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RESEARCH ARTICLE

Water Exctract of Purple Sweet Potato Increase Superoxide Dismutase, Catalase Genes and Decrease Mda Level in Multiple Organs of Diabetic Wistar Rats

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Abstract: Objective: The purpose of this study was to prove the water extract of purple sweet potatoes can increase superoxide dismutase (SOD) gene expression and catalase (CAT) and decrease malondialdehyde (MDA) in vital organs of diabetic rats. Method: This study was randomized experimental design with posttest only control group design. Sample divided into 2 groups: control and treatment groups, respectively 18 diabetic Wistar rats. The control group will be given streptozotocin. The treatment group was given streptozotocin and water extract of purple sweet potato 4 cc/day. This treatment is carried out for 2 months. Result: Water extract of purple sweet potato was proven significantly increased mRNA expression of aorta and kidney SOD but decreased SOD mRNA expression in the liver ($p < 0.05$). This water extract also increases SOD mRNA expression in cardiac but not statistically significant ($p > 0.05$). Water extract of purple sweet potatoes also found significantly increased renal and cardiac expression of CAT mRNA ($P < 0.05$), but not statistically significant ($p > 0.05$). Conclusion: Purple sweet potatoes extract to decrease the expression of liver CAT mRNA non-significantly, and decrease MDA in liver and kidney of diabetic Wistar rats significantly ($P < 0.05$).

Keywords: *MDA, mRNA CAT, mRNA SOD, Purple sweet potato extract.*

Introduction

Diabetes mellitus (DM) is a major health problem until today because it leads to various complications at various organs. These complications are caused by chronic hyperglycemia state which enhancing the formation of advanced glycation end products (AGEs) and free radicals [1,2]. Oxidative stress in DM occurs due to an imbalance of the number of free radicals and the number of endogenous antioxidants produced by the body such as superoxide dismutase (SOD) and catalase, resulting in oxidative stress [3]. Increasing of SOD and Catalase and other antioxidants may reduce complications of DM [4].

Supplementation of exogenous antioxidants in patients with DM will overcome microvascular complications, macrovascular and overcome tissue damage due to oxidative stress [1,5]. Flavonoids from various foodstuffs derived from plants have antioxidant effects which are protective against oxidative stress [6-8]. Anthocyanin is one of flavonoids and antioxidants can increase endogenous gene expression mediated activation of Nrf2 [9,10]. Anthocyanin from blueberries can improve SOD through Nrf2 activation [11] and regulates the expression of genes associated with inflammation [12].

Balinese purple sweet potatoes have a high level of anthocyanin [13] and proved to be able to cope the oxidative stress in diabetic rats [14]. Water extract of purple sweet potato can also maintain blood sugar levels and increase the total antioxidant in Wistar rats given high doses of glucose load [15]. Purple sweet potato water extract can lower blood glucose and MDA expression. In diabetic Wistar rats its effect also known to increase the antioxidant in the blood of diabetic rats[16] and increase SOD to a higher level in the normal rabbit [16]. Based on this evidence, water extract of purple sweet potato will increase endogenous antioxidant in various organs by

enhancement mechanisms of mRNA gene expression of SOD and catalase. To prove these allegations, then a study on diabetic Wistar rats with was done by administration of streptozotocin. Wistar rats suffering from diabetes are given a water extract of purple sweet potatoes. Evaluation of the expression of these genes in various vital organs was investigated by sacrificing the rats.

Materials and Methods

This study was experimental research design with randomized control group post-test only. The study was conducted for 60 days. Thirty-six Wistar rats were divided into diabetic (DM) and control groups. After adaptation for 2 weeks, each rat was administrated with intraperitoneal streptozotocin. Fasting blood sugar was checked on the 3rd day of administration. If the fasting blood sugar levels above 200 mg/dl, the rats were considering as DM rats and allocated randomly into two groups. Each group consists of 18 rats.

Research Procedure

The treatment group 1 (P1): a group of diabetic rats was given a standard diet and given water ad libitum, and water extracts of purple sweet potato as much as 4 ml/day. After the 30th day of treatment, 9 rats of the P1 group sacrificed as one group (O1). The rest of the group continued as O3, until 60th day for a final evaluation. The control group (P0) is a group of diabetic Wistar rats was only given standard food and water ad libitum.

In Po group after treatment for 30 days, 9 rats were sacrificed as a group (O2). The rest continued as an O4 group until the 60th day and a final investigation was done in many organs of them. After 30th day and 60th day of treatment, SOD mRNA was investigated in the aorta and kidney. mRNA PCR performed with the following procedures: All the rats were sacrificed with ether anesthesia and taken of aorta and kidney. The aorta was identified and taken along 5 cm. RNA isolation was conducted by trizol RT-PCR examination to determine the amount of mRNA of SOD and catalase.

Trizol RT-PCR Method

Examination of the mRNA was performed by PCR method following these procedures:

- All the rats were sacrificed with ether anesthesia and taken off the heart, aorta, liver, and kidneys. Aorta was identified and taken along the 5 cm.
- RNA isolation was conducted by the trizol RT-PCR examination to determine the amount of mRNA of SOD and catalase.
- The briefly trizol RNA isolation method is as follows:

- All samples were homogenate, then 750 µl trizol reagent added. After that homogenate solution was incubated for 5 minutes at room temperature.
- Add 200µL of chloroform, vortex for 15 seconds, then incubated for 15 minutes. The sample will centrifuge at 13.000 rpm for 15 minutes. Separate the top layer into a sterile tube.
- Then 500 microliter of isopropyl alcohol was added, vortex, incubated for 10 minutes at room temperature. Centrifuge the sterile solution at 13.000 rpm for 10 minutes. The supernatant was discarded, add 70% alcohol and did the second centrifugation.
- Add 20µL RNAse-free water and ready to do RT-PCR.

Data selections, editing, coding, and analysis, insert to file navigator using SPSS 17.0.

Results

Expression of SOD2 and Catalase mRNA in Heart

In this study, we found the relative expression of SOD2 mRNA between O3 and O1 group was 2.440 with p-value = 0.312 (95% CI: 0197-13655). Relative expression of catalase mRNA between O3 and O1 group was 1.872 with p-value > 0.05 (95% CI: 0158-8731 p = 0.576). All of the expression increased but not statistically significant as seen in fig. 1.

Relative expression of SOD2 mRNA between O4 and O2 group was 1.668 (95% CI: 0.858 to 4.625; p = 0.338), meanwhile relative expression of catalase mRNA between O4 and O2 groups was 1.309 (95% CI: 0.502 to 3.084; p = 0.574). Both groups were increased but not statistically significant (fig. 2).

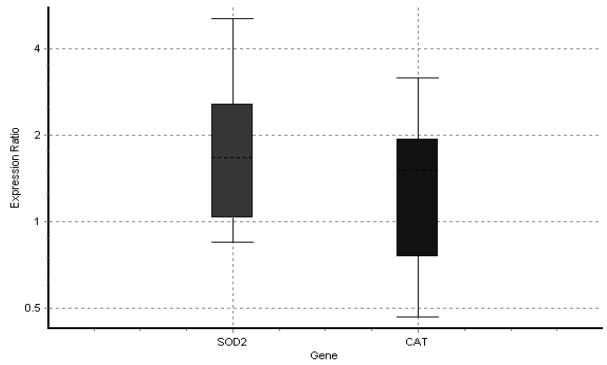

Figure 1: The O3 group vs O1 (after and before treatment)

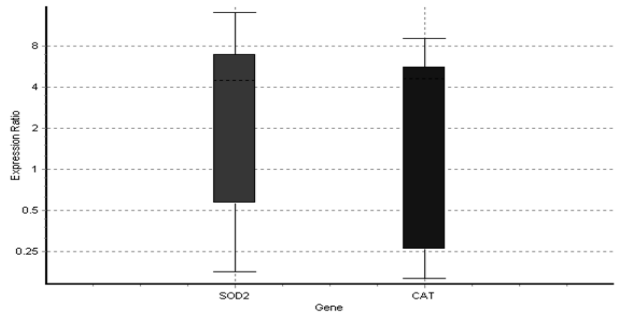

Figure 2: O4 group vs O2 (control group, after and before treatment)

Expression of SOD2 and Catalase mRNA in Aorta

In the other hand, evaluation of relative expression level of SOD2 mRNA between O3 and O1 group was 2.456 (95% CI: 1054-7975; p = 0.000). In these groups, we found a statistically significant increasing of the mRNA expression as seen in fig. 3. Catalase gene mRNA relative expression between O3 and O1 was 2,279 (95% CI: 0969-6505; p = 0172) increased but not statistically significant.

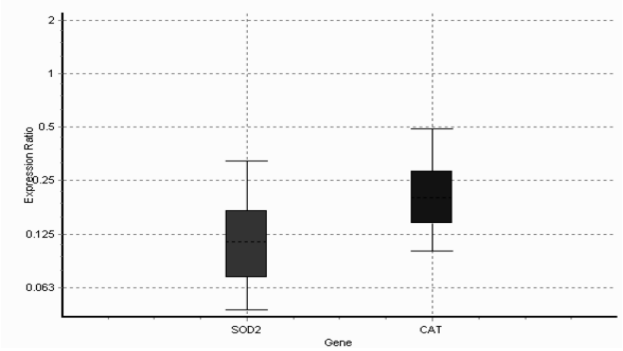

Figure 3: Relative expression of SOD2 mRNA level between O3 and O1 group

Relative expression of SOD2 gene mRNA between O4 and O2 group was 0.112 (95% CI: 0.050-0.294; p = 0.031). Its level was lower than 30th-day group and the number was statistically significant.

The same result also showed by catalase relative expression between O4 and O2 was 0.214 (95% CI: 0.108-0.474; p = 0.031) as seen in fig. 4.

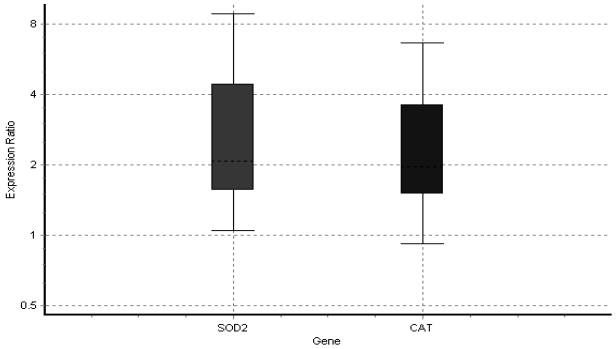

Figure 4: O4 group vs O2 (control after vs before)

Expression of SOD2 gene mRNA and Catalase in Hearts Tissue

SOD2 gene mRNA relative expression between O3 and O1 was 1.577 (95% CI: 0.776-2.996; p = 0.237) increased and the catalase relative expression between O3 and O1 was 1.298 (95% CI: 0.510-3.513; p = 0.431) increased but not statistically significant.

In the other group, we found that SOD2 gene mRNA relative expression between O4 and O2 was 3.300 (95% CI: 1.327-8.916; p = 0.048). It showed an increasing of expression and significant creation. CAT gene mRNA relative expression between O4 and O2 was 1.664 (95% CI: 0.828-3.666; p = 0.099) increased but not statistically significant (fig. 5 and fig. 6).

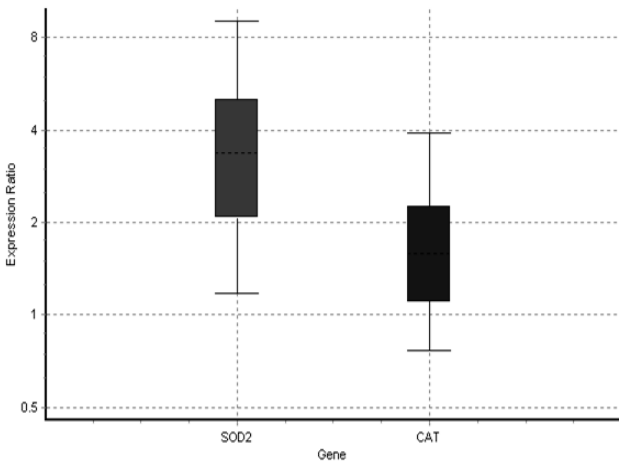

Figure 5: Evaluation of O3 group and O1 group (treatment group, after vs before)

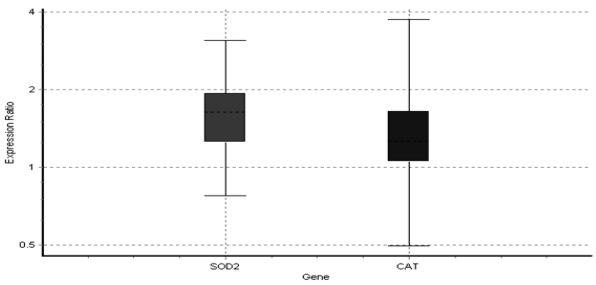

Figure 6: Evaluation of O4 group vs O2 (control group, after vs before)

Expression of SOD2 and catalase gene mRNA in kidney tissue

SOD2 gene mRNA relative expression between O3 and O1 groups found about 6.586 (CI 95%: 4.333-10.094; p = 0.050), it is a statistically significant increase. Catalase gene mRNA relative expression between O3 and O1 groups found about 64.433 (95% CI: 47.319-99.668; p = 0.000). This is statistically significant increasing of catalase gene expression.

SOD2 gene mRNA relative expression between O4 and O2 groups was 0.317 (95% CI: 0.250-0.418; p = 0.016), it decreased and statistically significant. In the other hand, catalase mRNA relative expression between O4 and O2 group which is getting the purple sweet potato treatment was 0.517 (95% CI: 0.261-1.152; p = 0.201) as seen in fig. 8.

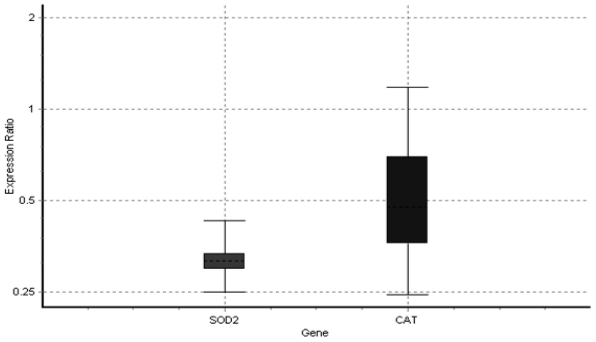

Fig. 7: Evaluation of SOD2 and catalase expression in treatment group (after - before)

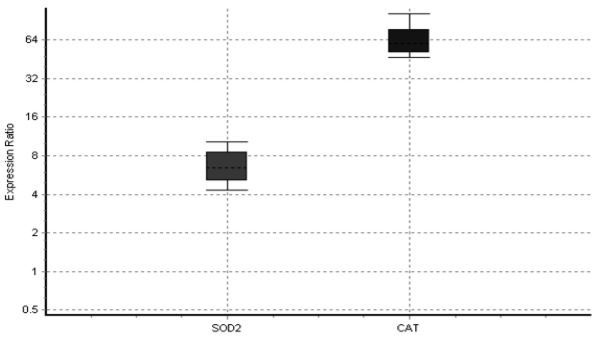

Fig. 8: Evaluation of SOD2 and catalase expression in control group (after - before)

Effect of Purple Sweet Potato Extract to mRNA Expression of SOD2 and Catalase

Analysis of the effect of purple sweet potato extract to SOD2 and catalase expression of both groups (control and treated group) in various organs presented in Table 1 below. Analysis of hepatic MDA level after administration for 30 days on diabetic Wistar rats was measuring by independent t-test which is presented in Table 2.

Table 1 shows the mean hepaticMDA expression after 60 days of treatment, in control group is 6,89±0,42 and mean of treated group is 3,99±0,28 (*t value* = 15,26 ; p = 0,001), it significantly different after 30th day of treatment between two groups (p<0,05). Table 2 shows the mean hepatic MDA measurement in control group was 8,06±0,22 and in treated group was 1,92±0,23. Independent-t test analysis found *t value* = 51,01 and p value = 0,001. It showed an significanlt different between two groups.

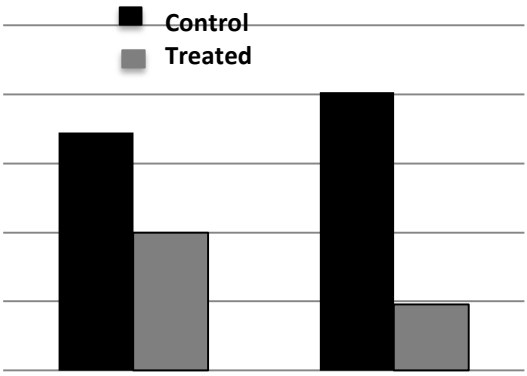

Figure 9: Comparison of liver MDA between the control group treatment group

Purple Sweet Potato Extracts Against MDA in Kidney Tissue After Treatment for 30 and 60 Days

Table 3 shows the difference between renal MDA mean between the control group and treated group after 60 days of treatment. Its shows that mean MDA in control group was 8.40±0,22 and in the treated group was 1.47±0.23. Independent –T-test shows that this difference was significant with *t values* = 80,57 and *p-value* = 0,001 (p<0,05).

Evaluation of the MDA levels after the 30th day of treatment also shows a significant difference between treated and untreated

Wistar rats. Mean MDA level in untreated Wistar was 5,91±0,19 and treated rats was 3,29±0,24 with *t value* = 22,49 and *p-value* = 0,001.

Table 1: Expression of SOD2 and catalase mRNA in heart, aorta, liver and kidney in both groups

	Heart		Aorta		Liver		Kidney	
	Treated	Control	Treated	Control	Treated	Control	Treated	Control
SOD2	2.440	1,668	2.456*	0.112*	1.577	3.300*	6.586*	0.317*
CAT	1.872	1.309	2.279	0.214*	1.298	1.664	64.433*	0.517

Table 2. Comparison between liver MDA mean in control and treated group after 60 days

Group	N	Mean of MDA in Hepar	SB	*T*	P
Control	**7**	8,06	0,22	**51,01**	**0,001**
Treated	**7**	1,92	0,23		

Table 3: Comparison of MDA mean in kidney tissue between control and treated group after 60 days

Group	N	Mean MDA in Kidney	SB	*t*	P
Control	**7**	8,40	0,17	**80,57**	**0,001**
Treated	**7**	1,47	0,15		

Discussion

Balinese purple sweet potato has high levels of anthocyanin [13] and proved to be able to cope the oxidative stress in diabetic Wistar rats [14]. Water extract of purple sweet potato can also maintain blood sugar levels and increase the total antioxidant in Wistar rats given high doses of glucose load [16]. In diabetic rats, its effect also is known to increase the antioxidant in the blood of diabetic Wistar rats [16].

Flavonoid quercetin can inhibit lipid peroxidation, directly or indirectly through increased antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione (GSH) in Wistar rats [17], while curcumin extract which also contains flavonoid can increase the levels of SOD and catalase as well as lower levels of MDA in liver tissue [18].

This study shows the water extract of purple sweet potato increased mRNA expression of SOD in aorta and kidney significantly but decreased SOD mRNA expression in the liver. In our investigation, water extract of purple sweet potato increased the expression of mRNA SOD2 in the heart but not statistically significant. Purple sweet potato water extract significantly increased the expression of mRNA and improve expression of catalase mRNA in the heart, aorta, but not meaningful.

This study showed the opposite results with curcumin which curcumin enhances SOD and catalase in the liver which exposed to oxidative stress, while the water extract of purple sweet potato decrease SOD2 level in the liver significantly. This result explained by the hepatoprotective effect of curcumin.

Other studies about the effects of anthocyanin from chokeberry fruits showed increased activity of SOD and catalase in cultures of pancreatic ß cells TC3 were exposed to hydrogen peroxide and high-dose glucose in vitro [19]. In this study, although examination of pancreatic tissue was not done, similar results was showed: there is an increase in mRNA expression of SOD2 and catalase in the heart, aorta, and kidney. Meanwhile, some of the results are not meaningful.

Purple Sweet Potato Extract Affects MDA Level in Liver and Kidney

Streptozotocin given to Wistar rats leads to hyperglycemia state and cause the oxidative stress in the kidney thereby increasing the MDA in kidney tissue [1.20,21], and lead to impaired renal function [22]. The results of MDA level in liver tissue of Wistar rats are presented in Table 1 and Table 2, while the results of the MDA expression in kidney tissue are presented in Table 3.

Administration of purple sweet potato extract in diabetic Wistar rats for 30 days and 60 days decreased MDA level significantly ($p < 0.05$) compared to control group. The results are consistent with research conducted by Tedgui and Mall (2006) which reported similar result [23].

Provision of purple sweet potato water extract contains anthocyanins can lower

blood sugar level that will minimize the formation of AGEs [23], increase SOD and decrease MDA level in DM rats. Water extract of purple sweet potato has nephroprotective activity by increasing SOD expression and decreasing MDA expression in kidney tissue, as well as increasing creatinine serum [16]. Administration of purple sweet potato water extract in this study proved to decrease MDA expression in kidney tissue in diabetic Wistar rats.

This study is consistent with study held by Herawati (2013), which reported that consumption of purple sweet potato anthocyanin extract can lower blood glucose, increase blood antioxidant status by increasing the value of ferric reducing antioxidant power (FRAP) and decreased levels of MDA thereby inhibit β cell damage [24]. The effect of purple sweet potato extract as well as flavonoids function which binds the free radicals so it can prevent the activity of its to form the AGEs [25,26]. Provision of anthocyanin extracted from purple corn in human kidney culture was proven can inhibit glomerulosclerosis [27].

The results are consistent with research declared by Kataya (2007) that reported the administration of red chili (high anthocyanin) in diabetic mice can lower MDA expression in kidney tissue significantly [1]. This study also consistent with an internal study conducted by Jawi et al. (2008) in pharmacology department of Udayana University. The study reported that the administration of water extract of purple sweet potato can reduce levels of MDA in the blood, the liver, the heart and the intestine after administration of the maximum load in mice. Purple sweet potato extract can be as exogenous antioxidants [14-16].

Conclusion

Water extract of purple sweet potato can reduced oxidative stress through the mechanism of reduction in MDA expression in renal and liver tissue of diabetic Wistar rats ($p < 0.05$), increasing expression of SOD mRNA in kidney, aorta and heart tissue, and also increasing gene expression of catalase in kidney, aorta and heart of diabetic Wistar rats. Meanwhile, it also decreased mRNA expression both of MDA and catalase in the liver organ. Further research is needed to investigate the activity of Nrf2 as basic molecular of SOD2 and catalase mRNA expression.

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