GUAVA (*Psidium guajava L.*) JUICE IS EFFECTIVE IN LOWERING BLOOD GLUCOSE LEVEL IN MICE (*Mus musculus*)

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ABSTRAK

Asia Tenggara, termasuk Indonesia, memiliki kebiasaan diet karbohidrat tinggi, yang akan menyebabkan konsentrasi tinggi glukosa darah. Glukosa darah yang terus-menerus tinggi dapat menyebabkan diabetes mellitus (DM), yang sering disebabkan oleh faktor genetik dan lingkungan. Saat ini, terapi DM meliputi latihan fisik, perencanaan diet, dan konsumsi obat-obatan. Obat-obatan yang tersedia sekarang memiliki cukup banyak efek samping dalam penggunaan jangka panjang. Oleh karena itu, kami meneliti terapi alternatif DM menggunakan jus buah jambu biji. Flavonoid (apigenin, tanin, myricetin, quercetin) dalam buah jambu biji diperkirakan menurunkan Blood glucose level. Tujuan dari penelitian ini adalah untuk mengetahui pengaruh Guava Juice biji (Psidium guajava L.) dalam mengurangi glukosa darah pada mencit (Mus musculus) menggunakan Glucose Tolerance Test (GTT). Percobaan dilakukan di Laboratorium Farmakologi, Fakultas Kedokteran. Universitas Airlangga, menggunakan mencit (Mus musculus), yang dibagi menjadi 5 kelompok, masing-masing terdiri dari 7 ekor. Pengukuran Blood glucose level dilakukan dengan memotong ekor tikus dan mengambil darah 0, 60, 120, 150 menit setelah pemberian glukosa. Hasil penelitian menunjukkan bahwa ada perbedaan yang signifikan (sig (p) <0,05) pada penurunan glukosa darah pada kelompok yang diberi Guava Juice konsentrasi 25% dibandingkan dengan kelompok yang menerima Distilled Water, metformin, 100% Guava Juice biji, dan 50% Guava Juice. Kesimpulannya, Guava Juice (Psidium guajava L.) konsentrasi 25% efektif dalam menurunkan Blood glucose level. ***(FMI 2012;48:174-179)***

Kata kunci: *Psidium guajava L. , penurunan gula darah, mencit, Glucose Tolerance Test*

ABSTRACT

Southeast Asia, including Indonesia, has a high carbohydrate diet, which will lead to a high blood glucose concentration. Continuous high blood glucose can lead to diabetes mellitus (DM), which often caused by genetic and environment. Nowadays, DM therapy covers physical exercise, diet planning, and medicine consumption. Available medicines right now have quite a lot side effect in long term usage. Therefore, we studied an alternate therapy of DM using guava fruit juice. Flavonoids (apigenin, tannin, myricetin, quercetin) in guava fruit were estimated to reduce blood glucose level. The objective of this study was to determine blood glucose-reducing effect of guava juice (Psidium guajava L.) in mice (Mus musculus) using Glucose Tolerance Test (GTT). The experiment was done at Pharmacology Laboratory, Medical Faculty. Airlangga University using mice (Mus musculus), which were divided into 5 groups, each consisting of 7 mice. Measurement of blood glucose level was done by cutting mice's tail and taking the blood 0, 60, 120, 150 minutes after glucose loading. Results showed that there were significant difference (sig (p) < 0,05) in reduced blood glucose in group given with 25% concentration of guava juice compared to the groups receiving Distilled Water, metformin, 100% guava juice, 50% guava juice groups. In conclusion, guava (Psidium guajava L.) juice in 25% concentration is effective in lowering blood glucose level. ***(FMI 2012;48:174-179)***

Keywords: *Psidium guajava L. , lowering blood glucose, mice, Glucose Tolerance Test*

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INTRODUCTION

Diet in Southeast Asia, including Indonesia, is generally quite high in carbohydrate content, which is between 70-80%. The carbohydrate source product as whole grains or cereals (wheat and rice), tubers (potatoes, cassava, sweet potato), and sugar (Suhardjo & Kusharto 1992). Blood glucose levels are constantly high will result in insulin resistance, in which the cell's response to insulin reduces and the absorption of glucose from the blood will decrease. If the situation continues, there will be type 2 diabetes mellitus predisposing factors that cause hyperglycemia include hereditary factors and obesity. Genetic and environmental influences contribute equally strong against the onset of diabetes mellitus type 2. People with obesity have a higher risk for diabetes mellitus type 2 because obesity is a risk factor for the development of insulin resistance and

diabetes mellitus type 2. Approximately 90% people with diabetes is type 2 diabetes (Gibney et al, 2005, Marfianti 2009, Mathur 2002).

Diabetes Mellitus (DM) is a group of metabolic diseases with characteristic hyperglycemia that occurs due to abnormal insulin secretion, insulin action or both. DM diagnosis of random blood glucose is over 200 mg/dl, fasting blood glucose more than 126 mg/dl, 2 hours after meals more than 200 mg/dl (Gustaviani 2006, Ligaray 2010). Results of Health Research (Riskesdas) in 2007, found that the proportion of the cause of death due to diabetes in the age group 45-54 years in urban areas was ranked 2nd, namely 14.7%. In rural areas, DM ranks as the 6th disease, comprising 5.8% (Agency for Health Research and Development, 2008). When this has been a lot of therapy to lower blood glucose levels, by adjusting diet, physical exercise, counseling, and medications associated with hyperglycemia such as sulfonylurea class of drugs, glinid, biguanide, alpha-glucosidase inhibitor, a thiazolidinedione. However, modern medicines are often negative side effects such as the occurrence of hyperinsulinemia (excess insulin levels in the blood) and finally low blood glucose levels (hypoglycemia), weight loss, increased blood cholesterol levels, light nausea, loss of appetite, diarrhea, indigestion, and can occur even more severe insulin resistance (Khardori 2011).

Guava fruit contains flavonoids like quercetin, myricetin, apigenin and tannins that contribute to antihyperglycemic activity. The research that has been conducted on onion (Allium ascalonicum) containing quercetin, and avocado seed containing tannin on hyperglycemia mice showed a decrease in blood glucose levels significantly. Research on *Abelmoschus moschatus* containing myricetin on alloxan-induced mice, and studies of celery and leaves *Cephalotaxus sinensis* containing apigenin in rabbits hyperglycemia showed a decrease in blood glucose levels were significantly (Liu et al 2006, Monica, 2006, Li et al 2007, Hapsari 2008, Wulandari 2010).

Based on above explanation, guava (*Psidium guajava L.*.) could be expected to lower blood glucose levels were tested in the form of guava fruit juice in mice with glucose tolerance test with a volume of 97.5% glucose solution giving as much as 0.01ml/gBW mice. The purpose of this study was to analyze the effect of guava (*Psidium guajava L.*) to decrease blood glucose levels.

MATERIALS AND METHODS

This research is an experimental laboratory study design the-post test only control group design and samples are taken by simple random sampling method. As experimental animals we used mice (*Mus musculus*) from the Laboratory of Pharmacology Faculty of Medicine, University of Airlangga male sex weighing 20-30 grams and 2-3 months old. Other requirements include the condition of healthy mice and no abnormalities were seen in the mice body. Before treatment, adaptation to the environment for at least one week and guarded condition (Jaya 2007). The sample size for each treatment is 7. The experiment was conducted in August 2011 in the Laboratory of Pharmacology and Biochemistry Laboratory, Faculty of Medicine, University of Airlangga.

The whole animal experiments were performed 1 week adjustment to the environment and guarded condition, as well as food and drink, we also considered, provided ad libitum. Mice were placed in a plastic tub the size of 40 x 30 cm2 were given chaff, each tub of 7 mice. Metformin hydrochloride dosage in humans is 500 mg. Based on the conversion factor, obtained dose for mice is 500 x 0.0026 = 1.3 mg/20g mice BW = 0.065 mg/gBW mice were then dissolved in distilled water and administered orally using oral sonde. Fruit juice guava (*Psidium guajava L.*) for this study was made at the Laboratory of Pharmacy Faculty of Medicine, University of Airlangga. Fruit juice guava (*Psidium guajava L.*) 100%: made with guava fruit to make juice without water mixture. To make preparations guava fruit juice (*Psidium guajava L.*) 50%, 2x dilution guava juice yield of 100%. To make preparations guava fruit juice (*Psidium guajava L.*) 25% dilution 4x guava juice yield of 100%.

Mice were fasted ± 18 hours before the treated (Jaya 2007) to avoid the effect of food on mice, but the drink still be given. Body weight of each mouse was weighed with scales and whole animal animals were randomly divided into five groups, each group consisting of 7 mice. After that, each mouse was treated in accordance with the group, namely: Group I as a negative control with distilled water provision, group II as a positive control with metformin HCl, group III treatment by administering guava juice content 100%, group IV treatment by administering guava juice content of 50%, and group V treatment by administering juice guava levels of 25%. Seven mice were treated with the appropriate group of 0.1 ml/20 g BW or 0.005 ml/g and 97.5% were given a glucose solution per sonde as 0:01 ml/g body weight. Giving substance treatment and glucose solution must not exceed the maximum limit of 20 grams murine gastric volume of 1 ml. Then do blood glucose tests at minute 0, 60, 120, and150. Blood sugar levels of the mice in the control group and each treatment groups were compared statistically with different test.

RESULTS

Prior to the study, the mice were subjected to environmental adjustment (acclimatization) for about a week, then fasted ± 18 hours. In early stages, measurement of body weight of mice and fasting blood glucose levels were performed, and, thereafter, the mice were treated according to the groups: the first group was given distilled water as a negative control, the second group was given with Metformin 0.065 mg/g body weight as a positive control, a third group given guava juice content 100%, the fourth group was given 50% guava, fifth group was given 25% guava.

Thirty minutes later measured their blood glucose levels are referred to as blood glucose levels minutes to 0, then after the oral glucose-induced glucose 97.5% per sonde as 0:01 ml/g body weight or as much as 0.00975 g/g body weight. Measurement of blood glucose levels back carried 60, 120.150 minutes after oral glucose administration. Measurement of blood glucose levels is done by cutting a little tip of mice by using scissors.

The results of measurements of blood glucose levels of mice were then analyzed in order to determine the working ratio of guava fruit juice (*Psidium guajava L.*) in lowering blood glucose levels when compared with controls. To assist this analysis, the test was used Analysis of Variants (ANOVA). Before analyzed by Anova, the data must be tested with the Kolmogorov-Smirnov normality test aimed 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 Variants/ANOVA) and advanced test LSD Post Hoc Multi Comparison (Least Significance Difference).

Data analysis using descriptive test was used to determine the mean and standard deviation of the data so that they can see if there are any effects of guava juice (*Psidium guajava L.*) in lowering blood glucose levels when compared with the control.

We can observe an increase in blood glucose at 60 minutes in distilled water group, metformin, 100% guava juice and guava juice 50% but rather a decrease in blood glucose levels in group guava juice 25%. At 120 minutes, the entire blood glucose levels decreased. In minutes to 150 groups of distilled water and guava juice 100% increase in blood glucose levels and the metformin group, 50% guava juice, guava juice 25% decrease in blood glucose levels.

Based test for normality using the Kolmogorov-Smirnov test obtained significance value (p) ? 0.05 for all of the data, which means that the data are normally distributed. The data distribution and ratio were normal, so that researchers can use ANOVA method.

Table 1. Mean and standard deviation

Minutes	Blood glucose level (mean ± standard deviation)				
	Distilled Water	Metformin HCl	Guava Juice 100%	Guava Juice 50%	Guava Juice 25%
0	156.71 ± 52.25	78.43 ± 20.82	113 ± 33.91	92.86 ± 13.95	327 ± 89.24
60	213.43 ± 54.18	160.43 ± 25.83	224.57 ± 62.29	209.14 ± 74.08	183.43 ± 53.7
120	132 ± 45.63	110 ± 43.86	96 ± 19.58	108 ± 25.81	77 ± 12.08
150	140.71 ± 39.79	100.57 ± 22.22	105 ± 23.61	95.29 ± 17.65	66.71 ± 12.8

Table 2. Results of the analysis of data normality with the Kolmogorov-Smirnov

Minutes	Asymp Sig (p)				
	Distilled Water	Metformin HCl	Guava Juice 100%	Guava Juice 50%	Guava Juice 25%
0	0.969	0.934	0.572	0.965	0.996
60	0.992	0.378	0.969	0.575	0.647
120	0.74	0.949	0.571	0.923	0.905
150	0.998	0.741	0.887	0.728	0.482

Table 3. Test Data homogeneity

	Test of Homogeneity of Variances			
	Levene Statistic	df1	df2	Sig.
p0	4.895	4	30	.004
p60	.770	4	30	.553
p120	4.729	4	30	.004
p150	2.413	4	30	.071

Table 4. Test the hypothesis of one-way analysis of variance/Anova

		ANOVA				
		Sum of Squares	df	Mean Square	F	Sig.
p0	Between Groups	287462.400	4	71865.600	29.053	.000
	Within Groups	74208.000	30	2473.600		
	Total	361670.400	34			
p120	Between Groups	11390.400	4	2847.600	2.737	.047
	Within Groups	31208.000	30	1040.267		
	Total	42598.400	34			
p60	Between Groups	18843.886	4	4710.971	1.486	.231
	Within Groups	95119.714	30	3170.657		
	Total	113963.600	34			
p150	Between Groups	19595.886	4	4898.971	7.878	.000
	Within Groups	18656.000	30	621.867		
	Total	38251.886	34			

Data P60 and p150 homogeneous group, but the data p0 and P120 groups are not homogeneous, the post hoc test can not be put on LSD. In the table, the value obtained significance (p) <0.05 for p0 and p150, showed no difference between the decrease in blood glucose group given juice guava (*Psidium guajava L.*) and control group was subjected to measurement of blood glucose levels to 0 minutes and minutes to 150, further, it can be tested further analysis of variance Post Hoc Multiple Comparison Dunnett T3. Of post hoc test data can be seen that there are significant differences in blood glucose levels measurement with distilled water administration, metformin, 100% guava juice, guava juice and guava juice 50% to 25% as indicated by the value of sig (p) <0.05.

DISCUSSION

The aim of this study was to obtain data on the effects of guava juice (*Psidium guajava L.*) in lowering blood glucose levels, using mice as experimental animals, as well as test dose of juice guava (*Psidium guajava L.*) in lowering blood glucose levels. It is intended to provide scientific information about the usefulness of juice guava (*Psidium guajava L.*) to decrease blood glucose levels

In this study, we used male mice as experimental animal weighing 20-30 grams and 2-3 months old. Other requirements include the condition of healthy mice and no abnormalities were seen in the mice body. Before treatment, adaptation to the environment was done for at least one week and guarded condition (Jaya 2007). Mice were also fasted ± 18 hours before the treated (Jaya 2007) to avoid the effect of food on mice. Mice were used as many as seven of each treatment groups.

This study performed glucose tolerance test (GTT) in mice with different starting treatment provision in each group per oral sonde (distilled, metformin HCl, guava fruit juice content of 100%, 50% and 25%). After that, wait for 30 minutes and 97.5% glucose was loaded to the mice by oral sonde and measured their blood glucose levels after sonde as measurement minutes to 0. Furthermore, mice blood glucose levels were measured again at minute 60, 120.150. How to measure the levels of mice blood glucose is to cut the little mice and shed his blood on the glucose test packs and measured with glucosemeter.

Data analysis showed that there was a significant difference in blood glucose levels decrease significantly in the administration of guava juice (*Psidium guajava L.*) content of 25% compared with distilled water, metformin HCl, guava juice levels of 100% and 50% in the measurement of blood glucose levels minutes to 0, 60,120,150, especially at minute 120 and 150 (can be seen from the sig (p) < 0.05). From the data, the minutes to 0 blood glucose levels distilled water group, metformin, guava juice 100%, 50% guava juice, guava juice 25% are not equivalent. Obtained blood glucose levels minutes to 0 on giving guava juice 25% higher than other groups.

Subsequently obtained an increase in blood glucose at 60 minutes in distilled water group, metformin, 100% guava juice and guava juice 50% but rather a decrease in blood glucose levels in group with guava juice 25%. At 120 minutes, the entire blood glucose levels decreased. In minutes to 150 groups of distilled water and guava juice 100% increase in blood glucose levels and the metformin group, 50% guava juice, guava juice 25% decrease in blood glucose levels.

Mechanism of action of guava in influencing blood glucose levels based on dose is not known with certainty, but there are possibilities that affect the data, among others: the variation of mice to tolerate glucose, measurement methods encounter some errors, omissions in the measurement, the destruction of the gauges. Decreased blood glucose levels by guava juice (*Psidium guajava L.*) influenced its flavonoid components, among others: Quercetin inhibits the enzyme Amylase which causes the breakdown and absorption of carbohydrates to be disturbed, so that the blood glucose levels in hyperglycemic state can be derived. The research that has been conducted on onion (*Allium ascalonicum*) containing quercetin in rats showed decreased levels of significant hyperglycemic blood glucose (Wulandari 2010).

Myricetin and apigenin which increases the amount of GLUT-4 transporter. Research on *Abelmoschus moschatus* containing myricetin on alloxan-induced mice, and studies of celery and leaves *Cephalotaxus sinensis* containing apigenin in rabbits hyperglycemia showed a decrease in blood glucose levels were significantly (Li et al 2007, Liu et al 2006). Tannin has the ability as an astringent that can precipitate protein on the surface of the mucous membrane of the small intestine and form a layer that protects the intestine, thus inhibiting the absorption of glucose and blood glucose increased rate is not too high. Research on avocado seed containing tannin on hyperglycemia mice showed a decrease in blood glucose levels significantly (Monica 2006). The benefits of guava juice can be seen from several studies using guava, among others: guava juice effect on the levels of nitrite ions in mice conditioned arthritis, ethanol extract of guava leaves can act as an antioxidant and has an antimicrobial effect (Adnyana et al 2004, Wiralis 2008).

CONCLUSION

The consumption guava (*Psidium guajava L.*) juice provides more significant reduction in blood glucose than that in control group.

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