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Original Article

Antioxidant Effects of Gude Bean (*Cajanus cajan*) to Homa-IR and IRS-1 of High Fat and High Fructose Diet Rats

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Abstract

This study aimed to determine the effect of antioxidants in Gude bean (Cajanus cajan) treatment on HOMA-IR level and IRS-1 gene expression on the rats induced with high fat and high fructose diet. This study used 25 male white rats (Rattus norvegicus) in two control groups and three treatment groups. The measurement of HOMA-IR was done before and after treatment using the blood plasma while IRS-1 measurement was after treatment using the muscle tissue. The results showed that rat blood glucose level after being induced high fat and high fructose diet reached average value higher ($p<0.05$) compared to the Normal group. The Gude bean treatment resulted the decrease of HOMA-IR level ($p<0.05$) and the increase of IRS-1 ($p<0.05$). Gude bean treatment can inhibit insulin signal interference by ROS synthesis inhabitation by decreasing HOMA-IR level and increase IRS-1 gene expression in rats induced high fat and fructose diet.

Keywords: - *Gude bean, antioxidant, HOMA-IR, IRS-1*

Introduction

Diet is important in both the growth and development of the human body. Its composition can determine the nutritional status. A modern dietary pattern, especially in western countries is dominated by high carbohydrate consumption including fructose, sucrose, and saturated fat (Jensen, *et al*, 2018). Consuming saturated fat and processed sugar, especially fructose can chronically initiate metabolic issues, such as insulin resistance, metabolic syndrome, and Type 2 Diabetes Mellitus (Taskinen *et al.*, 2019). A high fructose diet is one of the risk factors of hyperglycemia development (Kupsco *et al.*, 2019). Moreover, the development of hyperglycemia is one of the triggers of insulin resistance.

Insulin signal path has a number of kinase potential targets (substrate) activated, including insulin receptor protein and substrate insulin receptor family (Birbaum, 2001). IRS-1 is necessary in insulin cascade and its ability to form a signal complex by insulin receptor and other intracellular signals as the key to connect the process in plasma membrane to intracellular. Decreased IRS-1 protein reduces signal capacity in the system (Jensen *et al.*, 2018). IRS-gene is the gene that encodes IRS-1 protein synthesis that is widely expressed in the tissues responding insulin such as skeletal muscle, the liver, adipose, and cells such as pancreatic β to increase the affinity of glucose transporter molecule in the tissue membrane, increasing the glucose entering the cells (Wilcox G., 2005). Determining insulin resistance index can be indirectly conducted with Homeostasis Model of Assessment Insulin Resistance (HOMA-IR) method. The result can be regarded as a reference to manage DM and to prevent complication caused by oxidative stress condition due to hyperglycemia that causes insulin deficiency.

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To treat insulin resistance treatment, nutritional therapy and treatment should be combined because nutritional therapy is considered the most effective therapy. Nutritional interventions used in interference of insulin therapy are high fat and high fructose counting, energy intake reduction, and selection of functional food that give a positive effect to resistance insulin development. Functional food contains bioactive components such as micronutrients (selenium, zinc, vitamin C, vitamin D, calcium), fiber, and antioxidants. Antioxidant is necessary to control blood glucose and cholesterol levels and to give physiological effects to blood glucose regulation. As a result, the rate of the absorption of glucose and cholesterol has declined.

There are many spices and herbs which have great value as antioxidants (Sil, A., & Hore, J. K. 2023; Wijaya et al., 2023; Banerjee et al., 2022; Syamsul et al., 2022; Yuliani et al., 20200). One food ingredient that contains antioxidant in the form of anthocyanin is Gude bean (*Cajanus Cajan)* (Nurrahman et al., 2012). According to Ferramosca et al. (2017) Gude bean is an anthocyanin source as cyanidin-3-glucoside and delphinidin-3-glucoside that can combine blood cholesterol level and is functioned as antioxidant. In Yogyakarta, Indonesia, Gude bean is used only as processed vegetable combined with other types of vegetable. It is unique in taste and preferable due to its high anthocyanin and glutamate amino. Soon, it is expected that Gude bean can be widely produced as a local enterprise, especially in Yogyakarta.

In this research, the use of local food, Gude bean, was emphasized as functional food containing high antioxidant (anthocyanin) that is expected to be an alternative nutritional therapy, especially to maintain blood glucose and inhibit insulin signal interference. By the induction of Gude bean flour, it could have effect to HOMA-IR level and IRS-1 gene expression since ROS is inhibited.

The use of natural resources, especially local food needs to be optimized because they are easy to find for their abundant availability, easiness to grow, and the purity that can be assured. Due to these circumstances, the researcher explored the effectiveness of Gude beans in regulating genes related to metabolic diseases.

Conceptual Framework

25 rat models had 7-day adaptation of the cage and the laboratory environment, and the rats were divided into 2 groups consisting of 20 rats (for one control negative group and three treatment groups) induced high fat dan fructose diet and 5 rats (in control positive or normal group) given standard feed for 10 weeks. Then, data collection in the pretest to check blood glucose plasma, HOMA IR and resisting level. After the pretest, each group had intervention for 6 week The last step was data collection for post test and tissue sampling for IRS- 1 gene expression analysis.

Statement of the Problem

This study investigated Gude bean's (*Cajanus cajan*) effectiveness as local food that contains high antioxidants which is expected to be a new alternative nutritional therapy, especially to maintain blood glucose and inhibit insulin signal interference by ROS synthesis inhabitation. By inhibiting the synthesis, Gude bean flour could have effect to HOMA-IR level and IRS-1 gene expression. Control of genes related to metabolic diseases with biological ingredients or active compounds in natural sources of food can be one of the genomic- based nutritional therapies that lead to the choice of the right diet.

Based on the above problem statement, the research question was 'What are the effects of Gude bean *(Cajanus cajan)* treatment on Homeostasis Insulin Resistance Assessment Model (HOMA-IR) level and Insulin Receptor Substrate-1 (IRS-1) gene expression on the rats induced with high fat and high fructose diet?'

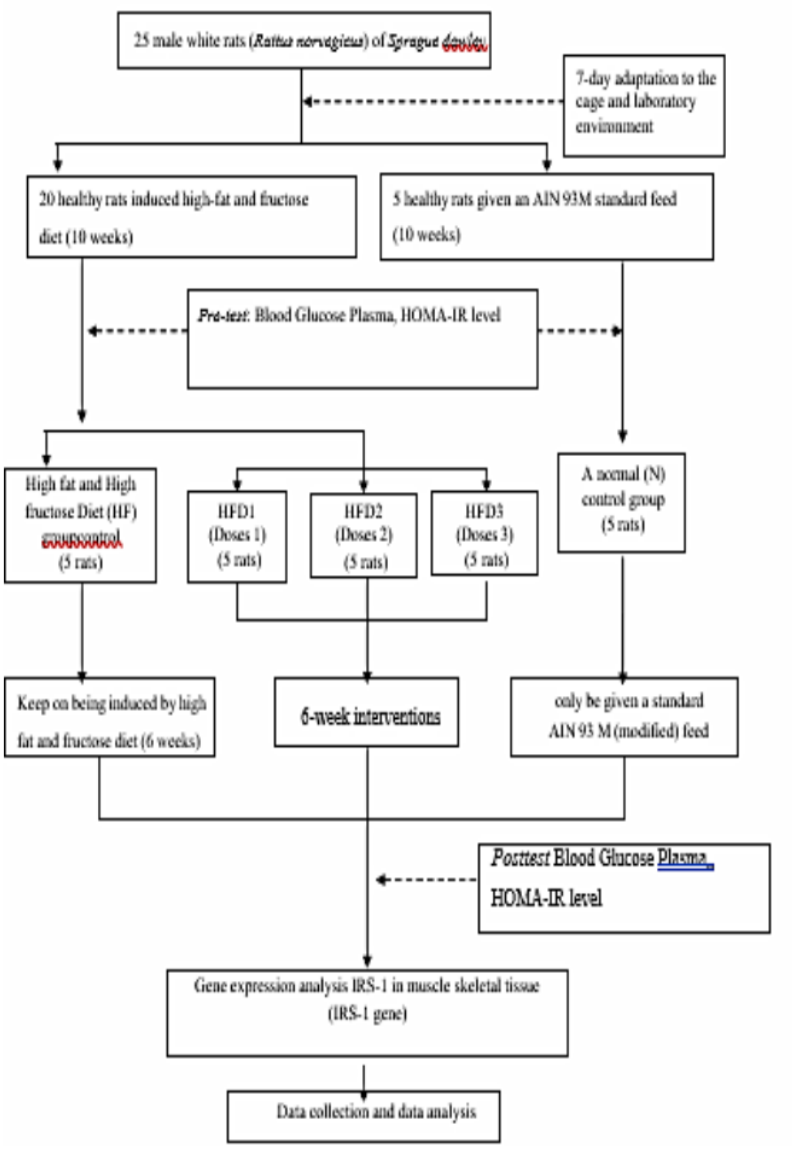


Figure 1. Conceptual Framework

Material and Methods

Research Design

This study used experimental method using a pre and posttest design using control groups: normal (N) and high fat and high fructose (HF) to compare the treatment groups HFD1, HFD2 and HFD3. The pretest was to check the HOMA IR level started by the induction of high fat and high fructose diet that

accelerate the condition of hyperglycemia. The next phase was the intervention for the treatment group with 15g of the feed with high anthocyanin from Gude bean flour every day.

At the end of the treatment, all the rats were anesthetized to take their blood as a sample of post-test measurements of HOMA-IR level. Then, the rats were dissected to take white adipose tissue, muscle tissue, and liver tissue as samples for determining the expression of IRS-1 gene.

Participants of the Study

Participants in the study were male white rats (*Rattus norvegicus*) of *Sprague Dawley* and sampled using purposive sampling where there were determined criteria in accordance with the research objective. In this research the determined criteria were sex, age, and weight of the rats. The inclusion criteria in this research were healthy rats, male white rats, 8-10 weeks of age, and on the average weight about 180-200g. The exclusion criteria were sick rats, female rats, more than 8-10 week of age and average weight less than 180g and more than 200g.

Instrumentation

The study utilized several instruments for data gathering. Firstly, the pretest and posttest using the collection and the storage of rat blood plasma sample, then posttest using the collection and the storage of rat tissue sample. The third instrument was the determination of HOMA- IR level by reference Rat Insulin (INS) ELISA Kit KTE101059 by Abbkine, and the last was The RNA isolation procedure in tissue samples by reference Invitrogen TRIzol™ Reagent from Thermo Fisher Scientific.

Data Gathering Procedures

Data gathering procedure of induction of high fat and high-fructose was started by acclimatization of the rats for one week before the experiments. After that they were induced standard food referring to the AIN 93M formula (Reeves et al., 1993) with a modification where *L-Cystine* and *Choline bitartrate* were replaced by *DL- Methionine* and *Choline chloride* respectively (El- sheikh & El- Fattah, 2011) and they were also provided water *ad libitum*. The criteria for hyperglycemia rats in this study was the rats with blood glucose levels ≥ 135 mg/dL (Kuzgun et al. 2020) after induction of high fat and high-fructose diet for 6 weeks. The rats were then divided into 5 groups with 5 rats each: the normal (Control +) group (N) with standard feeding referring to the AIN 93M formula, the rats group induced by a high fat and high fructose diet, the Control - group (HF), the rats group induced with a high fat and high fructose and treated using Gude bean (*Cajanus cajan*) flour with 33 g/kg feed (HFD1); 66 g/kg feed (HFD2), and 99 g/kg feed (HFD3).

Table 1. Rat blood glucose level before and after treatment with Gude bean (*Cajanus cajan*) flour

Group	Blood Glucose Level (GDP) (mg/dL)		Δ GDP Average (mg/dL)	p value
	Pretest	Posttest		
Normal (Control +)	99.67 ± 0.98^{a}	102.30 ± 3.55^{a}	↑ 3.63	0.092^{ns}
HF (Control -)	136.98 ± 9.47^{c}	149.81 ± 7.14^{b}	↑ **12.83**	0.164^{ns}
HFD1	122.41 ± 6.86^{b}	108.29 ± 4.06^{a}	↓ **14.12**	0.014$^{\#\#}$
HFD2	125.65 ± 9.17^{bc}	110.58 ± 5.36^{a}	↓ **15.07**	0.071^{ns}
HFD3	127.62 ± 6.407^{bc}	110.96 ± 7.30^{a}	↓ **16.66**	0.003$^{\#\#\#}$
p value	<0.000***	<0,000***		

The Collection and The Storage of Rat Blood Plasma Sample (pretest and posttest)

Rat blood plasma sample was collected through rat orbital sinuses which was previously anesthetized with injection of ketamine. Blood was drawn using a capillary tube that had been broken off, then accommodated in a 3 ml volume EDTA tube. Then the blood was placed in a cool box and the plasma was immediately separated. Because ELISA analysis was not done directly, the plasma was stored in -80°C for maximum of 6 months.

The Collection of Rat Tissue Sample (posttest)

The rats were decapitated, and then white adipose tissue, liver tissue, andmuscle tissue were taken out and stored in RNAase-free cryotubes. After that liquid nitrogen was added in the tube to maintain the tissue condition. Finally, the tissue was stored in -20^{0}C before it was used a month after that.

Data Processing for Level Determination

The determination of Homeostasis Model of Insulin Resistant Assessment HOMA-IR level by reference Rat Insulin (INS) ELISA Kit KTE101059 by Abbkine. The step standard solution of dilution procedure (Reference manual procedure Rat Insulin (INS) ELISA Kit from Abbkine No.KTE101059)

Data Analysis Approaches

The data that had been obtained was then tested for normality using *Shapiro- Wilk*. If the data were normally distributed, then data was tested using *Paired T- Test* to see the effect before and after the intervention for each treatment group. *One Way Anova* was used to analyze the effect of intervention in the normal, HF, HFD1, HFD2 and HFD3 group, and if p <α =0.05 was obtained, the test was continued with *post hoc multiple comparison testing*.

Ethical Approval

The research got approval from the Integrated Research and Testing Laboratory Ethics Commission, Universitas Gadjah Mada, Indonesia with the Ethical Clearance No.00014/04/LPPT/V/2020. This paper has been produced as a part requirements for the Doctor of Philosophy in Health Science at Lincoln University College Malaysia with the editing assistance provided by the university.

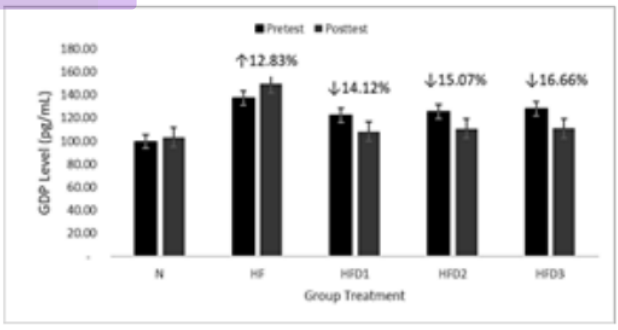
Results and Discussion

Blood Glucose Level

Rat blood glucose level after being induced high fat and high fructose diet reached average value to group HF=136.98mg/dL; HFD1=122.41mg/dL; HFD2=125.65mg/dL; and HFD3=127.62 mg/Dl that were higher (p<0.05) than Normal group that was 99.67 mg/dL. Based on the result, the rats had met the criteria of hyperglycemia with blood glucose level ≥135 mg/dL (Table 1).

Blood glucose level average value in each treatment group after being induced high fat and high fructose diet and treated using Gude bean (*Cajanus cajan*) flour was HFD1=108.29 mg/dL (p<0.05); HFD2=110.58 mg/dL (p>0.05); and HFD3=110.96 mg/dL(p<0.05) that were lower than HF group 149.81 mg/dL. The decrease of blood glucose level average on HFD2 group showed no significant value (p>0.05) (Table. 1). The biggest decrease of rats blood glucose level average was HFD3 group with 99 g/kg/feed Gude bean flour induction with a value of16.66% (Figure 2).

Figure 2. Blood glucose level difference before and after Gude bean (*Cajanus cajan*) treatment



HOMA-IR Level

Based on the analysis of the results using *one way* ANOVA test, the group induced high fat and high fructose diet and treated using Gude bean flour had lower HOMA-IR value compared to the group induced high fat and high fructose diet group without Gude bean flour (p=0.000). The lowest HOMA-IR average value was in the group induced HFD1 33 g/kg Gude bean flour (6.07 ± 0.62). The highest HOMA-IR average value was in the group induced HFD3 99 g//Kg Gude bean flour (7.35 ± 0.68) (Table 2).

Table 2. HOMA-IR value

Group HO	MA IR average value
Normal (control +)	5.54 ± 0.68
HF (Control -)	13.80 ± 2.17**
HFD1 33 g/kg Gude bean flour	6.07 ± 0.62*
HFD2 66 g/kg Gude bean flour	6.60 ± 0.77*
HFD3 99 g/kg Gude bean flour	7.35 ± 0.68*
P value	

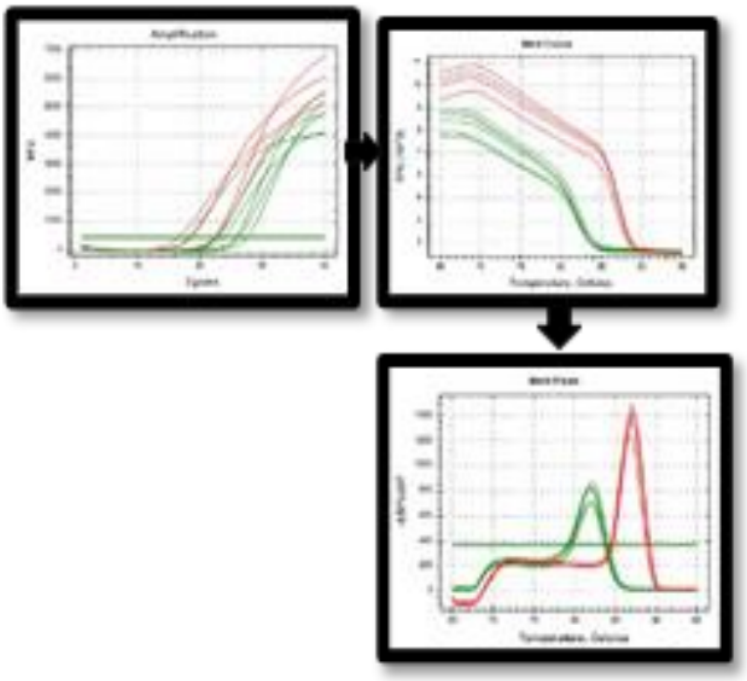
Note: * Significant difference from group HF.** Significant difference from group Normal, HFD1 33 g/kg Gude bean flour, HFD2 66 g/kg Gude bean flour and HFD3 g/kg Gude bean flour.

The next analysis using *post hoc* Tukey HSD test showed there was a significant difference of HOMA-IR average value between group induced high fat and high fructose diet and treated using AIN standard 95 modification diet and group induced high fat and high fructose diet without Gude bean flour treatment (p<0.005). There was also a significant difference of HOMA-IR average value between induced high fat and high fructose diet group without Gude bean flour treatment and group added induced high fat and high fructose diet and treated using Gude bean (*Cajanus cajan*) flour to all dose (HFD1, HFD2 and HFD3) (p<0.000).

In this research, the effect of intervention using Gude bean flour for 6 weeks could decrease HOMA-IR level of rat model. The treatment group with the addition of Gude bean flour resulted in HOMA-IR levels lower than the HF group. The biggest decrease was found in the HFD1 group, the group that was given a dose of 33 g/Kg Gude bean flour. Based on this, the feed with the lowest dose, that was 33 g/Kg has the ability to reduce plasma HOMA-IR levels and respectable antioxidant activity, so that it can protect the presence of free radicals. The content of antioxidant in Gude beans is thought to have a high and effective role as an antioxidant in the mechanism of HOMA-IR inhibition. Antioxidants derived from Gude bean can reduce the occurrence of oxidative stress resulting in a decrease in glucose intake in the body and reduce of hyperglycemia and decrease levels of HOMA IR in the body. The results of this study indicated that a high-fat and high-fructose diet could cause insulin resistance, which was characterized by an increase in the mean value of the HOMA-IR value. These results were supported by studies that had been conducted by Calvo- Ochoa et al., (2014) reporting that the administration of a high- fat and high fructose diet could cause insulin resistance which was characterized by high HOMA-IR values. According to Bonora et al., 2002, the higher the HOMA-IR value, the lower the insulin sensitivity, which could be due to a decrease in the target tissue response to insulin (DeFronzo and Tripathy, 2009). Similar to Amri et al., (2020) a significant increase in HOMA-IR can lead to reduced insulin sensitivity. Moreover, Sohouli, et al (2022) and Park et al. (2009) reported that the administration of antioxidants can increase insulin sensitivity which is characterized by a decrease in the value of HOMA-IR.

IRS-1 Gene Expression

The identification of IRS-1 gene used rat muscle skeletal tissue. The line graph of IRS 1 gene expression displays the result of PCR proses on amplification, melt curves and melt peak. There are two-line colors -- red is target gene and green is control gene and they form the same patterns which mean that Gude bean was effective in decreasing insulin interference. Based on the result of melt peak graphic, it was revealed IRS-1 can cross Ct linewhich mean that IRS-1 gene had been identified (Figure 3).



The bar graph of IRS 1 relative gene expression showed that after Gude bean treatment HFD1, HFD2, ad HFD3 group had higher relative gene expression then control negative group.

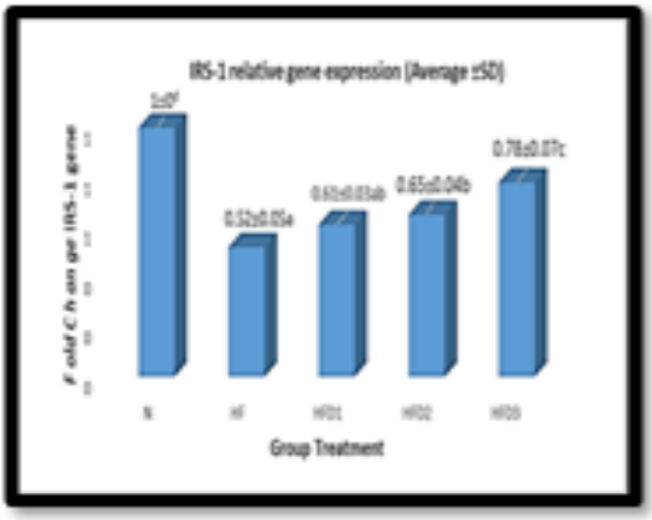


Figure 3. IRS 1 relative gene expression

The expression of the IRS-1 gene in skeletal muscle tissue of HF (Control -) group resulted in the lowest gene expression of 0.52 compared to other groups N= 1,00, HFD1=0.61, HFD2=0.65, HFD3=0.78. IRS-1 gene expression in skeletal muscle tissue that was given the addition of Gude bean flour (HFD2 and HFD3) had a significant increase ($p<0.05$) when compared to the HF group without the addition of Gude bean flour (mean fold change 0.52) and not significant compared HFD1 ($p>0.05$). The antioxidant effect of Gude bean flour in general and anthocyanin, in particular, was

influenced by variations in the dose given to rats (Zick., 2003). The results showed that the increase of gene expression of IRS-1 in HFD3 group (99g/Kg) was the highest compared to HFD1 and HFD2 although was still lower than the Normal group.
Based on the results of the expression of the IRS- 1 gene in skeletal muscle tissue, the HF group, the high- fat and high-fructose diet group without giving Gude bean flour, resulted in the lowest gene expression of 0.52 compared to other groups. The groups given a high-fat and high-fructose diet intervention and then treated with Gude bean flour resulted in hyperglycemia due to the induction of a high-fat and high-fructose diet and showed that IRS-1 gene expression result was higher compared to the HF group.

Conclusion

Based on the research result of the antioxidant treatment using Gude bean (*Cajanus cajan*) to the rats induced with high fat and high fructose diet, it can be concluded that Gude bean flour can decrease *Homeostasis Insulin Resistance Assessment Model* (HOMA-IR) level and increase *Insulin receptor substrate*-1 (IRS-1) gene expression in rats induced high fat and fructose diet.

Recommendations

In consideration of the findings and the conclusion of the study, there are some recommendations. First, further research is needed in patients with insulin disorders which refers to the results of this study as one of alternative diets to accelerate insulin signal interference therapy. Then, more study related to nutritional composition, the role of active compounds, and the potential of Gude bean to control of genes related to metabolic diseases with biological ingredients or active compounds in natural sources of food as one of the genomic-based nutritional therapies is needed. It is crucial for Indonesian government to give support and fund for researchers to conduct next research about Gude bean related to metabolic syndrome and to implement the research result. Furthermore, support and fund are also needed for local people to develop new functional food products from Gude bean.

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Conflict of Interest

The authors declare no conflict of interest.

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Missing "," You may need to place a comma after this word.

Article Error You may need to use an article before this word.

Article Error You may need to remove this article.

Proper Noun If this word is a proper noun, you need to capitalize it.

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