Aqueous Extract of Purple Sweet Potato Tubers Decrease MDA and Increase SOD2 in Kidney of Diabetic Rats

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Diabetes mellitus (DM) is a metabolic disease characterized by increase of blood glucose levels or hyperglycemia. Hyperglycemia results in oxidative stress and increase of AGEs. Oxidative stress and metabolic stress lead to reduction of endogenous antioxidant activity resulted in various organs injury especially kidney. The complication is caused by the formation of advanced glycation end products (AGEs), and free radicals. Increase free radicals that occur in diabetics accompanied with decline in endogenous antioxidant activity, such as superoxide dismutase (SOD) and catalase. One way to overcome the complications due to oxidative stress is through utilize the foods which possess natural antioxidant such as purple sweet potato. It has been proven in animals that purple sweet potato had antioxidant properties to prevent various complications in DM. Balinese purple sweet potato tubers contain high anthocyanin and it had been researched as antioxidant in rats in vivo. The aims of this research are to determine the effect of aqueous extract of purple sweet potato tubers to decrease malondialdehyde (MDA) and increase SOD2 in kidney of diabetic rats. This experimental study was conducted with randomized posttest only control group design. Research subject is 38 diabetic rats that divided into 2 groups, controlled group and treatment group, with 18 diabetic rats in each group. Treatment was given for 60 days to each group. Treatment group (P1) is diabetic rats group that was given a standard diet and ad libitum drinks, and 4CC/day/rat of purple sweet potato tubers aqueous extract for 60 days. Controlled group (P0) is diabetic rats group as control that was given a standard diet and ad libitum drinks only. After 60 days of treatment, evaluation was performed. The results of this research found that mean MDA in kidney of control and treatment group was 8.40±0.17 and 1.47±0.15, respectively. There was a significant decrease of MDA (p<0.05). Relative expression of SOD2 gene before and after treatment was 0.317 and 6.586, respectively. There was a significant increase of SOD2 gene (p<0.05). It can be concluded that aqueous extract of purple sweet potato tubers decrease MDA and increase SOD2 gene in kidney of diabetic rats.

Keywords: Aqueous extract of purple sweet potato tubers, MDA, SOD2, kidney, diabetic rats.

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INTRODUCTIONS

Diabetes mellitus (DM) is a major health issues nowadays, since it generates various complications to organs. The complications that occur are caused by chronic hyperglycemic state, which occur in DM, and lead to formation of advanced glycation end products (AGEs), and other free radicals. Increase formation of free radicals accompanied by decrease of endogenous antioxidant, for instance superoxide dismutase (SOD) and catalase, causing oxidative stress.

Administration of antioxidant to diabetic resolve macro vascular complications, microvascular complications, also tissue damage due to oxidative stress. Flavonoid, obtained from plant-based foods, possess antioxidant effect and is protective to oxidative stress.

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Anthocyanin is one of flavonoids, and increase endogenous antioxidant gene expression mediated by Nrf2 activation, and regulate expression of genes that related to inflammation. Purple sweet potato in Bali possess high anthocyanin levels, and proved to manage oxidative stress in mice. Beside its function as
Antioxidant, aqueous extract of purple sweet potato maintain blood glucose levels and increase total antioxidant in mice with high dose glucose load.\textsuperscript{13} It decreases glucose levels and MDA, along with increase total antioxidant in diabetic rats blood,\textsuperscript{14} and elevate blood SOD in normal rabbits.\textsuperscript{13} SOD elevation lower renal damage due to oxidative stress in transgenic mice’s renal. Administration of resveratrol flavonoid and various antioxidants resolve oxidative stress in renal tissue diabetics.\textsuperscript{16}

Flavonoid is a compound with low molecular weight, contained in plants, and generally act as the pigment that gives color to the leaves and other parts of plants.\textsuperscript{18} It also regulates signal transduction inside cells.\textsuperscript{18} Effects of flavonoid to biological system are different depending on the type and chemical structure of flavonoid. In accordance with its chemical formula, flavonoid genistein increases Glutathione-S-transferase levels in cells, protecting the cells from various genotoxic agents.\textsuperscript{19} Effect of flavonoid toward ROS occur by two mechanisms, which are endogenous antioxidant elevation and capturing/neutralizing free radicals. Increase of endogenous antioxidant by flavonoid has been proven in in vitro research through elevation of Nrf2 transcription factor, lead to increase of HO-1 protein expression.\textsuperscript{20}

**RESEARCH METHODS**

This experimental study conducted with randomized control group post- test only design. A total of 36 male Wistar rats aged 3-4 months adapted for 2 weeks, and intraperitoneal streptozotocin was given. Three days after administration of streptozotocin, fasting blood glucose examination was performed. Fasting blood glucose above 200 mg/dl considered as DM. Random allocation was performed to 36 diabetic rats, divided into 2 groups with 18 diabetic rats in each group. The treatments of each group are as follows: Treatment group (P1): diabetic rats group that were given a standard diet and ad libitum drinks, and 4CC/day/rat of purple sweet potato tubers aqueous extract for 60 days. Evaluation was performed after 60 days of treatment. Controlled group (P0): diabetic rats group as control that were given a standard diet and ad libitum drinks. After 60 days of treatment all rats were sacrificed with ether anesthetic. Its kidneys were taken and examination of SOD and MDA were performed on kidney. RNA isolation conducted with trizol method and RT-PCR was performed to determine the amount of SOD2.

**RESULTS**

Mean renal MDA in controlled and treatment group were 8.40 and 1.47, respectively (table 1). Analysis with independent t-test showed p = 0.001. This indicates significant decrease of renal MDA (p<0.05) in both groups after treatment.

| Table 1. Effect of Purple Sweet Potato Extract to MDA in Kidney of Diabetic Rats |
|-----------------------------|-------------------|------------------|------------------------------|
| **Subject groups** | **n** | **Mean renal MDA** | **p-value** |
| Control | 18 | 8.40 | 0.001 |
| Treatment | 18 | 1.47 | |

Relative expression of SOD2 gene before and after treatment were 0.317 and 6.586, respectively. Analysis with independent t-test showed p = 0.001. This indicates that mean renal SOD2 in both groups after treatment increase significantly (p<0.05).

| Table 2. SOD2 Gene Expression in Kidney of Diabetic Rats |
|-----------------------------|-------------------|------------------|------------------------------|
| **Subject groups** | **n** | **Mean renal SOD2** | **p-value** |
| Control | 18 | 0.317 | 0.001 |
| Treatment | 18 | 6.586 | |

**DISCUSSION**

There was significant change in blood MDA and SOD of diabetic rats group treated with aqueous extract of purple sweet potato tubers for 60 days. Mean renal MDA in controlled and treatment group were 8.40 and 1.47, respectively. Analysis with independent t-test showed p = 0.001. This indicates significant decrease of renal MDA (p<0.05) after treatment. Mean relative expression of SOD2 gene before and after treatment were 0.317 and 6.586, respectively. Analysis with independent t-test showed p = 0.001. This indicates that mean renal SOD2 in both groups after treatment increase significantly (p<0.05).

This study showed significant decrease of MDA and increase of SOD activity (p<0.05). Anthocyanin, possessed by purple sweet potato tubers, increase SOD levels and decrease MDA in blood of diabetic rats. Aqueous extract of purple sweet potato tubers had nephroprotective effect through SOD elevation and MDA reduction of diabetic rats renal tissue, and also increase serum creatinine and decrease BUN levels in blood.\textsuperscript{14} Anthocyanin in purple sweet potato tubers is an antioxidant that prevent oxidative stress in vivo and decrease MDA.\textsuperscript{12} This result is in accordance with research conducted by Tedgui and Mallat in 2006.\textsuperscript{21} They found that administration of aqueous extract of purple sweet potato tubers, which possessed anthocyanin, decrease MDA levels. Research conducted by Kataya and Hamza in 2007 also found similar results.\textsuperscript{1} It was observed that administration of red cabbage extract, which contain a high anthocyanin, decrease MDA in renal tissue of diabetic rats significantly.

Research by Herawati in 2013 found that consumption of purple sweet potato anthocyanin
Effects of flavonoids against ROS occur in two mechanisms, which are increase endogenous antioxidant and capture/neutralize free radicals. Increase of endogenous antioxidant by flavonoids has been proved by in vitro research through increase of Nrf2 transcription factor, lead to elevation of HO-1 protein expression. Flavonoid quercetin able to inhibit lipid peroxidation, directly or indirectly through antioxidant enzymes elevation such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione (GSH) in mice. Whereas curcumin extract, also contains flavonoid, able to increase SOD levels and catalase also decrease malondialdehyde levels in liver tissue that undergo reperfusion/oxidative stress. Other research that explore the effect of anthocyanin from chokeberry fruits showed SOD and CAT enzyme elevation in TC3 pancreatic β cells culture exposed with hydrogen peroxide and high dose glucose invitro. This research showed similar result although the pancreatic tissue was not examined, where there was increase of SOD2 expression. Administration of anthocyanin derived from purple corn able to prevent glomerulosclerosis in human renal tissue culture exposed with high dose glucose (Li et al., 2012).

CONCLUSIONS
Aqueous extract of purple sweet potato tubers decrease MDA and increase SOD2 expression in kidney of diabetic rats.

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