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Research Article

The effect of Soybean (*Glycine max* (L.) Merr.) Milk on blood glucose level in White Male mice strain ddY

Dadang Kusmana*, Evenia and Azizahwati

[1]Developmental Biology Laboratory, Department of Biology, FMIPA Universitas Indonesia
[2] Faculty of Pharmacy, Universitas Indonesia.
*Corresponding Author : *dadangksmn@gmail.com*

Keywords

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Abstract

Soybean is the most abundant source of isoflavones and one of its soy product is soybean milk. This study is aimed to investigate the effect of soybean milk on blood glucose content in male ddY mice. A completely randomized design was conducted using 25 males of DDY mice. Treatment were divided into 5 groups, which were normal control (CMC 0.5% ml/20 g b.w.), drug control (Metformin HCl 13 mg/20 g b.w.), and three different treatment doses (0.325 g soybean/20 g b.w.; 0.65 g soybean/20 g b.w.; 1.3 g soybean/20 g b.w.). Fasting blood glucose was measured on mice were treated based on their group. Thirty minutes treatment, blood glucose level was measured again and then mice were loaded with glucose 2 g / kg b.w., orally. Blood glucose level was measured at 30, 60.90 and 120 minutes postload glucose. Blood glucose level was measured by using ACCU-CHEC Active meter. Administration of soybean milk lowered blood glucose level in glucose loaded male ddY mice treated with 0.325; 0.65; 1.3 g soeybean/20 g b.w., but treatment with 0.325 g soybean/20 g b.w. showed the best reduction of blood glucose level.

Introduction

Diabetes mellitus (DM) is a group of metabolic disease characterized by high glucose level (hiperglikemia). DM is assosiated with metabolic abnormality of carbohydrate, lipid, protein and in the later stages that can cause cronic complications such as microvasculature, macrovasculature, and neuropatic disorder (Triplitt, Reasner, and Isley 2008). The disease increase over time and globally, particularly in the developing countries (WHO 2011). The prevalence of diabetes in Indonesia on 2030 is expected to continue to increase up to 21,3 million people. This growing prevalence of diabetes stayed at the 4th position over the world, after India, China, and United State (Wild, Roglic, Green, Sicree, and King, 2004).

Recent publication research proved that the female hormone 17β-estradiol protects pancreatic β-cell function against glucolipotoxicity, oxidative stress, and apoptosis. The female hormone 17β-estradiol also reported to protects lipogenesis in adipose tissue and liver. In addition, 17β-estradiol, that located in pancreatic β-cell, also stimulate insulin secretion and biosynthesis through the activation of estrogen receptors. The estrogen receptors modulate insulin sensitivity in muscle and hepatic tissue (Le May et al., 2006; Liu and Mauvais-Jarvis, 2010; Louet, Le May, Mauvais-Jarvis, 2004; Tiano et. al., 2011; Yamabe, Kang, and Zhu, 2010).

Isoflavon, the part of flavonoid subclasses, structurally have similarities with 17β-estradiol and can stick together with estrogen receptor's isoform α dan β, called fitoestrogen. One of plants that mainly become the most abundant isoflavon source is soybean (Aronson, 2009; Dixit, Antony, Sharma, and Tiwari, 2011). The soybean consumption is continue increasingly over time (Zakaria, 2010). Most of the soybean production are made to fulfill society-food supply, such as soybean milk, in addition to *tahu* and *tempe* (fermented soybean cake) (Jumadi, 2009). Soybean milk are the easiest production because it does not need fermentation process like *tahu* and *tempe*. Besides that, soybean milk also ready to be consumed powdery or milky.

Constituent and Method

Animals: White male mice strain ddY, 6th months old, approximately 18—25 grams body weight collected from Indonesia Science Center Institute (LIPI), Cibinong. The animals were adapted within a week in the laboratory.

Main Materials: Soybeans that have been dried within 4 months, collected from Balai Penelitian Tanaman Obat dan Aromatik, Bogor. Determination of plants has been done at Biology Research Center, Indonesia Science Center Institute

(LIPI), Cibinong. Other materials such as Metformin HCl was collected from Clinisindo Laboratories.

Chemical Materials: Monohydrathe glucose (Merck), CMC, and 70% alcohol (Brataco).

Preparation of Soybean milk: The threshold of soy intake, based on FDA recommendation is 25 g/ day as the cholesterol-lowering effects (Ulbricht dan Seamon, 2010). The preparation of making soybean milk are cleaning, blending, soaking, filtration, and heating. Heating at or near the boiling point (100°C) is continue for a period time, approximately 15 minutes, followed by removal of soy pulp fiber. The soybeans were chopping by blender followed by filtration. The filtrat produced were reheating while stirring but not until overheating (Jumadi, 2009).

Soybean milk treatment : This research used randomized complete design, 5 treatment with 5 replication. All the treatment groups were normal control group; comparison group; and 3 groups that were treated with variety dose of glucose. Total number of mice were counted by Frederer formula. Mice, in fasting condition (no foods but still drinking) within 16 hours, were collected its blood from the tail's vein and followed by estimated the fasting blood glucose level by blood glucose level testing. That fasting blood glucose level is mentioned as an early blood glucose level (T_0). After had been tested, a normal control group was given CMC 0.5% as much as 0.5 ml/20 g BW and for those dose-dependent groups were also given as much as 0.325 g, 0.65 g, and 1.3 g soybeans/20 g BW. Blood glucose level on each group were taken after 30 minutes. The level of blood glucose level in that time said as latest blood glucose level (T_{30}). After completed 2 phases blood glucose testing, mice were given 2 g/ kg BW of glucose. That mice were tested frequently on 30, 60, 90, and 120 minutes after treated with glucose (Tg_{30}, Tg_{60}, Tg_{90}, and Tg_{120}).

Statistical Analysis: Blood glucose levels were statistically tested with SPSS 19. The normality test (*Shapiro-Wilk* test) and homogenicity test (*Levene* test) were also taken. If the data had normal distribution and homogen, one-way ANOVA (*Analysis of Variance*) test was taken to observe the differences between groups and followed by LSD (*Least Significant Difference*) test. If the data had no normal distribution and not homogen, the parametric test *Kruskal-Wallis* test and followed by *Mann-Whitney* test were used to observe which group was has different significantly.

Results

The averages score of blood glucose level test in all experiment groups at each time were shown on Table 1 and the graph also can be seen on Figure 1.

Table 1: Average blood glucose level of each groups

Grou-ps	Mean of Blood Glucose Level ± SD (mg/dL)					
	T_0	T_{30}	Tg_{30}	Tg_{60}	Tg_{90}	Tg_{120}
NCG	62.4±12.92	80.8±10.69	252.8±34.67	210.4±27.81	156.4±15.06	133±14.66
CG	59.4±4.39	57.4±6.54*	57.6±24.34*	37±10.22*	39.4±10.88*	64±15.44*
1st Dose	68.8±13.12	98.6±16.65*	203.8±29.53*	166±23.65*	131.2±15.80*	102.2±11.21*
2nd Dose	72.2±11.30	107.2±14.62*	202±20.60*	186.6±16.13	155.4±29.44	137±26.33
3rd Dose	69±16.09	105.2±10.98*	229±22.55	181.2±8.32*	143.6±9.39	135.6±10.11

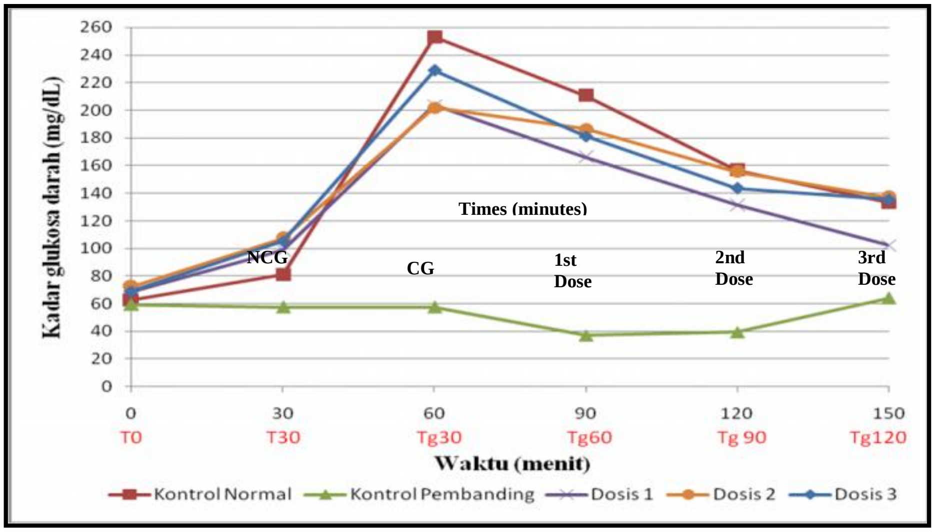
Addition*: = Significance < 0,05 are be considered with normal control group, NCG = Normal Control Group (0,5% CMC, 20 ml/ 20 g BW), CG= Comparison Group (suspension of Metformin HCl, 13 mg/20 g BW), 1st dose = 0.325 g soybeans/20 g BW, 2nd dose = 0.65 g soybeans /20 g berat, 3rd dose = 1,3 g soybeans /20 g BW, T_0 = early blood glucose level, T_{30} = blood glucose level within 30' after treatment, Tg_{30} = blood glucose level within 30' after treated with glucose, Tg_{60} = blood glucose level within 60' after treated with glucose, Tg_{90} = blood glucose level within 90' after treated with glucose, and Tg_{120} = blood glucose level within 120' after treated with glucose.

Statistical analysis showed that the blood glucose level data in all treatment groups at each time had normal distribution and homogen, except at Tg_{120}. The data at Tg_{120} had no normal distribution, so transformation of data were based on logarithm scale. For all data that had normal distribution and homogen were analyzed by parametric analysis, one-way ANOVA.

Early Blood Glucose Level (T_0)

The average score of fasting blood glucose level before treatment were between 59.4 and 72.7 mg/dL, approximately 12.8 mg/dL. One-way ANOVA test of T_0 showed that there were not significant differences between groups (p>0.05).

Figure 1: Graph of Average blood glucose level of each groups



Addition*: 1st Dose = 0.325 g soybeans/20 g BW, 2nd dose = 0.65 g soybeans /20 g berat, 3rd dose = 1.3 g soybeans /20 g BW, T_0 = early blood glucose level, T_{30} = blood glucose level within 30' after treatment, Tg_{30} = blood glucose level within 30' after treated with glucose, Tg_{60} = blood glucose level within 60' after treated with glucose, Tg_{90} = blood glucose level within 90' after treated with glucose, and Tg_{120} = blood glucose level within 120' after treated with glucose

Blood Glucose Level Within Thirty Minutes After Treatment (T_{30})

The average score of blood glucose level within 30' is from 57.4 to 107.2 mg/dL. On the 1st, 2nd, and 3rd doses showed significant increase in blood glucose level, from 29.8 to 36.2 mg/dL ($p<0.05$), so LSD test was taken. LSD test showed significant increase in blood glucose level ($p<0.05$) between 1st, 2nd, 3rd doses and normal control group (KN) neither comparison group (KP). Meanwhile, there were no significant differences ($p>0.05$) of blood glucose level of each dose.

Blood Glucose Level Within Thirty Minutes After Treated with Glucose (Tg_{30})

Blood glucose level in all groups had increased within 30' after treated with glucose. Normal control group showed that average of blood glucose level increase up to 252.8 mg/dL. It is because the glucose was absorbed by gastrointestinal tract. On treated groups with variety of doses it showed that less increase in blood glucose level than normal control group, which were 203.8; 202; 229 mg/dL for 1st, 2nd and 3rd dose group. It is compared to normal group, respectively the percentage score were 19.38%; 20.09%; and 9.41%.

Statistical analysis was followed by LSD test in case of significant differences of blood glucose level of each groups ($p<0.05$). The result showed that there were significant differences between normal and comparison group, 1st dose and 2nd dose group, either comparison group and 1st, 2nd, and 3rd dose group ($p<0.05$). Meanwhile, there were no significant differences ($p>0.05$) of blood glucose level of each dose. In conclusion, soybean milk on 1st and 2nd dose had same effect for lowering blood glucose level significantly ($p<0.05$) within 30 minutes after treated with glucose.

Blood Glucose Level within Sixty Minutes After Glucose Treatment (Tg_{60})

Sixty minutes after the administration of glucose, blood glucose levels normal control average dropped to 210.4 mg / dL, while the dose groups 1, 2, and 3, the blood glucose level average also dropped respectively by 166; 186.6; 181.2 mg / dL. Decreased levels of blood glucose average dose group 1, 2, and 3 when compared with the normal group were 21.10%; 11.31%; 13.88%, respectively. Based on statistical analysis processed with LSD test, blood glucose levels between normal control group with a control comparison, a dose of 1 and 3, as well as the control group comparison with a dose of 1, 2, and 3 were significant different ($p <0.05$).

Decreased blood glucose levels ($p <0.05$) were observed at doses of 1 and 3 group, but not so different in the dose group 2. The blood glucose average dose group 2 compared with dose group 3 had little difference, however significance difference values dose group 2 compared with the normal group was 0.059. The decline in blood glucose levels in dose group 2 were not significant may be due to biological variation in test animals used.

Blood Glucose Levels Ninety Minutes After Glucose Treatment (Tg_{90})

In Tg90 blood glucose levels average normal controls dropped to 156.4 mg / dL, while the dose groups 1, 2, and 3 131.2; 155.4; 143.6 mg /dL, respectively. Decreased levels of blood glucose average dose group 1, 2, and 3 when compared with the normal group respectively is 16.11%;

0.64%; 8.18%. Since there were significantly differences in blood glucose levels between groups ($p < 0.05$), the statistical test followed by LSD test. The difference in blood glucose levels were significantly between groups shown in normal controls with control comparators and dosage 1, as well as the control group comparison with a dose of 1, 2, and 3 ($p < 0.05$). Dose group 1 still has the effect of decreasing blood glucose levels significantly. Dose groups 2 and 3 did not have a decrease in blood glucose levels significantly, but seen a decrease in blood glucose levels average compared with the normal control group.

Blood Glucose Levels One Hundred and Twenty Minutes After Glucose Treatment (Tg_{120})

At 120 minutes after glucose loading, blood glucose average normal controls dropped to 133 mg / dL, whereas the dose groups 1, 2, and 3 increases respectively at 102.2; 137; 135.6 mg / dL. Because there are differences in blood glucose levels significantly between groups ($p < 0.05$), it must be followed by LSD test. It was concluded that there were significant differences in blood glucose levels between normal control group with control comparators and dose 1, as well as the control group comparison with a dose of 1, 2, and 3 ($p < 0.05$). Dose group 1 still showed a decrease in blood glucose levels significantly ($p < 0.05$) compared with normal controls. Decreased levels of blood glucose average dose group 1 when compared with the normal group was 23.16%. Dose groups 2 and 3 showed no big difference on blood glucose level compare to normal control group.

Discussion

Test animals used are male mice, as in this study wanted to see the effect fitoestrogenik of isoflavones contained in soy bean milk. Female mice were not used because their hormonal cycles can affect blood glucose levels measured.

This research used animal models oral glucose treatment is referred to as an oral glucose tolerance test. Oral glucose will given raise blood glucose levels and this situation will stimulate the release of insulin from the pancreas. Hepatic glucose production is pressed and glucose uptake into tissues are stimulated because insulin is released. Reasons of using an oral glucose tolerance test method in this research is to see an increase in glucose uptake into tissues or not by administration of soy milk. If there is an increase in glucose uptake into tissues, then feeding soy may be beneficial in lowering postprandial glucose.

Oral glucose tolerance test method referred to as an induction of physiological diabetes without damaging the pancreas and used test animals also have normal blood glucose levels, so it is not require drugs that stimulate the insulin secretion. Insulin is released when test animals loaded the oral glucose as a response of the body to normalize blood glucose levels. One mechanism of action of Metformin HCl is by increasing tissue sensitivity to insulin, so with the oral glucose loading glucose uptake can be seen in the network. Therefore, Metformin HCl is used as a control comparison in this study.

Blood samples were collected through mice tail vein because the oral glucose tolerance test method requires a blood collecting multiple times in a relatively short time intervals, so it does not enable retrieval of blood through the eye orbital sinus. Glucometer used as a measurement of blood glucose levels because the test strips only need a small sample of blood, which is approximately one drop of blood. In addition, blood from the tail vein can be directly measured blood glucose levels, thus preventing the occurrence of glycolysis.

In the initial blood glucose levels (T_0), obtained fasting blood glucose levels were more or less the same for the entire group as test animals were fasted and blood taken at nearly the same time before being treated. In the initial blood glucose levels (T_0), obtained fasting blood glucose levels average were normal in test animals. This is because in the fasting state, most of glucose production derived from the liver and the rest is produced by the kidneys. Glucagon is secreted within the fasting state to resist insulin and stimulates hepatic glucose production, so preventing hypoglycemia or restore normoglycaemia in case of hypoglycemia.

The rise of blood glucose levels average that occur at dose group 30 minutes after treatment (T_{30}) may be caused by the presence of soluble carbohydrates within soy bean milk. Most of the carbohydrate content in soybeans is a non-starch polysaccharide or fiber. However, soybeans also contained stachiosa and sucrose which is the major soluble carbohydrate contained in soybeans. Stachiosa cannot be digested by humans, but can sucrose be broken down into glucose and fructose which can cause an increase in blood glucose levels (Obendorf dan Kosina, 2011).

The active substance within soy milk are thought to contribute within reduction of blood glucose levels is isoflavones. Aglycone form of isoflavones present in small amounts within soybeans, but glycosides form present in an amount which is dominant. Isoflavone in the form of inactive glycosides, because they have to undergo hydrolysis and release of components aglycone are essential for isoflavones absorption in the gastrointestinal tract. For this reason, it can be said that the isoflavone aglycone is a form of biologically active isoflavones (Tepavcevic, Cvejic, Posa, dan Popovic, 2011).

All dose groups in Tg_{30}, Tg_{60} and Tg_{90} shows blood glucose levels on average lower than the normal control group. This might be due to the active substance which is thought to be isoflavones have reached concentrations that can give the effect, so can be seen an increase glucose uptake into tissues. Increase in glucose uptake into tissues especially noticeable at doses 1 which the decrease in glucose levels are significant at Tg_{30}, Tg_{60}, Tg_{90}, and Tg_{120}. Therefore, it can be stated that administration of soy milk is beneficial in lowering postprandial glucose. Decreased blood glucose levels average in dose group 2 and 3 at Tg_{30}, Tg_{60} and Tg_{90} are insignificant when compared with normal controls are shown in Table 1. The decrease in blood glucose levels were not significant this might be due to soluble carbohydrates and glycosides isoflavones within soy bean milk, coupled with the presence of glucose loaded.

In this research, there are factors that affect blood glucose levels, among other biological variation test animals, soluble carbohydrates and glycosides within soybean milk, stress effects of test animals, and blood sampling through mice tail vein. Further research may be suggested to extracting the soybean with a suitable solvent so the soluble carbohydrates does not filtered. Moreover, it can also performed the isolation aglycone isoflavones to test antidiabetic effect, since there may be other active substances within soy milk which can reduce the blood glucose levels. Repeatedly blood sampling also can make stress of the test animal that can affect measured blood glucose levels. Stress condition stimulates the secretion of epinephrine which inhibits insulin secretion through interaction with α_2 receptors, stimulates glucagon secretion through activation of β receptors which present in α-cells of the pancreas, and decrease glucose uptake by peripheral tissues, resulting in an increase in blood glucose levels.

Conclusion

Giving soy bean milk can lower blood glucose levels male albino mice DDy strain were loaded glucose at all doses (0.325; 0.65; 1.3 g soy / 20 g BW mice), but a decrease in blood glucose levels are best seen at doses 1 (0.325 g soybean / 20 g BW mice).

Recommendation

In the next research, it is recommended to perform the extraction or isolation aglycone isoflavones from soy beans.

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