BLOOD GLUCOSE REDUCTION IN MICE (*Mus musculus*) RESULTING FROM THE ADMINISTRATION OF *PARE* (*Momordica charantia*) FRUIT FLESH JUICE

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ABSTRAK

*Diabetes mellitus adalah penyakit degeneratif yang ditandai kadar glukosa darah melebihi nilai normal sehingga tidak dapat digunakan oleh tubuh. Obat antidiabetes, seperti metformin, merupakan terapi yang umum digunakan dewasa ini, namun terkait dengan diare, dispepsia, dan asidosis laktat. Buah pare (Momordica charantia) mengandung charantin yang memiliki fungsi mirip dengan Activator Adenosin 5-monophosphate activated protein kinase (AMPK) dan glukosa transporter tipe 4 (GLUT4), sehingga dapat menjadi solusi alternatif untuk mengurangi konsentrasi glukosa darah. Tujuan dari penelitian ini adalah untuk membandingkan efek buah pare (Momordica charantia) dan metformin untuk mengurangi konsentrasi glukosa darah pada mencit (Mus musculus L.). Penelitian ini menggunakan 35 mencit jantan (Mus musculus L.) berusia 2 sampai 3 bulan dan beratnya 20 sampai 30 gram. Penelitian ini menggunakan desain complete randomize controlled post test. Mencit dikelompokkan menjadi 7 kelompok. Kelompok I diperlakukan dengan aquades (kontrol); Kelompok II dengan Metformin 0,065 mg/g BB; Kelompok III dengan Metformin 0,0325 mg/g BB; Kelompok IV dengan Metformin 0.013 mg/g BB; Kelompok V dengan jus pare dalam konsentrasi 100%; kelompok VI dengan jus pare dalam konsentrasi 50%; dan kelompok VII dengan jus pare dalam konsentrasi 25%. Glukosa darah diukur dengan memotong 1 cm segmen ujung ekor dengan pisau bedah dan diukur dengan glucosemeter. Hasil penelitian ini menunjukkan tidak ada perbedaan yang signifikan antara varians (p> 0,05), yang mungkin timbul akibat cara mengekstrak charantin dari pare.**(FMI 2012;48:43-49)***

Kata kunci: *diabetes mellitus, metformin, buah pare, kadar gula darah*

ABSTRACT

*Diabetes mellitus is a degenerative disease characterized by blood glucose levels that exceed the normal values for blood glucose that cannot be used by the body. Antidiabetics, such as Metformin, is a current of therapy, but it is associated with diarrhea, dyspepsia, and lactic acidosis. Pare or bitter melon (Momordica charantia) contains charantin that has a function similar to Activator Adenosin 5-monophosphate activated protein kinase (AMPK) and glucose transporter type 4 (GLUT4), so it can be an alternative solutions to reduce blood glucose concentration. The purpose of this study was to compare the fruit pare (Momordica charantia) and metformin effects to reduce concentration of blood glucose in mice (Mus musculus L.). This research used 35 male mice (Mus musculus L.) aged 2 until 3 months and weighed 20 until 30 grams. This study used complete randomize controlled post test design. Mice were grouped into 7 groups. Group I was treated with distilled water (control); group II with Metformin 0.065 mg/g BW; group III with Metformin 0.0325 mg/g BW; group IV with Metformin 0.013 mg/g BW; group V was with Bitter Melon juice in a concentrate of 100%; group VI with Bitter Melon juice in a concentrate of 50%; and group VII with bitter melon juice in a concentrate of 25%. Blood glucose was measured by cutting off 1 cm segment of the tail tip with a scalpel and measured with glucosemeter. The result of this study showed there was no significant difference between the variance (p > 0.05), which might result from the way to extract charantin from the bitter melon. **(FMI 2012;48:43-49)***

Keywords: *diabetes mellitus, metformin, bitter melon, blood glucose concentration.*

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INTRODUCTION

Diabetes mellitus is a degenerative disease characterized by blood glucose levels that exceed normal values for blood glucose can not be used by the body due to insulin deficiency or insulin work uninterrupted. According to data from WHO (1993), Indonesia holds rank number 4 in Diabetes Mellitus patients in the world. Various epidemiological studies indicate a trend of increasing incidence rate and prevalence of type 2 DM in various parts of the world. WHO predicts an increase in the number of diabetics is large enough for years to come. For Indonesia, the WHO predicts rise in the number of patients from 8.4 million in 2000 to around 21.3 million in 2030. Therefore, diabetes mellitus has been one of the major threats to public health in Indonesia today (PERKENI 2006).

Drugs that are used as antidiabetic far are drugs that can trigger insulin secretion such as sulfonylureas and Glinid; increase the sensitivity to insulin Tiazolindindion crate; Metformin inhibits gluconeogenesis such as; and inhibit alpha-glucosidase. Each of these classes of drugs have side effects, ranging from mild to severe, such as administration of sulfonylureas, Glinid, and Insulin side effects gain weight and also cause hypoglycemia; metformin produce side effects such as diarrhea, dyspepsia, and lactic acidosis; administration of drug, a drug that inhibits alpha-glucosidase may cause flatulence. Therefore, it is necessary to develop other means of prevention that have minimal side effects and has positive results to lower blood glucose (PERKENI 2006)

Treatment with natural ingredients can be antihyperglycemic choice of drug development, especially for the people in Indonesia. This is supported by the fact that Indonesia is a country with natural resources (SDA) are abundant and diverse, therefore the people of Indonesia have facilities for obtaining natural ingredients. *Pare* (*Momordica charantia*) is a fruit that is often used by the people of Indonesia. Bitter melon/bitter melon is a tropical vine that the name originated from tropical Asia, especially the western regions of India, namely Assam and Burma. Peria grow well in lowlands and can be found growing wild in wastelands, fields, cultivated, or planted in the garden with a propagated on the fence. Indonesia is a tropical country so *pare* plants can grow well in Indonesia and readily available in Indonesia. *Pare* as antihyperglycemia is supported by the contents that are in *pare* itself (Xie et al 2011)

Pare frequently used by the community, especially people in China and other Asian countries as an herbal remedy believed to have the effect of lowering blood sugar. Bitter melon fruit contains several compounds like charantin, vicine and p-insulin (polypeptide). According to research conducted by Patel et al (2006), charantin have antihyperglycemic effects. This is supported by other studies conducted by Xie et al (2011), stating that such compounds charantin, vicine and p-insulin can cause inhibitory mechanism to the activity of Protein Tyrosine posphatase 1B (PTP1B), activates adenosine monophosphate activated protein kinase 5-(AMPK), increases the expression of glucose transporter type 4 (GLUT4), repair of pancreatic beta cells and have effects such as insulin, that all these things will result in antihyperglycemic effects.

Based on these descriptions, researchers conducted a study to determine the effectiveness of antihyperglycemic at *pare* (*Momordica charantia*) in lowering blood glucose levels in experimental animals, namely mice (*Mus musculus*) by measuring glucose levels to be com*pare*d at various concentrations. The treatment aims to find the dose that is effective in decreasing blood glucose in mice (*Mus musculus*). The purpose of this study was to determine whether the meat juice *pare* (*Momordica charantia*) has the effect of decreasing blood glucose in mice (*Mus musculus*).

MATERIALS AND METHODS

This research is an experimental laboratory with the-post test only control group design and samples are taken by simple random sampling method. As experimental animals we used mice (*Mus musculus*) of the Laboratory of Pharmacology of the Faculty of Medicine, University of Airlangga male sex weighing 20-30 g and aged 2-3 months (Yuliastuti et al 2000). Other requirements include the condition of healthy mice and no abnormalities were seen on the body of mice. Before treated, made adaptations to the environment for at least one week and guarded condition (Jaya et al 2009). According to WHO criteria, a minimum of five mice per treatment group (WHO 1993). The study was conducted in August 2011 at the Laboratory of Pharmacology of the Faculty of Medicine, University of Airlangga.

The whole animal experiments were conducted 1 week of adjustment to the environment and guarded condition, as well as food and drink noted ad libitum. Mice were placed in a plastic tub the size of 40 x 30 cm2 are given chaff, each tub of 5 mice. In this study, metformin will be given in 3 doses of 500 mg, 250 mg, and 100 mg. Based on the conversion factor, obtained dose for mice is 500mg x 0.0026 = 1.3 mg/20g BB mice, 0.65 mg/20 g mice BB, and 0.26 mg/20 g BW mice. Metformin HCl which then will be dissolved into distilled water that will be given orally using oral sonde.

Fruit Juice meat *Pare* (*Momordica charantia*) for this study were made at the Laboratory of Pharmacy Faculty of Medicine, University of Airlangga. To make preparation of meat juice *pare* (*Momordica charantia*) 100%: 2 ml supernatant was added to 8 ml of distilled water. To make preparation of meat juice *pare* (*Momordica charantia*) 50% of the supernatant of 1 ml of distilled water added to a volume of up to 8 ml. To make preparation of meat juice *pare* (*Momordica charantia*) 25% of the supernatant was added 0.5 ml of distilled water to a volume of up to 8 ml.

Mice were fasted ± 18 hours before treated (Jaya et al, 2009) to avoid the influence of diet on mice, but the drink still given. Body weight of each mouse was

weighed with scales and whole animal studies animals were randomly divided into five groups, each group consisted of 5 mice (Miya 1973). Thereafter, each mice were treated in accordance with the group, namely: Group I (control), consisting of 5 mice were given distilled water treated per sonde as 0:02 ml per gram of body weight. After that, wait for 30 minutes then examined blood glucose levels. Thereafter, the 5 mice were given as much as 97.5% per sonde 0:01 ml/g body weight. Blood glucose tests performed after 30, 60, 120, 180 minutes.

As a comparison group is a group II to IV. In group II to IV, in each group of 5 mice treated with metformin HCl treated with a solution of 1.3 mg/20 g BW (group II), 0.65 mg/20 g BW (group III), and 0.26 mg/20 g BW (group IV) per sonde. After that, wait for 30 minutes then examined blood glucose levels. Threreafter, the 5 mice in each group were given as much as 97.5% per sonde 0:01 ml/g body weight. Blood glucose tests performed after 30, 60, 120, 180 minutes.

The treatment group was the group V to VII. In each group of 5 mice given untreated meat juice *pare* (*Momordica charantia*) 100% (group V), 50% (group VI) and 25% (group VII) of 0:02 ml per gram of body weight. After that, wait for 30 minutes then examined blood glucose levels. Thereafter, the 5 mice were given as much as 97.5% per sonde 0:01 ml/g body weight. Blood glucose tests performed after 30, 60, 120, 180 minutes. Blood sugar levels of mice in the control group and each treatment group were com*pare*d statistically with a different test.

RESULTS

Prior to the study, we conducted environmental adaptation to the mice for about a week and fasted ± 18 hours. In the initial phase, mice body weight measurements, measurement of fasting blood glucose levels, and after the mice were treated in accordance with their respective groups: the first group was given distilled water as a control, a second group was given metformin 0.065 mg/g body weight, a third group was given metformin 0.0325 mg/g body weight, the fourth group was given metformin 0.013 mg/g body weight, the fifth group were given meat juice *pare* with a 100% level, the sixth group was given bitter melon fruit with meat juice content of 50%, and seven groups were given juice *pare* the meat content of 25%, 30 minutes and then measured their blood sugar levels are referred to as blood sugar levels to 0 minutes, and after oral glucose was induced as much as 0.00975 g/g body weight. Measurement of blood glucose levels back carried 30, 60, and 120 min after oral glucose administration. Sampling of blood glucose levels was done by cutting the tip of little mice by using scissors and measured blood glucose levels.

The results of measurements of blood glucose levels of mice based on table 5.1 and analyzed with the aim to com*pare* the meat juices work *pare* (*Momordica charantia*) in lowering blood glucose levels when com*pare*d with controls. To assist in this analysis, we used the test analysis of variants (ANOVA) to determine the statistical difference and use the Curve Dose Response for the presence of pharmacological effects. Before analyzing the ANOVA test, the data must be tested with the Kolmogorov-Smirnov normality test which aims to determine whether the data is normally distributed or not. After the normality test, the data were analyzed through the data homogeneity test (test Analysis of Variant/Anova) and the follow-up test Post Hoc Multiple Comparison LSD (Least Significance Difference).

Giving *Pare* 100% juice showed a decrease in blood glucose between the granting of the greatest *Pare* juice 50% and 25%, and also decreased the mean blood glucose when com*pare*d with the group with the provision of bitter melon juice other concentrations. Based on the average data of blood glucose levels, the effect of bitter melon juice 100% is almost the same as the effect of Metformin HCl 0,065mg/GBW. Based on tests for normality using the Kolmogorov-Smirnov test, the significance value was (p) $\geq$ 0.05 for all data, which means that the data are normally distributed. When the distribution and ratio data were normal, researchers can still use the method of ANOVA.

Test analysis of variants (ANOVA) for the measurement of blood glucose in the blood glucose increment tables minute 30 'and 0', the value of significance (p) = 0322, which means a homogeneous variants of 7 treatments. Then in the measurement of blood glucose in the blood glucose increment table 60 minutes 'and 0', the value of significance (p) = 0572, which means a homogeneous variants of 7 treatments. And also the measurement of blood glucose in the blood glucose increment table min 120 'and 0', the value of significance (p) = 0372, which means a homogeneous variants of 7 treatments. ANOVA test results also showed a significance value (p) over α (α = 0.05), namely 0188, 0344, 0142. Significance value (p) is over α. This indicates that there is no significant difference between each data treatment groups.

Table 1. Results of measurement of blood glucose levels in mice

Time	K1 (control)	KM1 (Met 0.065 mg/gBW)	KM2 (Met 0.0325 mg/gBW)	KM3 (Met 0.013 mg/gBW)	KP 1 (Bitter melon juice100%	KP 2 (Bitter melon juice 50%	KP3 (Bitter melon juice 25%
Fasting	67	84	95	115	90	63	76
	89	157	75	115	123	75	135
	102	95	121	121	10	95	117
	93	103	86	123	7	68	124
	89	76	75	118	115	86	126
Mean	88	103	90.4	118.4	69	77.4	115.6
Blood glucose	49	75	84	119	93	40	122
minute 0	94	162	63	102	104	68	138
	123	85	118	118	105	93	122
	86	93	93	122	104	45	140
	94	49	49	122	84	80	45
Mean	89.2	92.8	81.4	116.6	98	65.2	113.4
30 minutes	284	331	264	298	236	231	236
after forced	273	278	200	417	220	288	339
ingestion	416	331	211	349	171	358	323
	259	460	122	334	388	271	359
	342	147	298	340	211	358	339
Mean	314.8	309.4	219	347.6	245.2	301.2	319.2
60 minutes	193	206	183	225	170	162	249
after glucose	241	211	211	261	289	280	211
forced	336	225	261	236	243	302	225
ingestion	147	354	193	289	158	197	200
	299	105	264	183	249	280	162
Mean	243.2	220.2	222.4	238.8	221.8	244.2	209.4
120 minutes	142	85	113	113	114	132	153
after glucose	162	163	99	142	171	113	118
forced	153	133	113	122	109	122	104
ingestion	123	122	108	132	103	132	183
	166	84	132	113	89	104	153
Mean	149.2	117.4	113	124.4	117.2	120.6	142.2

Table 2. Mean blood glucose levels every minute

	p	0	30	60	120
Control	88	89.2	314.8	243.2	149.2
Metformin1	103	92.8	309.4	220.2	117.4
Metformin2	90.4	81.4	219	222.4	113
Metformin3	118.4	116.6	347.6	237.6	124.4
*Pare*1	106.2	98	245.2	221.8	117.2
*Pare*2	77.4	65.2	310.2	244.2	120.6
*Pare*3	115.6	113.4	319.2	209.4	142.2

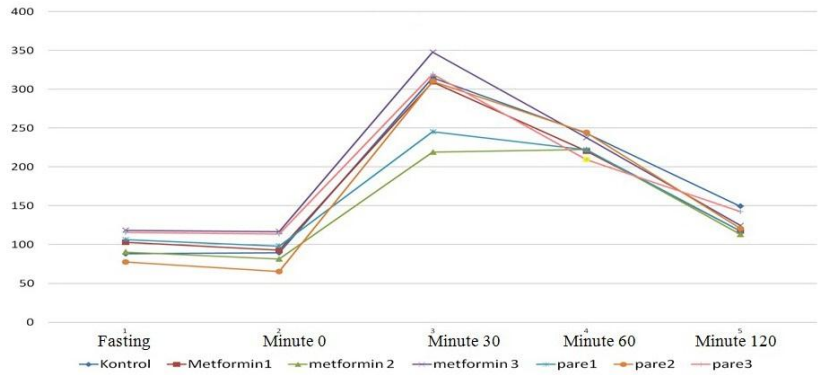

Figure 1. Blood glucose levels every minute

Table 3. Decrease in Blood Glucose

Groups	60	120
Control	22.7%	52.60%
Metformin1	28.3%	60.99%
Metformin2	-1.08%	33.67%
Metformin3	34.56%	70.90%
*Pare*1	7.43%	40.66%
*Pare*2	18.11%	57.37%
*Pare*3	34.88%	56.23%

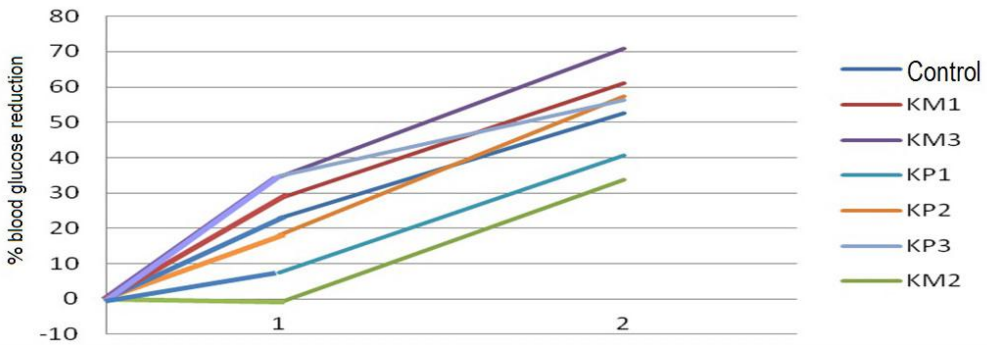

Figure 2. Dose response curve

At Dose response curves obtained pharmacological effect increases with increasing concentration *pare* the meat juices from 25% to 50%.

DISCUSSION

This study aimed to obtain data on the benefits of meat juice *pare* (*Momordica charantia*) in lowering blood glucose levels, using mice as experimental animals, as well as test doses of bitter melon juice pulp (*Momordica charantia*) in lowering blood glucose levels. It is intended to provide scientific information about the meat juices *pare* (*Momordica charantia*) against diabetes.

Bitter melon fruit contains several compounds like charantin, vicine and p-insulin (polypeptide). These compounds have been declared by Xie et al (2011) that compounds such as charantin, vicine and p-insulin can cause inhibitory mechanism the activity of Protein Tyrosine posphatase 1B (PTP1B), which will result in increased glycogen synthase, activates adenosine monophosphate activated protein 5- kinase (AMPK) and increases the expression of glucose transporter type 4 (GLUT4), which causes the glucose uptake into the muscle increased, improving the beta cells of the pancreas and has effects such as insulin, that all these things will result in antihyperglycemic effects.

The population was mice from the same colony, aged 2-3 months, weighing 20-30 grams. Other requirements

include the condition of healthy mice and no abnormalities were seen on the body of mice. Before treated, made adaptations to the environment for at least one week and guarded condition. Mice were also fasted ± 18 hours before treatment to avoid the influence of food in mice. In this study, the number of samples from each group was 5 male mice. The overall number of samples required 35 mice.

Before starting the experiment, the weights of all the treatment groups of mice were weighed. Then, fasting blood glucose of mice was measured first. Furthermore, all experimental groups of mice were treated in accordance with the group, the first group as a control, a second group was given Metformin HCl 0,065mg/GBW, a third group was given metformin HCl 0.0325 mg/GBW, the fourth group was given 0,016 mg metformin HCl/GBW, the fifth group, sixth and seventh *pare* given meat juice with a concentration of 100%, 50%, and 25%. 30 minutes later each group measured their blood glucose levels and blood glucose is called min-0. Once the blood glucose is measured, each group was given an oral glucose of 9.75 mg/g body weight per sonde and re-measured blood glucose after 30, 60, 120 minutes later. The data obtained by the fasting blood glucose and postprandial glucose levels of mice. The data were analyzed descriptively to determine the mean and standard deviation. Furthermore, the normality test with the Kolmogorov-Smirnov test, a score of significance (p) ≥ 0.05 for all data, which means that the data are normally distributed.

Then test the homogeneity of the data, seen in the ANOVA test. Based on the analysis of test analysis of variants (ANOVA), Based on the analysis conducted, it can be concluded that the administration of distilled water, Metformin HCl 1.3 mg/20g BW, Metformin HCl 0.65 mg/20g BW, Metformin HCl 0.26 mg/BW 20g, bitter melon fruit juice 100% meat, bitter melon fruit juice 50% meat, meat juice *pare* 25% did not show significant results in a decrease in blood glucose. However, it appears the decrease in blood glucose was greater in the treatment com*pare*d to the control. The largest decrease is given by the 100% juice *pare* the decline effect is almost the same as Metformin HCl 0.65 mg/20g body weight. Statistical test results are not meaningful because the data range is too wide. Range of data that is too wide can be seen in the large standard deviation values in each group, especially in the data difference in blood glucose levels 30 and 0; and the data is the difference in blood glucose levels 60 and 0. This wide range may be due. Factors glucose meters are less accurate when used repeatedly, which in this experiment are likely to show less accurate results in some mice.
Internal factors of mice are unknown and can not be controlled, such as a genetic disorder or disease, may also affect the results of the study. For example, mice that are used can have abnormalities of glucose metabolism. It can also be due to the body's response to different mice on treatment administration.

Another experiment on antihyperglycemic activity is "Traditional Chinese Medicines in Treatment of Patients with Type 2 Diabetes Mellitus" by Xie et al (2011). In a study that used ethanol extract of bitter melon fruit flesh and the results more meaningful decline when com*pare*d with the current study only uses the juice of bitter melon fruit flesh. In another study by Lola et al (2008), used *pare* juice with garlic and combined with the research provides meaningful results to a decrease in blood glucose, which may also have the effect of garlic antihyperglycemic thus produced a greater decrease than the provision of pulpy juice *pare* only.

CONCLUSION

No effect of meat juice *pare* (*Momordica charantia*) in various concentrations to significantly decrease blood glucose in mice (*Mus musculus L.*). But still found a decrease in blood glucose. This is due to the dose given is not optimal, the lack of dose variation, the lack of experimental animals. Increased pharmacological effects obtained from concentrations of 25% to 50%.

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