

High Antioxidant Level in *Cajanus sajan* Reduces Blood Glucose Level and Improves Blood Lipid Profile of Rats as Diabetes Mellitus Models



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Manuscript submitted: 27 November 2020, Manuscript revised: 09 December 2020, Accepted for publication: 18 January 2021

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Keywords

blood glucose;
cholesterol;
lipid profile;
pigeon bean tempe;
triglycerides;

Abstract

Hyperglycemia in DM was able to increase glucose auto-oxidation, protein glycation, and polyol pathway activation. As a result, it can accelerate the formation of reactive oxygen compounds acting as the main factor causing DM complication. Before we evaluated pigeon bean Tempe with formulations of 25%, 50%, and 75%, we tested the number and activity of its antioxidant. Then we substituted it on standard feed for 14 days to find the effects on glucose level and lipid profile. The effects were then compared to that before the intervention. The result showed that the total and activity of antioxidants in pigeon bean Tempe (*Cajanus sajan*) was 15% and 41.30% respectively. On the other side, pigeon bean Tempe with a formulation of 75% was more able to reduce blood glucose level, total cholesterol, triglycerides, and LDL-cholesterol by 44.08%, 34.38%, 35.80%, and 43.22% respectively ($p < 0.05$) if compared to pigeon bean Tempe with formulations of 25% and 50%. Meanwhile, pigeon bean Tempe with the formulation of 75% provided better effects for an increase of HDL level 56.20% ($p < 0.05$). All test results on Tempe formulation used were compared to that before the intervention.

International Journal of Health Sciences © 2021.

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1 Introduction

Diabetes mellitus is a group of carbohydrate, lipid, and protein metabolic diseases characterized by hyperglycemia due to absolute or relative insulin deficiency. Hyperglycemia can increase glucose auto-oxidant, protein glycation, and polyol pathway activation. It accelerates the formation of reactive oxygen compounds as the main factor causing DM complication. It is due to imbalanced cellular antioxidant defense capacity; such as superoxide dismutase (SOD), glutathione peroxidase, and catalase from oxidative damage (Packer et al., 2000; Naudi et al., 2012).

The prevalence of DM always increases every year. In 2000, the number of people suffering from DM was estimated at around 171 million and predicted to increase to 336 million in 2030 (Wild et al., 2004). Basic Healthy Research data (2013) shows that the prevalence of DM in Mataram is quite higher if compared to that in other regions in NTB that was 1.7%. Based on data from Mataram Health Office, there were 1689 cases related to DM in 2013 and (January-December) and 1653 in 2014 (January-November). It shows that DM cases, particularly in Mataram are still quite high.

Hyperglycemia condition also affects the transports of triglycerides and cholesterol in the circulation and tissue. Decreased insulin production disrupts enzymes important for metabolism; such as lipoprotein lipase enzyme and hormone-sensitive lipase. As a consequence, fat level in the circulation increases, but decreases in the adipose tissue. It will increase lipid peroxidation and trigger oxidative stress in people with DM (Tjokroprawiro, 2003; Inawati et al., 2006). Hence it is necessary to obtain DM treatment and management.

DM treatment can be carried out optimally by combining nutritional therapy, physical activities, and medication. Nutritional therapy is considered the most effective therapy, especially for the onset of DM. Nutritional interventions used in DM therapy are *carbohydrate counting*, decreased energy intake, and selection of functional foods providing positive effects for the development of DM (Franz & Alison, 2012). Functional food contains bioactive components i.e. micronutrients (selenium, zinc, vitamin C, vitamin D, and calcium), fiber, antioxidants, and prebiotics. Dietary fiber food, especially soluble dietary fiber food has functions to control the levels of cholesterol and glucose and gives physiological effects on glucose regulation (Hirsch & Brownlee, 2005; Kim et al., 2006; Partama et al., 2018). The food has a high viscosity that can slow glucose, cholesterol, and triglyceride absorption in the small intestine. Exogenous antioxidants from functional food have functioned as coenzyme interacting and synergizing with cellular antioxidants to protect the body from free radical damage (Maritim et al., 2003; Tensiska, 2009). The selection of functional food is expected to be found from local food, such as pigeon bean Tempe (*Cajanus sajan*), easing to find and process it.

Pigeon beans (*Cajanus sajan*) are a source of anthocyanin pigments; such as *cyanidin-3-glucoside* and *delphinidin-3-glucoside* that can reduce cholesterol levels and are functioned as antioxidants. In this research, pigeon beans were processed into Tempe using Tempe yeast (*Rhizopus sp*), hence a daily consumption. Besides, it is also used as nutritional therapy for people with DM. Pigeon beans processed into Tempe are inspired by a local business in Lombok, particularly in Mataram. People there produce Tempe and tofu from soybeans. However, the research used gude beans with lower carbohydrates and fat than soybeans. In addition to that, pigeon beans have never been widely produced as functional food providing positive effects especially for people with DM (Haliza, 2009; Nurrahman et al., 2012).

We selected pigeon beans in the form of tempe because, after the fermentation process, Tempe gave more advantages than unprocessed tempe. In terms of nutrient composition in general, Tempe has a higher digestibility of protein and essential amino acid, lower anti-nutritional substances; such as antitrypsin and

phytic acid, and decomposing enzymes that digest protein, fat, and carbohydrate more easily. Besides, it contains genistein and daidzein, isoflavones serving as antioxidants to increase cellular antioxidant activities. The significance of using pigeon beans as tempe is that the beans have lower carbohydrate and fat than Tempe made of soybean. Therefore, it is good to be consumed by people with DM (Nurrahman et al., 2012).

Pigeon beans in West Nusa Tenggara are only used as processed vegetables combined with other vegetables with a distinctive and favored taste. Furthermore, the beans contain not only higher anthocyanins, but also higher glutamate amino acid than soybeans, making the food more savory. Therefore, in addition to nutritional therapy, gude beans can be widely produced as a local business, especially in Mataram. The result of previous research shows that the use of black soy yogurt can reduce the levels of cholesterol, triglyceride, and HDL of white rats with hypercholesterolemia. It also shows that the use of purple sweet potatoes with a 10% concentration can reduce the effects of free radicals caused by liver necrosis on rats given paracetamol and maximum activity load (Jawi et al., 2008). Meanwhile, we emphasize the use of local food, Tempe as a functional food. Before given to the rats with DM, Tempe had been tested. We conducted a proximate test to find the levels of carbohydrate, lipid, and protein; soluble and insoluble fiber tests; resistant starch level test; and test on the activity as well as the capacity of antioxidant Tempe produced contained. Therefore, it is expected that Tempe can be used as an alternative nutritional therapy, especially for the management of glucose and lipid profiles in people with DM.

2 Materials and Methods

Pigeon bean Tempe

The Process of Pigeon bean Tempe (Cajanus sajan) Production (Nurrahman et al., 2012)

Firstly, foreign objects mixed with pigeon beans were removed. Then, the beans were washed using clean water and boiled for 30 minutes before skinned and soaked for 36 hours. Soaked pigeon beans were drained and steamed for one hour. Finally, the beans were inoculated with tempe yeast (2%) and incubated at 25-27°C for 36 hours (Figure 1).

The Test on Activity and Total Antioxidant in Pigeon Tempe (Cajanus sajan)

Antioxidant activity in pigeon Tempe was measured using the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) method; while the total antioxidant was analyzed using the colorimetric method with FRAP Ferric reducing activity potential (FRAP) (Xu & Chang, 2007; Selawa et al., 2013).

Experimental Animals and Diets

Experimental animals used were 25 Wistar white rats (*Rattus norvegicus*) (eight weeks old, 80-22 g) obtained from Immunology Laboratory University Mataram. The research had been approved by the Ethics Commission of Faculty of Medicine, University Mataram (Approval Number: 56/UN18.8/ETIK/2016). All experimental animals were categorized into five groups i.e. normal rats (N), rats with DM (P1), rats with DM + pigeon beans with a formulation of 25% (P2), rats with DM + pigeon beans with a formulation of 50% (P3), and rats with DM + pigeon beans with a formulation of 75% (P4). P1, P2, P3, and P4 groups were injected with low dozed streptozotocin (STZ) and Nicotinamide (NA) for seven days intraperitoneally. The rats were considered hypertriglyceridemic if they had glucose levels in plasma > 100 mg/dL. Pigeon bean Tempe was given with standard food (AIN93) in certain formulations to achieve isocaloric. The standard food combined with 25%, 50%, and 75% calorie formulations was served in pigeon bean Tempe calculated based on the result of proximate analysis test (See Table1).

Table 1
Calculation of the Formulation of Pigeon Bean Tempe with
Standard Diet AIN 93 (g/kg Rat Weight) Given to Rats with DM

| Ingredient | Standard Diet | Tempe Diet | | |
|------------------------|---------------|------------|--------|--------|
| | | 25% | 50% | 75% |
| 1. Casein | 140 | 105 | 70 | 35 |
| 2. Corn starch | 620.7 | 605.94 | 591.18 | 576.42 |
| 3. Tempe flour | 0 | 69.63 | 139.25 | 208.88 |
| 4. Corn oil | 42.79 | 31.71 | 21.15 | 10.57 |
| 5. Sucrose | 100 | 100 | 100 | 100 |
| 6. CMC | 50 | 46.35 | 42.70 | 39.05 |
| 7. Vitamin mix AIN93 | 10 | 10 | 10 | 10 |
| 8. Mineral mix AIN 93 | 35 | 35 | 35 | 35 |
| 9. L-cystin | 1.8 | 1.8 | 1.8 | 1.8 |
| 10. Choline bitartrate | 2.5 | 2.5 | 2.5 | 2.5 |

[Nurrahman et al., 2012](#)

Rats with DM (P2, P3, P4) were given pigeon bean Tempe with formulations of 25%, 50%, and 74% for 14 days. During the intervention, N group (normal control) and P1 (DM control) were given standard food for 14 days.

Analysis of the Levels of Glucose and Lipid Profile in Blood

The analysis of the levels of glucose and lipid profile (triglycerides, total cholesterol, LDL-cholesterol, and HDL-cholesterol) was performed after the rats were given the formulations for 14 days ([Warabi et al., 2004](#); [Attie et al., 2002](#)). The result was then compared to that of the result given by the rats that did not obtain any formulation. Moreover, glucose and lipid profiles were measured using the enzymatic colorimetric method with Kit Dialysis.

3 Results and Discussions

The Examination on Total Antioxidant and Activities of Pigeon Bean Tempe (Cajanus sajan)

The result of the test on antioxidant activities of pigeon bean Tempe was 15%; while its total antioxidant and anthocyanin level were 41.30% and of 65.58 ppm/100 g sample respectively. By high active substances (antioxidant) of pigeon bean Tempe the result showed, we could use it as a choice of processed food. Also, it created an opportunity to make local food more profitable.

The Measurement of the Level of Glucose and Lipid Profile in Rats with DM Before and After the Intervention of Pigeon Bean Tempe (Cajanus sajan)

The result of the measurement of the levels of glucose and lipid profile in rats with DM after the intervention of pigeon bean tempe formulation for 14 days showed a decrease lower than that before DM intervention and control ($p < 0.05$). The measurement results before and after the intervention of pigeon bean tempe in rats with DM are presented in Table 2.

Table 2
The level of glucose and lipid profile in Rats with DM before and after the intervention of Pigeon Bean Tempe

| Treatments | Before Pigeon bean tempe administration (mg/dl) | | | | | After Pigeon bean tempe administration (mg/dl) | | | | |
|------------|---|--------|--------|-------|-------|--|--------|--------|-------|-------|
| | Glu | Chol | Tg | HDL | LDL | Glu | Chol | Tg | HDL | LDL |
| t0 | 67.75 | 82.44 | 91.09 | 66.30 | 30.80 | 67.77 | 85.66 | 92.38 | 65.61 | 31.91 |
| t1 | 214.12 | 177.04 | 131.09 | 25.33 | 71.52 | 216.25 | 178.81 | 132.24 | 24.70 | 74.22 |
| t2 | 216.11 | 171.00 | 133.28 | 25.47 | 69.90 | 157.10 | 143.56 | 114.16 | 37.58 | 67.87 |
| t3 | 219.39 | 171.13 | 136.64 | 25.61 | 73.80 | 133.07 | 126.94 | 97.51 | 58.08 | 44.19 |
| t4 | 222.67 | 177.46 | 134.16 | 25.88 | 72.46 | 124.52 | 112.51 | 86.12 | 59.09 | 41.14 |

Data are shown as mean (n=5)

Glu : Blood Glucose

Chol : Total of Cholesterol

Tg : Triglycerides

LDL : Low Density Lipoprotein

HDL : High Density Lipoprotein

t0 : Normal rats + standard diet

t1 : Rats with DM + standard diet

t2 : Rats with DM + pigeon beans tempe with a formulation of 25%

t3 : Rats with DM + pigeon beans tempe with a formulation of 50%

t4 : Rats with DM + pigeon beans tempe with a formulation of 75%

The intervention of pigeon bean tempe with a formulation of 75% more effectively reduced the levels of glucose, cholesterol total, triglyceride, and LDL_cholesterol by 44.08%, 34.38%, 35.80%, and 43.22% respectively ($p < 0.05$) than pigeon bean Tempe with formulations of 25% and 50%. Also, pigeon bean Tempe with a formulation of 75% provided better effects for the increase of HDL level that was 56.20% ($p < 0.05$). All results of the examination on Tempe formulation used was compared to that before the intervention.

Discussion

Total antioxidants and their activities of pigeon bean (*Cajanus sajan*) tempe respectively were 15% and 41.30%. 2,2-Diphenyl-1-picrylhydrazil (DPPH) test was effective to measure the antioxidant activity of gude beans able to keep free radicals away. During a hyperglycemic condition, free radicals increased. Hence external additional antioxidant that could be obtained by consuming dietary food was required. Pigeon bean tempe contained high antioxidant 41.30% and activities 15%. Consuming antioxidants from dietary food would help cellular antioxidant activity prevent the glucose antioxidant process known as a cause of increased production of free radicals and inhibit advanced glycation end products (AGEs) to bind with their specific receptor (RAGE). It could inhibit the formation of free radicals that spurred oxidative stress crucial for pathogenesis and complication on DM (Elmarakby et al., 2012; Karunakaran & Keun-Gyu, 2013).

Hyperglycemic conditions due to STZ and NA inductions led to glucose auto-oxidant, protein glycation, and polyol pathway activation, accelerating the formation of free radicals. The formation of free radicals was able to increase lipid, DNA, and protein modification in various tissues. It could eventually cause oxidative stress that led to a complication on people with DM (Nuttal et al., 1999). Table 2 shown the level of glucose considerably declining after the intervention of pigeon bean tempe with a formulation of 75%. It was because the high fiber level of pigeon beans inhibited glucose absorption by blood vessels, inhibiting the rate of the increase of glucose level. It supported previous research stating that the intervention of *Gracilaria verrucosa* extracts with a high fiber level in different doses was able to reduce glucose level in white rats (Candratama et al., 2012). Decreased glucose level was also because of high antioxidant level and activity in pigeon bean tempe able to minimize the formation of AGEs in the polyol pathway. As a result, the enzymatic glycation process was suppressed. Antioxidant was an electron-donor compound inhibiting oxidation reaction by binding free radicals with highly reactive molecules, so lipid peroxidation as one of cell damage causes could be prevented. By consuming antioxidants from dietary food, the function of cellular antioxidants would

be balanced. Therefore, it could provide protective effects on DM by reducing lipid peroxidation and Nitrite Oxide (NO) production and increasing cellular antioxidant activities (Coskun et al., 2005; Dykes et al., 2006).

Our research result also showed a more decreased lipid profile level; such as total cholesterol, triglyceride, and low-density lipoprotein (LDL), and increased high-density lipoprotein (HDL). The highest decrease and increase in lipid profile were found in the intervention of pigeon bean Tempe with a formulation of 75%. It was because the fermentation process in pigeon beans reduced both fat and carbohydrate levels in pigeon bean Tempe and slowed down glucose absorption due to high fiber levels. Therefore, glucose production declining decreased triglyceride level as fat reserves.

In DM cases, the consumption of pigeon beans with high antioxidants was able to increase cellular antioxidant activity that could prevent lipid peroxidation to reduce oxidative stress as one of the complications causes in people with DM (Coskun et al., 2005; Dykes et al., 2006). The result of our research and the previous research also stated that flavonoid level or high antioxidant type, as well as high fiber in dietary food, were able to prevent lipid peroxidation and reduce glucose level in DM-conditioned or hyperlipidemic-conditioned experimental animals (Setyawan et al., 2005; Isdamayani & Panunggal, 2015).

4 Conclusion

High total antioxidant and its activity of pigeon bean (*Cajanus sajan*) Tempe were able to reduce glucose level and improve lipid profile in rats with DM. The intervention of pigeon Tempe with a formulation of 75% provided better effects than that with formulations of 25% and 50%.

Acknowledgments

This research was supported by Mataram Health Polytechnic and Human Resources Development and Empowerment Agency, Ministry of the Health Republic of Indonesia.







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