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Research Article

HYPOGLYCEMIC AND ANTIOXIDANT ACTIVITIES OF BALINESE PURPLE SWEET POTATO (*IPOMOEA BATATAS L*) IN INDUCED-DIABETIC RATS

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ABSTRACT
Anthocyanins from plants are potent antioxidants, which may have beneficial effects against oxidative stress-related diseases, such as diabetes mellitus. Balinese purple sweet potato (*Ipomoea batatas* L.) is an anthocyanin-rich food source. This study was aimed to investigate the effect of water extract of Balinese purple sweet potato on blood glucose level and oxidative stress in diabetic rats. Subject of this study were 20 male adult rats divided into 2 groups with randomized pre-test and post-test control group design. Blood glucose, malondialdehyde (MDA), and total antioxidant levels of all rats were measured before and after treatment. After obtaining pre-test data, all of the groups were treated with streptozotocin (STZ) at 40 mg/kg body weight intraperitoneally to induce diabetic condition. Three days later, the treatment group of rats was treated with 3 ml/day of water extract of purple sweet potato for 60 days. The control group of rats was given 3 ml of water per day. Blood glucose, MDA, and total antioxidant levels were measured to obtain post-test data at day 30 and 60. The results showed that in control group, there was a significant ($p<0.05$) increase of blood glucose and MDA level, and significant decrease in the total antioxidant level when compared to treatment group. These results suggested that the water extract of purple sweet potato might be used to reduce the blood glucose, increase the antioxidant level and reduce the oxidative stress in rats with STZ-induced diabetes mellitus.

***Key Words**: Purple Sweet Potato, Blood Glucose, Total Antioxidant, Diabetic Rats*

INTRODUCTION
The ccomplication of diabetes mellitus (DM) is one of important human health problems. The prevalence of DM complication was estimated around 40% of the cases. The cause of this complication was the presence of oxidative stress as a result of hyperglycemia (Kataya, 2007). Hyperglycemia, when accompanied by oxidative stress, will increase the genesis of advanced glycation end products (AGEs). The increase of AGEs production will cause a decrease in endogenous antioxidants function and promotes the production of other free radicals. This will ultimately lead the body to the state of oxidative stress, thereby increasing the damage of macromolecules in various tissues (Tedgui and Mallat, 2006). Antioxidants treatment in diabetic patients may reduce oxidative stress (Lean, 1999 and Kataya, 2007).
Several studies revealed that oxidative stress can be prevented by various types of fruits and vegetables (Sanchez-Moreno, 2003; Prior, 2003 and Micallef, 2007), because they contain different types of natural antioxidants that are categorized as flavonoids, one of which is the pigment anthocyanins (Ghosh, 2007). Anthocyanins in addition to having antioxidant properties, is also believed to reduce the blood glucose level because it promotes insulin secretion (Jayaprakasam, 2004).
Purple sweet potato (*Ipomoea batatas* L.) of Bali is rich in anthocyanins (Suprapta et al., 2004), and has antioxidative effects *in vivo* in mice, rats, and rabbits with oxidative stress (Jawi, 2008; Jawi and Budiasa, 2011). This foodstuff is of medical and economic importance to be studied because it has been developed as commercially-available products such as syrup, juice and wine. The aim of this study was to prove the ability of water extract of Balinese purple sweet potato in attenuating the oxidative stress and lowering the blood glucose levels in rats with streptozotocin (STZ)-induced DM. To our knowledge, this is the first

study on the effect of Balinese sweet purple potato on blood glucose and endogenous antioxidant level in diabetic rats.

MATERIALS AND METHODS

Preparation of the Extract

Water extract of purple sweet potato tuber was prepared by the following procedure: purple sweet potato tubers obtained from the farmers in the area of Tabanan Bali, were washed with tap water and then peeled. The tuber was cut into small pieces (approx. 2 cm x 2 cm x 2 cm) steamed for an hour. The steamed tuber was blended in a blender with distilled water (1:2, w/v). Filtration using three layers of cheese cloth to obtain the filtrate. The filtrate was boiled for 30 minutes and kept under room temperature before use. The content of anthocyanin in this filtrate was measured and account for 146 mg/ml of filtrate.

Induction of Diabetes

Wistar male rats (150 to 200 g) were obtained from animal house facility of Gadjah Mada University, Yogyakarta, Indonesia. They were maintained under standard laboratory conditions at 25 ± 2°C, with relative humidity at 50 ± 15%, and normal photoperiod (12 hours light-dark cycle). Commercial pellet diet and water were provided *ad libitum*. After 18 h of fasting, the rats were injected intravenously through the tail vein with a single dose of 40 mg/kg STZ (Sigma, St. Louis, Mo, USA), freshly dissolved in citrate buffer (pH 4, 5). After injection, the rats had free access to food and water, and were given 5% glucose solution to drink overnight to counter hypoglycemic shock. Diabetes mellitus in rats was observed by moderate polydipsia and marked polyuria. Three days after the STZ injection, the fasting blood glucose levels were determined by orthotoluidine method. The rats showing fasting blood glucose more than 200 mg/dl were considered diabetic and selected for the experimentation.

Experimental Design

Diabetic rats were randomly divided into 2 groups (n = 10 for each group) with the following treatments: group 1 was treated with STZ + water (3 ml/day); group 2 was treated with STZ + water extract of Balinese purple sweet potato (3 ml/day). The water and the extract were given orally for a period of 60 days, beginning from 3 days after STZ administration.

Blood Collection and Biochemical Analysis

Blood samples were obtained from the tail vein of both groups of rats on day 1, 3, 30, and 60 after diabetes mellitus induction with STZ. The specimens were collected for the measurements of blood glucose, MDA, and total antioxidant level. Blood glucose level was examined using GlucoDr with stick merk easy touch. MDA level was determined by measuring Thiobarbituric Acid Reactive Substances concentration. Briefly, 750 µL of phosphoric acid were pipetted into 13 mL polypropylene tube. Fifty microliters of TEP standards were then added. The mixture was thoroughly mixed and 250 µL of 40 mM TBA solution was added. Finally, 450 µL of distilled water was added to each tube, and the tubes then covered tightly. This mixture then boiled for 1 hour. After boiling, the tubes were then placed into an ice bath to cool the samples. The cooled samples were mixed well and applied to Sep-Pak C_{18} column. The column was prepared by washing it with 5 ml of methanol followed by distilled water wash. The TBARS were eluted from the column with 4 ml of methanol. Total antioxidant level was measured using ABTS-containing RANDOX kit (RANDOX Laboratories Ltd., Ardmore, UK, and BT29). ABTS (2, 2´-Azino-di-[3-ethylbenzthiazoline sulphonate]) was incubated with a peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation ABTS. This has a relatively stable blue-green color, which was measured at 600 nm. Antioxidants in the added sample cause suppression of this color production to a degree which is proportional to their concentration.

Statistical Analysis

Statistical analysis was carried out using SPSS for Window (version 17.0). All data were expressed as mean ± SD. Groups of data were compared by using *t*-test. Values were considered statistically-significant when $p < 0.5$.

RESULTS

Administration of STZ to rats resulted in significant increase of blood glucose level and MDA level in one hand, while on the other hand reduced the level of total antioxidant. The blood glucose in the rats of treatment group tend to decrease on the day 30 and 60 (Figure1). On the day 30, the blood glucose level accounted for 178 mg/dl, and then decreased significantly to become 100 mg/dl on the day 60 (Table 1). When compared to control group, treatment with the water extract of Balinese sweet potato reduced the blood glucose level about 52.83%. A similar trend was also observed on the level of MDA, in which the MDA level in the blood of treatment group decreased significantly on the day 30 and on the day 60 (Figure 2). The MDA level of the treatment group on the day 30 was 5.9 mmol/l and decreased significantly to become 2.9 on the day 60. When compared to control, the decrease of MDA level at the day 60 was about 65.88%.

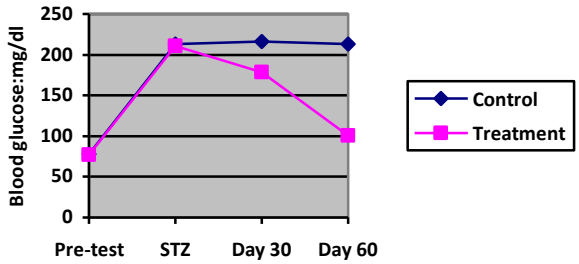

Figure 1: The comparison of blood glucose level between control and treatment groups

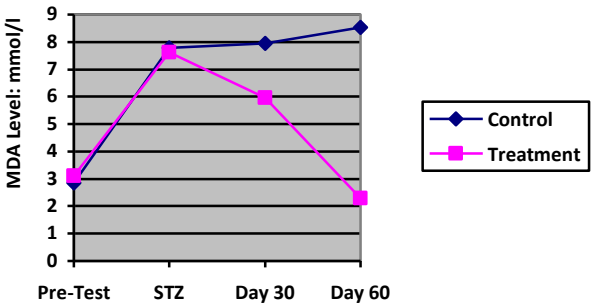

Figure 2: The comparison of blood MDA levels between control and treatment groups

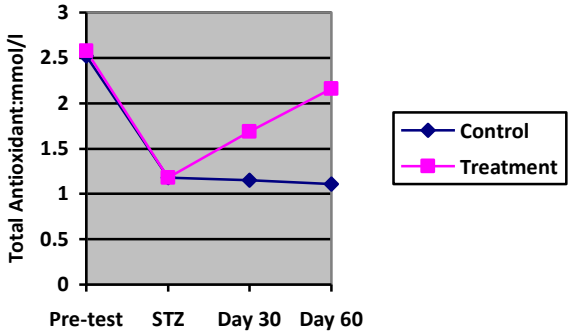

Figure 3: The comparison of blood total antioxidant level between control and treatment groups

Table 1: Change in glucose, MDA and total antioxidant levels in blood of STZ diabetic induced-rats treated with water extract of Balinese purple sweet potato

Group	Glucose level (mg/dl)		MDA level (mmol/l)		Total antioxidant level (mmol/l)	
	Day 30	Day 60	Day 30	Day 60	Day 30	Day 60
Control	216±4,9	212±4,6	7,9±0,3	8,5±0,4	1,1±0,04	1,1±0,05
Treatment	178±3,4 (17.59%)*	100±1,9 (52.83%)*	5,9±0,2 (25.31%)*	2,9±0,3 (65.88%)*	1,6±0,04 (45.45%)**	2,2±0,05 (100%)**

** Percentage of decrease compared to control group.*
*** Percentage of increase compared to control group.*

On the other hand, treatment with the water extract of Balinese purple sweet potato significantly increased the total antioxidant level in the blood of rats of treatment group from 1.6 mmol/l on the day 30 to 2.2 mmol/l on the day 60 (Figure 3, Table 1), while the total antioxidant level in the blood of rats of control group remained 1.1 mmol/l. This result indicated that 100% of the total antioxidant level increased in the treatment group when compared to control group.

DISCUSSION

Purple sweet potato grown in Bali has been known to contain relatively high anthocyanin which was ranged from 100 to 210 mg/100 g fresh weight (Suprapta *et al.,* 2004). This pigment can regulate the blood glucose levels through several mechanisms. A research on the ability of anthocyanins in regulating blood glucose level had been carried out *in vivo* in animals and *in vitro* in tissue culture, and showed that there were two types of anthocyanins that could increase insulin secretion of β cells of the pancreas in tissue culture, namely cyanidin-3-glucoside and delphinidin-3-glucoside (Jayaprakasam, 2004). This study suggested that consuming fruits and vegetables containing anthocyanins may reduce the incidence of DM (Ghosh, 2007), because anthocyanins increase insulin secretion and may protect β cells of the pancreas from apoptosis (Nizamutdinova *et al.,* 2009). Anthocyanins also have inhibitory effect on α-glucosidase enzymatic activity, so it can prevent the increase of postprandial blood sugar (Ghosh, 2007). Anthocyanins may also increase the phosphorylation of insulin receptor, thereby increasing the entry of glucose into the tissues and lowering blood glucose level (Nizamutdinova *et al.,* 2009).

In the present study, treatment with water extract of purple sweet potato resulted in hypoglycemic effect. Previous study also proved that water extract of purple sweet potato could maintain blood sugar level after a high oral glucose load in healthy rats (Sutirta-Yasa and Jawi, 2010). Anthocyanins are natural pigments that are water-soluble, and cause a variety of colors in fruits, leaves, and flowers of plants. Balinese purple sweet potato is rich in anthocyanins (Suprapta, 2004), so the levels of anthocyanin in the water extract of this foodstuff is also high, allowing the occurrence of anthocyanin-mediated effects to lower blood glucose level significantly. These findings were consistent with the study on the incidence of type 2 DM, in which there was a decline in the incidence of diabetes in people who consumed anthocyanin-rich foods from various plants (Wedick *et al.,* 2012).

Source of ROS production under hyperglycemia condition is glucose oxidation that produces superoxide ions and the interaction of glucose with proteins to form Advanced Glycation End-products (AGEs) (Maritim *et al.,* 2003). Increased AGEs production will lead to increased enzymatic activity of NAD (P) H oxidase so that superoxide ion formation is also increased (Gao and Mann, 2009). Water extract of purple sweet potato which contains anthocyanin could reduce the blood glucose level in this study, so it will minimize the formation of AGEs (Tedgui and Mallat, 2006), and will ultimately reduce blood MDA level and increase the total antioxidants level. Group of rats treated with the water extract of purple sweet potato for 30 days showed significant change in blood MDA and total antioxidant level. Anthocyanin found in purple sweet potato (Suprapta *et al.,* 2004), is one of many types of antioxidants that can prevent oxidative stress. The results were consistent with studies conducted *in vitro* against the purple sweet

potato extract that showed good antioxidant properties (Padda, 2006). These results correspond well with *in vivo* studies conducted in mice that suffered oxidative stress due to strenuous physical activities. Water extract of Balinese purple sweet potato was able to reduce heavy physical activity induced-MDA level in mice blood and various organs, such as liver and heart (Jawi *et al.*, 2008). Based on the results of this study can be concluded that the treatment with the water extract of Balinese purple sweet potato significantly could reduce the blood glucose and MDA levels, and increase the total blood antioxidant level in rats with STZ-induced diabetes mellitus.

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