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Hypoglycemic Effect of Lesser Yam (*Dioscoreahispida* Dennst) Extract on Hyperglycemic Rats in Postprandial Condition

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Abstract—Hyperglycemia is a condition when the blood glucose levels exceed normal levels. Hyperglycemia can lead to the occurrence of Diabetes Mellitus (DM). The use of natural ingredients in lowering blood glucose levels has been carried out. However, studies on the benefits of lesser yam (*Dioscorea esculenta*) in reducing blood glucose levels have never been reported. This research aims to analyze the effect of administration lesser yam extract to hyperglycemic rats. Data were collected using experimental laboratory pre-post test randomized design. Data were analysed using analysis of variance one way statistical test and advanced Tukey. Lesser yam contains Inulin as PLA (Water Soluble Polysaccharide) which serves to inhibit gastric emptying thus it can help manage a diet of people with hyperglycemic. The results obtained by the control group (not given treatment) shows no change in fasting blood glucose levels interesting as well as post prandial. The administration of lesser yam extract affects blood glucose levels to decreased in hyperglycemic rats in all groups. Significant decrease occurs in the testing conditions first, second and third day post prandial group. The decrease is caused by the influence of the extracts after 2 hours of administration. Lesser yam (*Dioscoreahispida*Dennst) is capable of lowering blood glucose levels of hyperglycemic rats in post prandial condition.

Keywords: Dioscorea Esculenta; Hyperglycemic; Polysaccharide; Diabetes Mellitus

1. INTRODUCTION

Hyperglycemia is a disposition of Diabetes Mellitus (DM) thus hyperglycemia is needed to be managed. One of medications to lower blood glucose is metformin. Herbal medicine is often used to lower blood glucose of people with hyperglycemia.

Dewoto (2007) stated that in Indonesia clinical test for herbal medicine has not been done because it is expensive to conduct clinical trial. The trial will only be conducted when an herbal medicine has been proven efficacious and safe in preclinical trial. In addition, a long process also contributes to the complexity of clinical trial procedure. In order to conduct a clinical trial, it is necessary to standardise the tested ingredients. It is also not easy to find the right dose of herbal medicine, and the concern of the producers about negative results also does not support the conduct of clinical trial. If clinical trial is not conducted, herbal medicines will not be standardised. This will affect the users of herbal medicines. They will be affected by a more dangerous side effect.

The treatment of DM can be done medically using pharmaceutical drugs. However, the high cost, irregularity of drug use, and long term side effect make this type of medication sometimes difficult to be done. A research by Putri and Muhammad (2013) shows that most people with DM do not take their medicines regularly. This will affect the success of DM medication. The main purpose of DM treatment or medication is to lower the rate of blood glucose. People with DM also use herbal medicines to lower their blood glucose. The fixed standard of the use of herbal medicine in the market has not been determined so that there will be side effect if it is consumed (Dewoto, 2007).

Research by Sunarsiht *et al.* (2007) shows that Asiatic bitter yam (*Dioscoreahispida*) can lower the level of blood glucose proportionally as much as 20%. Asiatic bitter yam (*Dioscoreahispida*) contains cyanide that if consumed too much, it will be toxic. In order to consume it safely, it is suggested to cut the size of the yam into smaller pieces, to wash, to soak, to heat, and to dry it. Another method is to make the yam into flour. This method is effective in reducing the cyanide substance up to 85% (Ardiansari 2012). Applying the right procedure in processing the yam starting from the peeling, cutting, and cooking, both glucosidecyanogenic and hydrogen cyanide can be removed or reduced before the yam is consumed (Janagamet *et al.*, 2008).

Asiatic bitter yam contains bioactive compounds namely water-soluble polysaccharide (PLA). Water - soluble polysaccharide contained in Asiatic bitter yam is beneficial for health, especially for lowering the level of blood glucose (Aprianita *et al.*, 2009). This is in accordance with a research by Rahmawati (2010) stating that soluble polysaccharide extract of Asiatic bitter yam has the effect of lowering blood glucose on people with hyperglycemia. Saputro and Teti (2015) reported that Asiatic bitter yam contains 31.99% of PLA. Water-soluble polysaccharide can cause the decrease of carbohydrate absorption efficiency. The decrease contributes in the decrease of insulin response so that pancreas work in producing insulin becomes easy (Aprianita *et al.*, 2009).

A research by Tetiet *et al.* (2012) stated that PLA in Asiatic bitter yam has a distinct characteristic. It depends on the substance added during the extraction. Extraction in the research was conducted using water, papain, and tempeh yeast. The result shows that extraction using water produced 18% of amylose, 1.97% of protein, 1.49% of crude fibre, 101.10 ppm of glucose, 109.91 ppm of mannose, and 16.06% of HCN. Extraction using papain produced 32% of amylose, 2.33% of protein, 1.42% of crude fibre, 840.29 ppm of glucose, 872.14 ppm of mannose, and 2.29% of HCN. Extraction using tempeh yeast

produced 28% of amylose, 1.21% protein, 0.72% of crude fibre, 1924.62 ppm of glucose, 344.08 ppm of mannose, and 6.88% of HCN.

Water-soluble polysaccharide (PLA) has an ability to hold water and form thick liquid or membrane in digestive tract. This ability can delay gastric emptying, inhibit the mixing of digestive tract content with digestive enzyme so that the absorption of food substance in proximal part will decrease. According to Lunn and Burtiss (2007), the thick and jelly character of PLA can inhibit the absorption of macronutrient and decrease the response of post prandial glucose.

Glycoprotein content or polysaccharide protein in Asiatic bitter yam or *Dioscorea* difficult to remove. The right method is needed in order to obtain pure polysaccharide. The method used was maceration using water as solvent using addition of tempeh yeast. Extraction using tempeh yeast addition has aminolytic and proteolytic activities. The aminolytic activity of the yeast can degrade starch so that water-soluble polysaccharide (PLA) can be separated more easily (Harijono *et al.*, 2012). The biscuits added with WSP, extracted from tubers of either wild or lesser yam, or their mixtures with alginate, have significant hypoglycemic effects (Harijono *et al.*, 2013).

This research was conducted in order to analyse the effect of Asiatic bitter yam extract as antidiabetic. The result of this research will inform people about the benefit of local yams. This research was aimed at reducing the number of people with hyperglycemia significantly.

2. RESEARCH METHODOLOGY

This research is conducted in Animal Physiology Laboratory and Biochemistry Laboratory, Biology, Faculty of Science and Mathematics, Universitas Negeri Semarang. The research used experimental laboratories with pre-posttest control group randomized design

2.1 Materials

Asiatic bitter yam tuber was obtained from Kelompok Swadaya Masyarakat Mekarsari, Dusun Gegunung, Sendangsari, Pengasih, Kulon Progo, Yogyakarta (Mr. Kemin). The White Wistar Rats and alloxan were obtained from PAU Food and Nutrition Universitas Gadjah Mada, Yogyakarta. Weight of male rats is 150-200gr and two months old, as many as 24 rats. Rats food formulation were obtained from PAU Food and Nutrition Universitas Gadjah Mada, Yogyakarta.

2.2 Ekstrak Gadung

Asiatic bitter yam tuber extraction is made based on Harijono *et al.* (2012) method. The flour of Asiatic bitter yam tuber was weighed 1,5 kg, then 1L water added or until the flour is submerged, after that it was blended until the texture is porridge look alike. Next, the porridge is filtered using gauze until the filtrate is obtained. The filtrate is mixed with tempe yeast as much as 1,5 g and being incubated. The incubation occurred in 30°C for 12 hours. After that, the extract is diluted and used in treatment phase.

2.3 Experimental Animals

As many as 24 male white wistar rats are used in this research and divided into four groups. Blood sampling is carried out based on blood sampling standards for testing blood glucose levels. The first steps, the rats were fasted for 10 hours. During fasting, the rats only given drink. After fasting for 10 hours the rats were taken blood through the lateral vein and dripped on glucostrip to then read on the glucometer as KGD0. Then the rats were injected with alloxan monohydrate at 24 mg / 200 gBB intra peritoneally to make hyperglycemic rats and to help accelerate damage to the pancreas of rats and then given standard feed with the weight of each rat's feed equal to 15 g / 200gBB and added with sugar water dose of 500g / 1.5 liters of water for 3 days the rats were only given standard feed and sugar water. After 3 days of taking blood again as KGD1 if there has not been an increase in blood sugar levels then the administration of sugar water is continued until there is an increase in blood sugar levels by checking the sugar levels every day. Rats are said to be hypoglycemia when fasting blood glucose levels reach 126 mg / dl and post prandial reaches 200 mg / dl (American Diabetes of Association 2016). The hyperglycemic micewere distributed in 4 groups randomly and treated as follows: 1. First group are given standard food, 2. Second group are given Asiatic bitter yam extract 200 mg/kg BB and standard food, 3. Third group are given Asiatic bitter yam extract 400 mg / kg BB and standard food, 4. Fourth group are given Asiatic bitter yam extract 800 mg / kg BB and standard food. After treatment the rats blood were taken samples as fasting KGD21 then given standard food, after 2 hours, blood samples were taken again as post prandial KGD21. Then rats were given standard food and drinking water until 22.00, after that rats were fasted until 08.00. After fasting rats blood samples were taken without given Asiatic bitter yam extract first as fasting KGD22, then given standard feed and 2 hours afterwards blood samples were taken as KGD23 post prandial. Blood sampling as fasting and post prandial KGD23 was the same as fasting and post prandial KGD22.

2.4 Experimental Design And Data Analysis

The data obtained were analysed using Analysis of Variance One Way and continued with Tukey's Post-Hoc Test and were considered statistically significant when $p < 0.05$. Data was analysis by SPSS versi 1.6.

3. RESULT AND DISCUSSION

From this research on antidiabetic activity of Asiatic bitter yam on hyperglycemic rats, observation data for each group is obtained. The level of fasting blood glucose before alloxan (KGD0) was around 89 – 106 mg/dl. After alloxan (KGD1) was given, the level of blood glucose increased with mean value 133 mg/ dl in fasting condition and 206 mg/dl in post prandial condition. Rats were identified hyperglycemic when their level of fasting blood glucose reaches 126 mg/ dl, and their level of post prandial blood glucose reaches 200 mg/dl (American Diabetes of Association 2016). The level of blood glucose identified had attached with hyperglycemia criteria of experimental rats having similar characteristics to type diabetes in human. Data of rat blood glucose level after the treatment of Asiatic bitter yam (*Dioscoreahispida*) is shown in Figure 1 and 2 below:

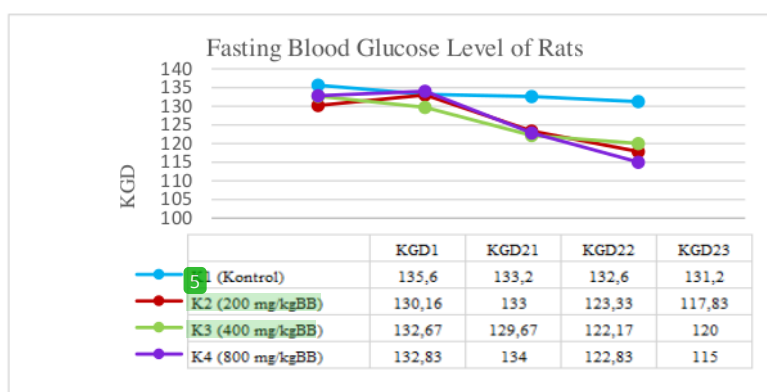


Figure 1. Fasting Blood Glucose level of Rats. KGD1 is Level of post alloxan blood glucose. KGD21 is the level post treatment blood glucose on the first day. KGD22 is the level of post treatment on the second day, and KGD23 is the level of blood glucose on the third day. Group 1 is control, group 2 is dose of 200mg/kgBW, group 3 is dose of 400mg/kgBW, and group 4 is dose of 800mg/kgBW.

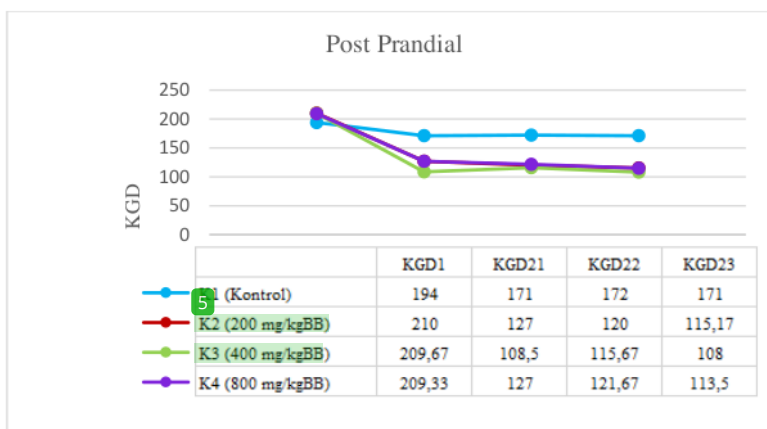


Figure 2. Blood Glucose Level of Post Prandial Rats. KGD1 is post alloxan blood glucose level. KGD21 is first day post treatment blood glucose level. KGD22 is second day post treatment blood glucose level and KGD23 is third day blood glucose level. Group 1 is control. Group 2 is dose of 200 mg/kgBW. Group 3 is dose of 400 mg/kgBW. Group 4 is dose of 800 mg/kgBW.

After alloxan (KGD1) was administered to all groups, fasting blood glucose level shows high mean value reaching criteria for fasting hyperglycemia (>126 mg/dl) (American Diabetes of Association 2016). In group 1, blood glucose level decreased although this group was control group and the extract of Asiatic bitter yam was not administered. However, the decrease was still above the level of hyperglycemia criteria. In the figure above, in both group 2 (dose of 200 mg/kgBW) and group 4 (800 mg/kgBW), the level of blood glucose increased from KGD1 to KGD21. This occurred because the test of blood glucose level was conducted right after the extract was administered. It was assumed that Asiatic bitter yam extract administered during the time had not given any effect. In group 3, the level of blood glucose decreased. The decrease of blood glucose level in the first day (KGD21) to the second day test (KGD22) was bigger than that of in the second day (KGD22) to the third day (KGD23) test. This also occurs in group 2 and group 4. The test of blood glucose level in the first day (KGD21) shows a more significant decrease because, before the blood test, rats were administered Asiatic bitter yam extract. Meanwhile, the second day (KGD22) to third day (KGD23) test was less significant because Asiatic bitter yam extract was not administered to the rats before blood glucose test.

The test of post prandial blood glucose level shows that there was decrease in group 1, group 2, group 3, and group 4 after Asiatic bitter yam was administered on the first day (KGD21).

The result of One Way Anova analysis shows that there is a meaningful comparison among the treatment groups. The comparison is provided with significant value of 0.020 in fasting KGD23. Because the result of the analysis shows meaningful difference, Tukey's Post-Hoc Test was used in order to identify which groups have difference. The result of Tukey's Post-Hoc Test can be seen in Table 1.

In general, people with diabetes can not control their blood glucose level because they consume carbohydrate. The food consumed is supposed to, besides providing nutrition, control glucose level in the blood. One of bioactive compounds that can postpone the emptying of food in the gastric and give longer effect of being full is water soluble polysaccharide (PLA) (Aprianita *et al.*, 2009). Water soluble polysaccharide (PLA) is one of bioactive compounds contained in Asiatic bitter yam. Data analysis shows that Asiatic bitter yam affected the decrease of blood glucose level on the first day of the test. Meanwhile, on the second and third day, the test did not show a significant result because the administering of the extract was only conducted on the first day. This concludes that the extract should be consumed daily according to the way it works, which is by inhibiting the emptying of gastric. According to Badan Standarisasi Nasional (National Standardisation Institution) (2006), consumption of Asiatic bitter yam with dose below 1 ppm per day is still in the safe limit of consumption, meaning that if Asiatic bitter yam extract is consumed twice a day, it is still in the safe limit of consumption.

Table 1. Rats Glucose level while Fasting and Post Prandial on Treatment Groups

	Group	Fasting	Post Prandial
		Glucose Blood levels (mean ± SD)	Glucose Blood levels (mean ± SD)
KGD21	K1	133.2±17.8 ^a	171.2±3.3 ^c
	K2	133±4.9 ^a	127±7 ^a
	K3	129.6±9.6 ^a	108.5±10.5 ^b
	K4	134±7.2 ^a	127±13.7 ^a
KGD22	K1	132.6±16.9 ^a	172.6±4.3 ^a
	K2	123.3±8.2 ^a	120±3.5 ^b
	K3	122.2±13.9 ^a	115.6±2.9 ^b
	K4	122.8±4.9 ^a	121.6±10.4 ^b
KGD23	K1	131.2±9.4 ^a	171.6±5.5 ^a
	K2	117.8±4 ^a	115.2±12.7 ^b
	K3	120±11.8 ^a	108±8.9 ^b
	K4	115±3.7 ^b	113.1±1 ^b

Superscript ^{a,b} shows significant difference

Asiatic bitter yam has a bioactive compound containing glycoside cyanogenic that, when hydrolysed, it becomes hydrogen cyanide (HCN) (Teti *et al.*, 2012). Cyanide content in Asiatic bitter yam can be removed by processing the yam properly. Asiatic bitter yam which is not processed tends to contain more cyanide than the one which is processed.

The making of experimental hyperglycemic rats' model was conducted by injecting alloxan monohydrate. The injection of alloxan monohydrate before the treatment aimed to accelerate the damage of pancreas β cell so that insulin is not produced well leading to the increase of blood glucose level (Li *et al.*, 2009). The administration of alloxan will cause blood glucose to fluctuate for 24-36 hours consisting of hyperglycemia and hypoglycemia phase occurring alternately before

permanent hyperglycemia occurs (Hikmah *et al.*, 2016). Alloxan is a diabetogenic compound that is cytotoxic to pancreas through the forming of free radical and oxidative stress (Nugroho, 2006). The induction of alloxan to the tested animal damages pancreas tissue leading to the decrease of insulin production by pancreas (Li *et al.*, 2009). Alloxan works by damaging essential substance in pancreas β cell causing the decrease of granules which bring insulin in pancreas β cell. This effect is especially for pancreas β cell, thus alloxan with high concentrate does not affect another tissue (Adam, 2000).

Hyperglycemic rats experienced polyuria. Polyuria is an excessive production of urine (Hikmah *et al.*, 2016). This can be seen from the condition of the cage that is damp every day. One obvious symptom of metabolism disorder such as in diabetes is the loss of weight, despite having a good appetite. This occurs because glucose can not be metabolised rapidly into energy needed by the body so that it causes depression of fatty and protein cells in order to fulfill the need of body energy (Wikanta *et al.*, 2008).

Doses of Asiatic bitter yam used in this research were 200mg/kgBW, 400mg/kgBW, and 800mg/kgBW. Among the three doses, 400mg/kgBW was the most effective dose. The significance difference between control group (K1) and group 3 (K3) a dose of 400mg/kgBW in post prandial sample test. Meanwhile, the analysis of group 2 (K2) and Group 3 (K3) does not show any significant difference. It is assumed that dose of 200mg/kgBW and dose of 800mg/kgBW are not effective in lowering blood glucose level. This result is relevant with a research by Tetiet *al.* (2012). On the first day of sample test during the fasting, there had not been any effect of the extract yet because the test was conducted immediately after the extract was administered. This has caused no significant difference occurred in fasting blood glucose level test.

Control group also experiences decrease in their blood glucose level although not as much as the treatment groups. The decrease is still above hyperglycemia criteria. It is assumed that the decrease occurred because all rats were fasted although they were not given any food. The diet given did not contain excessive carbohydrate so that blood glucose level could be lowered although not much.

4. CONCLUSION

Extract of Asiatic bitter yam affects hyperglycemic rats. Asiatic bitter yam can lower blood glucose level significantly at 400 mg/kgBW. Statistic analysis shows that post prandial test has a significant result.

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