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The Effect of Edamame Milk on Triglyceride Levels in Diabetes Wistar Rats

Original Article

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Abstract
Type 2 diabetes is a hyperglycemic disease caused by cell insensitivity due to increasing blood glucose levels due to reduced insulin secretion. Hyperglycemia conditions have the risk to increase triglyceride levels in type 2 diabetes. Edamame milk contained isoflavones that had potential to reduce triglyceride level. To find out the effect of given edamame milk on triglyceride levels in type 2 diabetes wistar rats. A true experimental with pre-post randomized control group design towards 24 type 2 diabetes wistar rats in 4 groups. Groups K(+), P1 and P2 were induced Streptozotocin 45 mg/kgBW and Nicotinamide 110 mg/kgBW. P1 and P2 groups were given edamame milk at a dose of 1,8 ml/200grBW and 3,6 ml/200grBW for 28 days. Blood samples were taken through a retro-orbitalis plexus. Triglyceride levels were tested pre and post intervention using the GPO-PAP method. Data analysis had used Paired T test and One Way ANOVA test with Post hoc follow-up test. Triglyceride levels in P1 and P2 groups had significant differences ($p<0,05$) against the control groups. Edamame milk could reduce triglyceride levels of groups P1 and P2 at dose 1,8 ml/200grBW and 3,6 ml/200grBW. P2 was the highest group reduced in triglyceride levels up to 29,41±3,55 mg/dL. Edamame milk could reduce triglycerides levels in 28 days. Given an edamame milk at a dose 3,6 ml/200grBW more efficient to reduce triglyceride levels in type 2 diabetes rats significantly.

Keywords: *diabetes type 2, triglyceride, edamame milk, edamame soybean*

INTRODUCTION

The World Health Organization (WHO) estimates that people with diabetes mellitus in Indonesia will increase from 8.4 million (2000) to around 21.3 million in 2030 [1]. The prevalence of people with diabetes mellitus in Indonesia has increased from 6.9% in 2013 to 10, 9% in 2018. In 2018, Central Java Province still ranks above the Indonesian average, which means that the figure is still relatively high [2].

Type 2 diabetes mellitus is closely related to the condition of hypertriglyceridemia. Hypertriglyceridemia occurs because triglyceride levels exceed the normal limit of 150 mg/dl. In the condition of diabetes mellitus, elevated triglyceride levels can be more than 200 mg/dl. The increase in triglyceride levels is directly proportional to the increase in blood glucose levels where high blood glucose levels can accelerate the formation of triglycerides in the liver. Triglycerides are one of the fat compositions in the body consisting of three fatty acids esterified into glycerol. In the state of insulin resistance, there is also an inability of the endothelium lipoprotein lipase enzyme which causes plasma VLDL clearance to increase. This can increase the occurrence of complications in patients with type 2 diabetes mellitus [3].

High levels of triglycerides in patients with type 2 diabetes mellitus can be prevented by one way, namely diet regulation. Dietary arrangements that can be made include eating foods that are low on the glycemic index, high in fiber, low in saturated fat, and high in antioxidants [4]. Edamame is a type of vegetable plant that belongs to the legume family such as soybeans. One of the advantages of edamame is the content of isoflavones with higher levels than other soybeans. There are three main isoflavones present in edamame, namely genistein, daidzein and glycitin. Based on previous research, isoflavones as one of the antioxidants that play a role in reducing triglycerides [5]. Edamame juice is an innovative product from processed vegetable food extracts that is able to reduce the risk of dyslipids with isoflavones in it.

MATERIAL AND METHODS

This research is included in the scope of clinical nutrition using the True Experimental design method with Pre-Post Test Control Group Design. The research was carried out in September-October 2020 at the Inter-University Center for Food and Nutrition Laboratory (PAU) UGM, Yogyakarta for the treatment of experimental animals. The implementation of this research has been approved by the Health Research Ethics Commission no. 304/IX/2020/Commission for Medical/Health Research Bioethics, Faculty of Medicine, Sultan Agung Islamic University.

The samples in this study were male wistar white rats aged 8-12 weeks weighing 150-200 g obtained from the Experimental Animal Development Unit (UPHP) Universitas Gajah Mada. The number of samples in this study were 24 rats in 4 groups. The sample groups in this study were the negative control group (K-), the positive control group (K+), the treatment group 1 (P1) and the treatment group 2 (P2). Determination of the minimum sample size according to WHO is 5 rats per group. To anticipate the possibility of dropping out, a 10% sample is added.

All rat samples were adapted for 5 days, given standard AD II Comfeed feed of 20 g/head/day ad libitum. After that the sample was divided into 4 groups. In the positive control group (K+), P1 and P2 were induced by DM using Streptozotocin (STZ) 45 mg/kgBW and Nicotinamide (NA) 110 mg/kgBW. Diabetes mellitus conditioning was carried out on the 6th day for 3 days. Prior to intervention with edamame extract, an initial blood sample was performed. At the intervention stage, groups P1 and P2 were given edamame juice at a dose of 1.8 ml/200grBW and 3.6ml/200grBW rats, while the negative and positive control groups were still given standard AD II comfeed. The intervention in this study was carried out for 28 days. Determination of the dose based on daily human consumption of 1 cup (200 ml) and half a glass (100 ml) per day. Then it is calculated by multiplying 0.018 based on the Laurence and Bacharach conversion table so that 200 ml of the human dose is equal to 3.6 ml of the rat dose and 100 ml of the human dose is equal to 1.8 ml of the rat dose.

Edamame juice was made before giving treatment to experimental animals. The production of edamame juice is made using a soybean milk maker machine with a ratio of 1:1 edamame juice and water. The edamame used is fresh edamame packaged in frozen form with the Deluxe brand originating from Mitratani Jember, East Java which has passed the HACCP and Halal MUI. Fresh edamame is peeled from the outer skin then washed thoroughly with running water and separated from the epidermis in a separate container. After obtaining the edamame seeds without the outer skin or epidermis, the edamame seeds were weighed using a digital scale as much as 100 grams and then put into the soybean milk maker machine with the addition of 100 ml of water. Press the on button on the machine to start the process of making edamame juice and the machine will automatically cook the edamame seeds for 12-15 minutes with a heating temperature of 80oC to produce liquid edamame juice. The machine will emit a beep when the process is complete.

Data collection on triglyceride levels was carried out 2 times, namely before the intervention and after the intervention. Blood was taken through the retro orbital plexus in wistar rats as much as ±1% of body weight. Examination of triglyceride levels using the glycerol-3-phosphate oxidase-phenol aminophenazone (GPO-PAP) method with spectrophotometry measured at a wavelength of 546 nm. Rat body weight was also measured before and after the intervention in all groups as one of the supporting data.

Testing the normality of the data using the Shapiro-Wilk test because the number of samples 50. The analysis to determine the difference in triglyceride levels before and after in each group used the Paired t-test because the data were normally distributed. While the analysis to determine the difference in influence between groups using the One-way Anova test followed by the Post Hoc LSD test because the variation is homogeneous and normally distributed.

RESULTS

The body weight of the sample was weighed before and after the intervention. The results of the average weight measurement of each sample group can be seen in Table 1. there are differences in sample weight during the study. All groups before the intervention had body weight according to the inclusion criteria, namely 150-200 gr. The results of the paired t test showed that there was a significant increase in body weight in the K(-), P1 and P2 groups and a significant decrease in the K(+) group during the edamame extract intervention.

Table 1. Average Body Weight Before and After Intervention

Group	Before Intervention (g)	After Intervention (g)	Δ Average Chane	*p*
K(-)	188.83±3.66	217.17±3.82[a]	28.83±0.82	0.000
K(+)	185.33±3.67	165.50±3.33[b]	-19.83±2.3	0.000
P1	185.50±4.23	206.17±4.58[c]	20.67±0.82	0.000
P2	185.17±2.93	214.00±3.03[a]	28.33±0.75	0.000

Table 2. Effect of Edamame Extract on Triglyceride Levels

Group	Triglyceride Levels (mg/dL)		*p*	Δ Average Change
	Before Intervention	After Intervention		
K(-)	72.68±4.08[a]	73.72±3.70[a]	0.009	1.05±0.62
K(+)	121.91±2.64[b]	125.55±1.73[b]	0.001	364±1.21
P1	120.96±2.16[b]	99.76±1.28[c]	0.000	-21.21±2.28
P2	119.43±2.65[b]	90.02±3.11[d]	0.000	-29.41±3.55

Measurement of triglyceride levels in the sample was carried out before and after the intervention. Analysis of the mean triglyceride levels in each group can be seen in Table 2. Based on the results of the paired t test analysis in table 2 above, it shows that there are significant differences in triglyceride levels in all groups. The results of the test of different mean triglyceride levels between groups showed that there were differences in changes in triglyceride levels between the treatment groups (p 0.000). Descriptively, the decrease in triglyceride levels occurred in all treatment groups, namely P1 and P2, which indicated that the intervention of edamame juice could reduce the subjects' serum triglyceride levels. The highest decrease occurred in the P2 treatment group which was given edamame juice at a dose of 3.6 ml/200grBW.

Based on the ANOVA test analysis, there was a significant difference in triglyceride levels between groups before and after the intervention with edamame extract with p value = 0.000. Significant differences between groups after the intervention can be known by the Post Hoc LSD test ($p<0.005$). In the Post Hoc LSD test after the intervention, there were significant differences between all groups, both the control group and the treatment group.

DISCUSSION

Based on the table of changes in body weight of rats during the study, it showed that there was weight gain in the negative control group, treatment 1 and treatment 2. While the positive control group experienced weight loss at the end of the study.The negative control group experienced weight gain which illustrates that the sample is in healthy condition because it is not induced by diabetes so that glucose metabolism runs normally where the energy used comes from glucose reshuffle without any disturbances in protein and fat metabolism [6].

Weight loss in the positive control group was due to the rats being in a diabetic condition. The study on wistar rats induced with STZ 45 mg/kgBW and NA 110 mg/kgBW had a lower body weight of 200 g compared to control rats weighing 300 g. This is consistent with the theory which states that STZ-induced diabetic rats will experience weight loss due to pancreatic beta cell damage which causes hyperglycemia and hyperinsulinemia due to insulin resistance. As a result, there is a disruption in the gluconeogenesis process where fat and protein are used as energy producers so that the amount of muscle tissue and adipose tissue will decrease [7,8].

Changes in body weight in the treatment group given 1.8 ml/200grBW of edamame juice and 3.6 ml/200grBW of rats showed an increase in body weight until the end of the study. The decrease in blood glucose is in line with the decrease in gluconeogenesis, lipolysis and glycogenolysis. The presence of dietary fiber in edamame juice can help stabilize blood glucose levels. Fiber is able to absorb water and bind glucose and slow gastric emptying. In the small intestine, fiber increases the viscosity of the intestinal contents which results in a decrease in the -amylase enzyme and slows the absorption of blood glucose so that it can reduce the increase in blood glucose levels [9,10].

In this study, the sample was conditioned to diabetes by giving induction Streptozotocin (STZ) at a dose of 45 mg/kgBW and Nicotinamide (NA) at a dose of 110 mg/kgBW intraperitoneally. STZ that enters the cytosol of β cells will cause cell DNA damage that leads to apoptosis and cell necrosis [11]. The main cause of STZ toxicity is the ability of STZ to alkylate DNA. DNA alkylation occurs during STZ decomposition which will cause the DNA chain to break. DNA damage will trigger the process of ADP ribolation which results in increased oxidative stress and β cell death due to depletion of NAD+ and ATP and inhibition of insulin synthesis and secretion. This condition causes an increase in blood glucose. The addition of NAD in mice aims to inhibit poly ADP-ribose synthase (PARP) and prevent further cell damage due to the toxicity of STZ. According to the theory, the induction of STZ at a dose of 45 mg/kg and NA 110 mg/kg can increase fasting blood glucose levels >300 mg/dL in 7 days [12].

The increase in fasting blood glucose is directly proportional to the increase in triglyceride levels. Excess glucose levels can be used in de novo fatty acid production which is stored in the form of triglycerides [13]. STZ induction causes an increase in fasting blood glucose levels which results in damage to pancreatic beta cells, resulting in hyperglycemia and hyperinsulinemia due to insulin resistance. This condition causes VLDL secretion and free fatty acid flow to the liver by reducing the insulin-inhibiting effect on insulin-sensitive hormones in adipose tissue, resulting in a decrease in lipoprotein lipase activity [14]. This causes an increase in triglyceride levels. There were significant differences in triglyceride levels between groups in this study. The group that had the highest triglyceride levels at the end of the study was K(+) due to STZ-NA induction which caused hyperglycemia. Based on research on experimental animals in the form of rats induced by STZ 45 mg/kgBW and NA 110 mg/kgBW showed that there was an increase in triglyceride levels of 160 mg/dL in 21 days [15].

Table 2 shows that the K(+) group induced by STZ/NA with the same dose according to the previous theory had the highest triglyceride levels until the end of the study, but were still within the normal range (<150 mg/dL). This is because the effect of the STZ-NA induction period is only 3 days. Based on the results of the meta-analysis of previous studies, many positive effects of isoflavone administration in reducing triglyceride levels in the condition of type 2 diabetes mellitus have been shown. Giving isoflavone supplementation for 2 months can significantly reduce triglyceride levels in women with type 2 diabetes mellitus in China [16]. In a study on the antidyslipidemic activity of tempeh flour and soy milk on the lipid profile of diabetic rats, there was a positive correlation between the isoflavone content of soybeans and the reduction of triglyceride levels [17].

P1 and P2 groups with a significant difference with the K(+) group. This was because the P1 and P2 groups were given the intervention of edamame juice containing 87.35 g/ml isoflavones in 100 ml of edamame juice with different doses. The isoflavone content in this study was greater than the previous study, which was 41.94 g/ml (5). The improvement in conditions in groups P1 and P2 was caused by isoflavone compounds that have a hypolipidemic effect by decreasing the synthesis and esterification of cholesterol and triglycerides, increasing bile acid secretion, inhibiting the conversion of hepatic glucose into lipids and increasing the activity of LDL receptors for lipid metabolism so as to reduce triglyceride levels [18].

The daidzein component in isoflavones plays a role in reducing triglyceride accumulation. Genistein affects lipolysis by stimulating the lipolytic enzyme hormone-sensitive lipase or by increasing the lipolytic effect of epinephrine [19]. Research on isoflavones in diabetic rats showed that soybean extract containing low doses of isoflavones as much as 0.5-3 mg mg/kgBW or equivalent to 0.1-0.6 mg/200grBW rats had an effect in reducing triglyceride levels from 257 mg/dl to 224 mg/dl for 30 days [20]. In groups P1 and P2, this study showed that administration of edamame juice with isoflavones 0.3 mg/200grBW and 0.15 mg/200mgBW was able to significantly reduce triglyceride levels by 25% and 18%, respectively. The P2 group had 25% lower triglyceride levels than the K(+) group because they were given edamame juice in the amount of 3.6 ml/200grBW rats with isflavone content 1.55 mg/kgBW rats per day.

The protein contained in soy juice can also help in lowering triglyceride levels. A meta-analysis study suggested that soy protein can reduce triglyceride levels by 10.5% with a daily consumption of 47gr. In addition, the protein in edamame can induce adiponectin and reduce triglyceride levels through insulin.23 Protein can accelerate fat degradation through adiponectin signaling in the liver. Adiponectin functions to improve insulin sensitivity in the liver. Increased insulin sensitivity can increase the excretion of lipoprotein lipase which has an effect on reducing triglyceride levels [7].

CONCLUSION

There is a significant relationship between subscapular and suprailiac subcutaneous fat thickness, while there is no relationship between triceps and biceps fat thickness.

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CONFLICTS OF INTEREST

Conflict of interest : Authors state no conflict of interest.

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