 45 mg/Kg bw), K3 = kersen leaf extract (300 mg /Kg bw), K4 = combination (metformin 45 mg/Kg bw + kersen leaf extract 300 mg/Kg bw).

Data were normally distributed and homogeneous in delta groups. One-way ANOVA test was conducted to compare changes in blood sugar levels from each group. The results show that delta blood sugar levels differ significantly ($p = 0,000$). The results of the ANOVA test were followed by post hoc Bonferroni test because the data variance was normal, as listed in Table 2.

Table 2. Benferoni post hoc analysis of blood sugar levels delta

Groups	K1	K2	K3	K4
K1	#			
K2	0.000	#		
K3	0.000	1.000	#	
K4	0.000	0.000	0.000	#

The table above shows that K1 compared to K2, K3 and K4 are different in decreasing blood sugar levels ($p < 0.05$). K3 compared to K1 is different in reducing blood sugar levels ($p < 0, .05$). K3 and K2 show no significant differences ($p = 1$). K4 against K1, K2 and K3 also showed a significant difference ($p < 0.05$).

DISCUSSION

This study was carried out in 15 days of treatment showing that *Muntingia calabura* and metformin leaf extracts were effective in reducing blood glucose levels in alloxan-induced mice, i.e. 87.08 mg/dl. The positive control group (K2) shows blood glucose level reduction. It is because metformin has a way of reducing glucose production in the liver and increasing the sensitivity of muscle tissue and adipose to insulin. This effect occurs because of the presence of cell kinase activity (AMP-activated protein kinase) [5.6]. *Muntingia calabura* extract group (K3) shows blood glucose level reduction where in *Muntingia calabura* there is a content that focuses on inhibiting cell damage due to oxidative stress [9].

Administering metformin of 45 mg/kgBB combined with *Muntingia calabura* extract of 300 mg/kgBB (K4) shows effective results with glucose reduction values reaching 131.77 ± 3.57 mg/dl compared to the group of metformin of 45 mg/kgBB (K2) only reaching glucose reduction of 92.68 ± 3.10 mg/dl and *Muntingia calabura* extract of 300 mg/kgBB (K3) only reached a glucose reduction of 91.70 ± 4.40 mg/dl with p-value of <0.05 . This happened because the combination of metformin and *Muntingia Calabura* extract had a different mechanism of action which helped reduce glucose levels effectively.

Metformin is a medicine that has a way of reducing glucose production and increases the sensitivity of muscle tissue and adipose to insulin. Stimulation of muscle cells and adipose tissue will affect glucose regulation and transport. Glucose will enter through glucose transporters (GLUT) which can diffuse glucose into cells, i.e. Na^+ independent. Insulin stimulates glucose transport by inducing energy to translate GLUT 4 and GLUT 1 from intracellular vesicles to the plasma membrane, thereby increasing glucose uptake in peripheral tissue [5,6]. Whereas, *Muntingia calabura* leaf extract has flavonoid compounds [8].

Flavonoids are one of the compounds contained in the leaves of *Muntingia calabura*. Flavonoids can protect the body from Reactive Oxygen Species (ROS) follow-up by blocking propagation reactions and stimulating the formation of endogenous antioxidants such as Glutathione Peroxidase (GPx), superoxide dismutase (SOD), and Catalase (CAT) and reducing Malondialdehyde (MDA) [12–14]. Flavonoids are divided into several types consisting of flavones, flavanones, and flavonols. The examples of flavonol compounds include camphor, quercetin and myricetin [15]. The flavonol compound which is thought to have activity in reducing blood glucose levels is quercetin [10]. Quercetin prevents oxidative injury and cell death through several mechanisms including capturing oxygen radicals by donating H^+ ions. Thus, radical reactions that will damage the normal cell deoxyribonucleic acid (DNA) around can be stopped [16]. Quercetin immunohistochemical examination also shows that quercetin can improve β cell degeneration. It increases adequate insulin secretion and ultimately affects the process of transporting glucose in the blood can be channeled to peripheral tissues [11–13,17].

This research has been carried out, but the authors feel that they still have limitations, i.e. this research does not describe microscopically in the pancreatic and liver organs to assess whether there is an effect of the combination.

CONCLUSION

This study shows that administering *Muntingia calabura* leaf extract of 300 mg/KgB + Metformin of 45 mg/KgBB is more effective in reducing glucose compared to metformin and *Muntingia calabura* monotherapy. Research can be used as better therapy for diabetes. For further research, it is suggested to assess microscopic pancreatic β and liver cells against the effects of these combinations.

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