

Assay for hypoglycemic functional food of cocoyam (*Xanthosoma sagittifolium* (L.) Schott.) tuber

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Abstract. Diabetes Mellitus (DM) type II is a degenerative disease that is a major killer in many countries. It is characterized by an increase of the blood glucose level above normal. It is important to choose an appropriate food sources using glycemic index (GI) concept in order to prevent blood glucose increase. One of Indonesian traditional carbohydrate source is cocoyam (*Xanthosoma sagittifolium* (L.) Schott.) tuber. The tuber is assumed having a higher carbohydrate content with lower GI. The research aims to measure GI of cocoyam tuber (CT) and determine glucose and glycogen level in animal model after CT fed. Experimental research was carried out by using completely randomized design. We used twenty four male rats as animal models. They were grouped in to 4 different treatments. Group I was treated with standard feed, group II was treated with standard feed and glucose, group III was treated with steamed CT, and group IV was treated hypoglycemic agent standard, glibencamide. The research results that GI of steamed CT was low. It was 54. Blood glucose of diabetic rats after fed by CT decreased significantly ($p < 0.05$), similar to diabetic rats after treated by glibencamide. Whereas glycogen level in diabetic rats after fed by CT was higher than in diabetic rats after fed by standard feed. Cocoyam tuber increased glycogen level in diabetic rats significantly ($p < 0.05$). Glycogen level in diabetic rats fed by CT was as high as in healthy rats. Therefore CT is potential consumed for DM type II patients.

1. Introduction

Nowaday, philosophy on food needs has much shifted. Food does not only overcome hunger, but also the most important food is good to the physical health. In the past nutrients on food were only expected give benefit for basic physical needs and sensory satisfaction. Otherwise now, food is expected to be functional. Food has advantages improving physical health and preventing any chronic diseases. Several chronic diseases caused death and dissability worldwide. One of them is diabetes mellitus (DM).

Diabetes mellitus is the sixth biggest cause of mortality in all age groups. It is a collective symptoms which characterized by hyperglycemic in the blood and glycogenesis failure due to insulin metabolic disorder. In a normal physical condition, excessive glucose will be stored as glycogen. Otherwise glycogen will be broken down into glucose in the glucose lack condition. Long term DM may cause a series of metabolic disorder which causes macrovascular and microvascular pathological disorders [1]. The number of DM patients increase rapidly worldwide and it is now a global epidemic



[2]. Wild *et al.* [3] estimate that in 2030, the number of DM patients in Indonesia reaches out 21.3 million. It is fourth position below China, India, and United States [4].

An action to prevent this chronic disease is healthy diet. It can be performed by selecting carbohydrate sources based on glycemic index (GI) concept. According to Rimbawan and Siagian [5], GI concept emphasizes to understand the ability of carbohydrate source on the blood glucose level speed increase. Generally, food which increases blood glucose level quickly has high GI, while food which increases blood sugar level slowly has low GI. Low GI food is potentially to develop as a functional food. According to Miller *et al.* [6] and Pruett [7], GI score is categorized into three classes, i.e. low (<55), medium (55 to 70), and high IG (>70). Carbohydrate sources with low GI should be explored to use in DM prevention.

One of Indonesian traditional food material is cocoyam (*Xanthosoma sagittifolium*) tuber. It is also known as kimpul (Javanese) or tannia. Cocoyam tuber is consumed as an alternative staple food because of the high carbohydrate content, around 70-80%, and have a digestible characteristic [8]. However, it is less information about CT potency as functional food source. Therefore, it is necessary to study the potency of CT as functional food source. The study aims to determine the GI score of CT and its hypoglycemic activity.

2. Experimental

This study was performed experimentally using completely randomized design. Twenty four adult male rats (*Rattus norvegicus*) aged 2–3 months was used as animal models. Cocoyam tuber originated from Wonogiri. After peeled and cleaned up, it was steamed at temperature 70° for 20 minutes. The study consists of two stages. Firstly, we measured blood glucose level of normal rats and determined GI value of CT. Secondly, we measured blood glucose level and liver and muscle glycogen levels of DM rats.

In the first stage, twelve rats were grouped into three different treatment groups. In the first group rats were treated pellet diet *ad libitum*, in the second group rats were treated 6.75 g/kg bw pure glucose orally, and in third group rats were treated steamed CT *ad libitum*. The blood glucose level of the rats was determined by using glucose oxidase-phenol + aminophenazone (GOD-PAP) method [9]. It was performed three times at the 0th, 1st, and 2nd hours after treatment. The blood was collected from vena orbitalis.

Glycemic index was calculated based on the blood glucose level in individuals treated cocoyam tuber obtained by comparing the Area under Curve (AUC) of individuals treated CT and the AUC of individuals treated standard glucose which had GI 100. Area under curve was determine by using trapezoid method [11]. According to Venn and Green [12], GI was determined by using formula as follow:

$$GI = \frac{AUC_{cocoyamtuber}}{AUC_{standardglucose}} \times 100 \quad (1)$$

In the second stage, twelve DM rats which induced by 40mg/kg BW streptozotocin (STZ) interaperitoneally [13] were used. In the first group, DM rats were treated pellet diet *ad libitum*. In the second group or positive control group, DM rats were treated 0.9 mg/200g bw of glibenclamide. Whereas in the third group DM rats were treated steamed CT *ad libitum*. Blood was collected 4 times on day 0th, 1st, 3rd, and 6th. The blood glucose level was determine by using GOD-PAP method similar to the previous stage [9]. On the last days, rats in the first and third groups were sacrificed and dissected. Each of one gram liver and gastrocnemius muscle samples were preserved to determine the glycogen levels by using *Anthrone* method [14].

Data analysis were performed statistically by using *Statistical Product and Service Solutions* (SPSS) ver.17. Significant difference among treatment groups was examined by using *Analysis of Variance* (ANOVA) and followed by *Duncan Multiple Range Test* (DMRT).

3. Result and discussion

3.1. Blood glucose level of normal rats and glycemic index of cocoyam tuber

Blood glucose level of rats which treated pure glucose increased rapidly within 1 hour after treatment. It was higher than the blood glucose level of rats treated CT (Table 1). The increase level of blood glucose in rats which treated CT was rather similar to the control group. After 2 hours, the blood glucose level decreased slightly in rats treated pure glucose. Nevertheless it was higher than the blood glucose level before treatment. Meanwhile, the blood glucose level of rats which treated CT decreased close to the normal level. Blood glucose data of control group and CT group were used to determine AUC and calculate GI then. Cocoyam tuber had lower GI. It was 54. According to Rimbawan and Siagian [5], this score is categorized as low GI. Low GI food (<55) is considered as slow release carbohydrate. This carbohydrate can be broken down into monosaccharide slowly so the glucose releases into bloodstream last longer. This leads a gradual release of insulin to correspond glucose in the blood [15].

Table 1. Blood Glucose Level and Glycemic Index Score

Treatment	Average Blood Glucose Level (mg/dl) at hour			Blood Glucose Level Increase at hour		AUC (mg.hour/dl)	GI
	0	1	2	1	2		
	Control	115.8	148.6	188.5	32.8		
Pure Glucose	103.5	275	259	171.5	155.5	456.7	100
Cocoyam Tuber	104.4	141.9	113.4	37.5	9	250	54

According to Lenner et al [16], DM patients should consume low GI food. It would help patients improving their blood glucose control. Comparing to other tubers, CT has lower GI than potato, but higher GI than yam and taro. Glycemic index of steamed potato, yam and taro are 62, 41, and 47.7 respectively [17]. Most DM patients consume potato for daily diet though it has a medium GI.

3.2. Blood glucose level on DM rats

Rats were treated with STZ intraperitoneal in order to induce DM. The blood glucose rose in highly level over 150 mg/dl. It showed that all animals were in hyperglycemic condition. Barrett-Connor et al [18] stated, hyperglycemic condition is attained when blood glucose level is greater than or equal to 109 mg/dl. Blood glucose level increases because beta cells of pancreatic gland which produces insulin are damaged by STZ [19].

Table 2. Blood glucose, liver and muscle glycogen level on DM rats

Treatment	Blood glucose level (mg/dl)			Blood glucose level increase/decrease (mg/dl)		Glycogen level (µg/25 mg)	
	1 st day	3 rd day	6 th day	3 rd day	6 th day	Liver	Muscle
	Na CMC (placebo)	158.9	347.6	475	188.7	127.4	266.4
Glibenclamide (positive control)	288.7	369.7	434.9	81	65.2		
Cocoyam tuber	249	118.3	83.5	-130.7	-34.8	531.2	169.8

Blood glucose were changed in different level after treatments (Table 2). At the end of day 6, the blood glucose level of DM rats treated placebo and glibenclamide still rose. However, the blood

glucose increase in placebo group was insignificant ($p < 0.05$). Glibenclamide is a standard drug for DM treatment. Glibenclamide has hypoglycemic effect which stimulates insulin release in any glucose input [20]. Meanwhile, the blood glucose of DM rats treated CT declined in a significantly level back to normal glucose level (< 109 mg/dl). It was similar to the Folasire et al's [21] study. They concluded that blood glucose level of DM rats induced by alloxan declined as well after treated CT.

There are several possibilities why CT has hypoglycemic activity. Firstly it is because of CT has lower GI as previously described. Secondly, CT has antioxidant activity [22]. Vitamin C content on the CT is considered to this activity. Cocoyam tuber contains 2 mg/100 g vitamin C [23]. This is the most important antioxidant soluble in the plasma. It is clearer free radicals in the plasma. Regarding DM, Azrimaidaliza [24] stated that vitamin C acts as an antioxidant. It reduces oxidative damage which caused by free radicals, thus vitamin C prevents to the DM. In the DM which is induced by STZ, there is very reactive free radicals formations, e.g. NO, O₂ and H₂O₂. They damage several parts of pancreatic beta cells, such as cell membrane, protein and cause DNA fragmentation. Vitamin C from CT prevents beta cell damage, thus beta cell produces insulin as normal function.

Cocoyam tuber also contains flavonoid [25]. Any certain flavonoids have an antioxidant function [26] by acting as atom binder or free radicals scavenger [27]. Furthermore, several flavonoids capable to regenerate pancreatic beta cells. These flavonoids can stimulate progenitor cells in the pancreatic gland to differentiate and form new Langerhans Islet cells. Diosgenin is the most predominant phytochemical of CT [28]. It is known have anti-cancer activity which prevent cell proliferation and have hypoglycemic activity [29]. It reduces blood glucose level by decreasing lactase and maltase activity which play a role in carbohydrate breaking into simple sugar (monosaccharide). Breaking down of disaccharide in the intestines will be lower and glucose release into blood will be slower [30]. Diosgenin is an inhibitor for α -amylase and α -glucosidase enzymes which act in starch breaking down. Therefore this compound is the potential in DM treatment [31].

3.3. Glycogen levels of DM rats liver and muscle

In the DM rats which induced by STZ, the liver and muscle glycogen level increased (Table 2). The increase level was higher than normal individuals. The glycogen levels of the livers and muscle in normal rats are 227.56 μ g/25 mg and 79.91 μ g/25 mg respectively [30]. In this study, glycogen level of placebo group did not increase as high as CT group. The glycogen level of placebo group was rather similar to normal level. Whereas glycogen level of CT group increased significantly ($p < 0.05$), twice of normal level.

Liver and muscle have important roles on the body glucose homeostasis by involve in glycogen synthesis. In DM which induced by STZ, the glycogen synthase phosphatase enzyme becomes defective. Therefore its activity is lower [32]. Suarsana et al [33] stated that less glucose enters to the cell in DM patients. Cell lacks of glucose. It interferes glycogenesis and glycogen storage in liver and muscle as well. Otherwise, glycogen in liver and muscle will be catabolized. Glucose will be released to the bloodstream and it results hyperglycemia condition and low glycogen status in hepatocytes and muscle cells [34]. Table 2 shows that diabetic rats treated Na-CMC had lower glycogen level than DM rats treated CT. It might CT has a high glycogenesis activity, but there is no strong evidence supporting study yet. It should be further study to determine on the biochemical mechanism of CT.

4. Conclusion

The research results that blood glucose level of normal rats which treated CT decreased close to the normal level. Blood glucose of DM rats after fed CT decreased significantly ($p < 0.05$), similar to DM rats after treated by glibenclamide. Whereas glycogen level in DM rats after treated by CT was higher than in DM rats after fed standard feed. Cocoyam tuber increased glycogen level in DM rats significantly ($p < 0.05$) as high as in healthy rats. Glycemic index of CT was low. It was 54. Therefore CT is potential consumed as a functional food source for DM type II patients

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