

ANTIOXIDANT EFFECT OF PURPLE EGGPLANT FLOUR (*SOLANUM MELONGENA L.*) AGAINST OXIDATIVE STRESS IN HYPERGLYCAEMIC RATS

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received: July 09, 2017 accepted: August 29, 2017

available online: September 15, 2017

Abstract

Background and Aims: Oxidative stress in diabetes mellitus (DM) occurs due to an increase in free radicals and decreased antioxidant defenses including superoxide dismutase (SOD). It causes the occurrence of lipid peroxidation as indicated by the levels of malondialdehyde (MDA). Healthy foods that are rich in antioxidants are needed to reduce oxidative stress, such as eggplant (*Solanum melongena L.*). Until now there is no scientific evidence about the effects of purple eggplant against oxidative stress in hyperglycemia. The aim of this study is to determine the antioxidant effect of eggplant flour (TTU) against oxidative stress in hyperglycemic rats by induced Nicotinamide-Streptozotocin (NA-STZ). **Materials and Method:** This experimental study was designed using posttest only. Thirty-six male Sprague Dawley rats, aged 10-12 weeks, body weight (bw) 207.25 ± 26.76 grams were divided randomly into 5 groups, namely 2 control groups (normal and hyperglycemic) and 3 groups of treatment. Hyperglycemic rats were induced by NA-STZ (230-65 mg/kg bw). Administration of TTU through feed for 28 days varied with dose of 2.36 grams, 4.71 grams, and 7.07 grams in groups P1, 2, and 3, respectively. Examinations of blood glucose levels were conducted before intervention, whereas the antioxidant activity of SOD and MDA plasma levels were examined after intervention. **Results:** The antioxidant activity of SOD in groups which were given TTU was higher than the hyperglycemic rats ($31.19 \pm 1.98\%$), but lower than the normal group ($220.83 \pm 23.68\%$). Levels of plasma MDA in groups which were given TTU were lower than the hyperglycemic rats (220.47 ± 5.24 nmol/L), but higher than the normal group (1.55 ± 0.20 nmol/L). The antioxidant activity of SOD in the P3 group ($69.29 \pm 3.82\%$) was higher than the P1 group ($41.84 \pm 3.82\%$) and the P2 group ($55.10 \pm 3.23\%$), while the levels of plasma MDA in the P3 group (1.89 ± 0.17 nmol/L) was lower than the P1 group (3.79 ± 0.24 nmol/L) and the P2 group (3.17 ± 0.53 nmol/L). **Conclusion:** The results of this study demonstrate that administration of TTU significantly prevents oxidative stress in hyperglycemic rats.

key words: hyperglycemia, purple eggplant, oxidative stress, nicotinamide, streptozotocin

Background and Aims

Diabetes Mellitus (DM) is a metabolic disease characterized by hyperglycemia due to impaired insulin secretion and insulin action, or both, which results in disturbance of carbohydrates, fat, and protein [1]. According to International Diabetes Federation (IDF), there were approximately 415 million individuals with DM in 2015 and that number is predicted to rise to 642 million in 2040. In 2015, Indonesia was the seventh highest country in the world where the numbers of individuals with diabetes were approximately 10 million and is predicted to be the sixth highest country in the world (16.5 million) in 2040 [2].

According to the data from Indonesian Basic Health Research Survey in 2013, the proportion of individuals ≥ 15 years with DM was approximately 6.9%. This proportion was obtained by blood glucose testing and DM symptoms observation i.e. polyuria, polydipsia, polyphagia, and weight loss. On the other hand, the prevalence of DM based on doctor's diagnosis was approximately 2.1% [3].

In Type 2 DM, a metabolic disease where the body is able to produce insulin but becomes resistant, the insulin becomes ineffective, which is called insulin resistance. Insulin resistance is linked to reduced stimulation of glucose transport in muscle and adipose tissue, defects in insulin signaling as well as defects intrinsic to the glucose transport system. When the insulin signal is disrupted, the disruption of glucose transport into the cells happens because of impaired GLUT-4 translocation to the plasma membrane [4]. This condition will cause a decrease in glucose utilization in peripheral tissues leading to increased blood glucose levels (hyperglycemia) [5] and if occurring continuously will lead to increased free radicals, which reduce the antioxidant defense system [6,7]. This condition is called oxidative stress

and leads to the modification of lipids, DNA, and proteins in different tissues [8-10]. The increase of oxidative stress in DM will cause the formation of lipid peroxidation characterized by increased production of MDA [11].

The body has mechanisms known as the antioxidant defense system to counteract the free radicals that are formed. When the amount of free radicals is excessive, the body will require exogenous antioxidants, and one of these is flavonoids [12]. One food that is known to contain a variety of phytochemicals which potentially act as antioxidants such as phenolic and flavonoid is eggplant [13,14].

The purple eggplant (*Solanum melongena* L.) is one of the plants in Indonesia which is rich in antioxidants and is believed to reduce blood glucose levels. Previous studies show that the ethanol extract of the skin and fruit extracts of purple eggplant when infused with the flesh have the effect of reducing the blood glucose levels of hyperglycemic rats [15-17]. Another study demonstrated that the eggplant extract can reduce the levels of serum MDA in DM rats [18].

Eggplant is one of the horticultural commodities which are easily damaged if not handled appropriately and correctly. Proper management is required to maintain freshness and increase the shelf-life, such as the making of flour. Another benefit of flouring is it is easy to create a composition that is malleable and can be enriched or fortified with nutrients [19-20].

This research aimed to determine the effect of TTU in preventing oxidative stress in hyperglycemic rats induced by NA-STZ.

Materials and Methods

Animals

Thirty-six (36) male *Sprague Dawley* rats (10-12 weeks old and body weight 207.25 ± 26.76 grams) were used in the study, and were obtained from the Laboratory of Food

Engineering and Nutrition of the Center for Food and Nutrition Studies (CFNS) *Pusat Antar Universitas* (PAU) Universitas Gadjah Mada (UGM). They were housed in individual cages in an animal room (temperature 20-25°C, humidity 70-80%). They were quarantined for four days to acclimatize them to laboratory conditions. The rats were provided food using standard diet AIN-93M (15 grams during the acclimatization and conditioning after induction of Type 2 DM and 20 grams during experimental phase) and tap water *ad libitum*. This study was approved by the Ethics Committee of Faculty of Medicine UGM.

Induction of Type 2 Diabetes Mellitus

Rats were fasted overnight for approximately 12 hours before injection. They were induced with nicotinamide (230 mg/kg) and streptozotocin (65 mg/kg) by intraperitoneal (i.p) injection. Streptozotocin (STZ) was

dissolved in 1 M buffered sodium citrate at pH 4.5 for this injection. Nicotinamide (NA) was dissolved in saline and administered 15 min before STZ. Fasting blood glucose levels were measured five days after induction. Criteria of hyperglycemia rats were if fasting blood glucose was >150 mg/dL [21].

Preparation of purple eggplant flour for treatment

Purple *mustang* eggplant varieties were used in this study from *Prambanan Klaten, Dukuh Kebondalem Lor*. They were cabinet dried at 40°C for ±45 hours and ground into powder to produce the purple eggplant flour (TTU). The purple eggplant flour was given to the rats after mixing with AIN-93M and substituting corn starch with TTU. Each group was given a completely different feed combination as listed below in [Table 1](#).

Table 1. Composition of Standard Feed and Feed Modification of TTU.

Composition	Composition (g/kg diet)			
	AIN-93M*	Feed P1	Feed P2	Feed P3
Corn starch	620.692	554.129	487.565	396.002
Casein	140	124.304	108.607	92.911
Sucrose	100	100	100	100
Soybean oil	40	38.486	36.973	35.459
Fiber	50	25	0	0
Mineral mix	35	25.977	16.954	7.930
Vitamin mix	10	10	10	10
L-cystine	1,8	1,8	1,8	1,8
Cholin Bitartate	2.5	2.5	2.5	2.5
TBHQ	0.008	0.008	0.008	0.008
TTU	-	117.797	235.593	353.390
Total (g)	1000	1000	1000	1000

*Source: [22]; TBHQ= Tert-butylhydroquinon; TTU= purple eggplant flour

Experimental Studies

All rats were randomly divided into five groups, with each group consisting of six rats. Group 1 was normal control (normal rats without DM induction), group 2 was hyperglycaemia control (DM rats without treatment TTU), and groups 3, 4, and 5 were treatment with TTU in

concentrations of 2.36 grams (P1), 4.71 grams (P2), and 7.07 grams (P3), respectively. All treatments were administered orally (20 grams) for a period of 28 days.

At the beginning (before treatment), blood samples were collected from the *reorbital plexus* after the rats fasted for 12 hours. Blood samples were collected to analyze blood glucose levels.

At the end of the experimental period (28 days), the rats were fasted overnight and blood samples were collected to analyze the antioxidant activity of SOD and the level of MDA plasma. Plasma was obtained from blood samples collected in Eppendorf tubes containing an anticoagulant (EDTA) and then were centrifuged at 4000 rpm for 15 minutes.

Blood Biochemical Examination

The fasting blood glucose levels were determined by the GOD-PAP method [23], the antioxidant activity of SOD by colorimetric methods [24], and plasma levels of MDA with the Thiobarbituric Acid Reactive Substances (TBARS) method using spectrophotometry [25].

Statistical Analysis

Statistical analysis was performed using STATA 12 program. The results are presented as the mean \pm SD (standard deviation). The statistical significance of data analysis was assessed by one way analysis of variance (ANOVA) and followed by Bonferroni post hoc test to determine the extent of any significance. The results were considered statistically significant at p value less than 0.05 ($p < 0.05$).

Results

The mean body weight of rats before treatment showed that there was no significant difference between groups ($p > 0.05$). Based on this statistic it can be concluded that the condition of the rats by weight was the almost the same before treatment (see [Table 2](#)).

Table 2. Subject Characteristics Before Intervention.

Groups	Body Weight (grams)	Blood Glucose Levels (mg/dL)
Normal Control (n=6)	220.83 \pm 23.68 ^a	66.90 \pm 1.63 ^a
Hyperglycemia Control (n=5)	196.20 \pm 5.89 ^a	220.47 \pm 5.24 ^b
P1 (n=6)	191.33 \pm 16.63 ^a	218.93 \pm 4.41 ^{b,c}
P2 (n=5)	204.20 \pm 25.23 ^a	216.17 \pm 4.26 ^{b,c}
P3 (n=6)	221.33 \pm 39.97 ^a	212.78 \pm 4.03 ^c
p ¹	0.1775	<0.001

The results are presented in mean \pm SD

¹ The results of the analysis of one-way ANOVA between groups in the same column

^{a, b, and c} The notation is different in the same column indicating significant difference $p < 0.05$

Table 3. The Effect of TTU on the Antioxidant Activity of SOD and Levels of Plasma MDA

Groups	Parameter	
	Antioxidant Activity of SOD (%)	Levels of Plasma MDA (nmol/L)
Normal Control (n=6)	72.45 \pm 4.42 ^a	1.55 \pm 0.20 ^a
Hyperglycaemia Control (n=5)	31.19 \pm 1.98 ^b	7.90 \pm 0.19 ^b
P1 (n=6)	41.84 \pm 3.82 ^c	3.79 \pm 0.24 ^c
P2 (n=5)	55.10 \pm 3.23 ^d	3.17 \pm 0.53 ^d
P3 (n=6)	64.29 \pm 3.82 ^e	1.89 \pm 0.17 ^e
p ¹	<0.001	<0.001

The results are presented in mean \pm SD

¹ The results of the analysis of one-way ANOVA between groups in the same column

^{a, b, c, d, and e} The notation is different in the same column indicating significant difference $p < 0.05$

[Table 2](#) shows that there are differences in blood glucose levels that are statistically significantly between groups ($p < 0.001$), and hyperglycaemic rats induced by NA-STZ

(hyperglycaemia control, P1, P2, and P3) were different with the normal rats (normal control). Blood glucose levels of hyperglycaemic rats were higher than that of normal control rats.

Treatment of hyperglycemia rats with TTU demonstrated significantly ($p < 0.001$) reversed levels of the antioxidant activity of SOD and plasma MDA levels. The antioxidant activity of SOD rats with hyperglycaemia that were given TTU (P1, P2, and P3) were higher than the hyperglycaemia control rats, but lower than normal control rats. Plasma MDA levels of hyperglycemic that rats were given TTU (P1, P2, and P3) were lower than hyperglycaemia control rats, but higher than normal control rats (see [Table 3](#)).

Discussions

Diabetes mellitus (DM) is a metabolic disease characterized by high blood glucose concentrations (hyperglycaemia) due to impaired insulin secretion, insulin action, or both [26]. The blood glucose levels of hyperglycemic rats induced by NA-STZ were higher than normal rats. The findings in this study are in line with other studies, namely that the blood glucose levels of DM rats are higher than normal control rats [27]. Induction with NA and STZ can cause partial damage of pancreatic β -cells accompanied by metabolic disorders in these cells so that DM is caused by the presence of insulin resistance (Type 2 diabetes) [28]. Insulin resistance is linked to reduced stimulation of glucose transport in muscle and adipose tissue, defects in insulin signaling as well as defects intrinsic to the glucose transport system. When the insulin signal is disrupted, the disruption of glucose transport into the cells happens because of impaired GLUT-4 translocation to the plasma membrane [4]. This condition will cause a decrease in glucose utilization in peripheral tissues leading to hyperglycaemia [5].

Hyperglycaemia continuously will lead to an increase in free radicals and therefore reduces the antioxidant defense system [7]. Superoxide dismutase (SOD) is one of the enzymatic

antioxidant system and is the main (primary) defense against oxidative stress conditions [12]. The increase of oxidative stress in DM will cause the formation of lipid peroxidation which will lead to increased production of MDA [11].

The antioxidant activity of SOD of hyperglycemia rats that were given TTU was higher than the hyperglycaemia control rats, but still lower than normal control rats. Unlike the case with the levels of plasma MDA, the levels of plasma MDA of hyperglycemic rats that were given TTU was lower than hyperglycaemia control rats, but still higher than the normal control rats. Increasing antioxidant activity of SOD and reducing levels of plasma MDA were indicators of oxidative stress due to hyperglycaemia and these conditions can be reduced by administering TTU. The antioxidant foods (green grass jelly drink) can reduce plasma MDA level. However, tomato juice, papaya, and tea can also reduce plasma MDA level, but not statistically significant [29].

Prevention of oxidative stress in hyperglycemia may be caused by the content of antioxidant compounds found in TTU, such as phenols, flavonoids, anthocyanins and vitamin E, among others. They have antioxidant capabilities, acting as a scavenger for free radicals by donating a hydrogen atom (H) of the hydroxyl groups in the radical chain reaction [30-33]. Phenol (ArOH) phenol will donate H atoms in Reactive Oxidative Stress (ROS) that will produce ariloxil radical (ArO*) and a stable molecule [30]. Flavonoids (FIOH) will donate a H atom of the hydroxyl groups in the ROS cycle that will produce flavonoids phenoxyl radical (FIO*) and a stable molecule. Flavonoids phenoxyl radicals will react further with a by breaking the radical chain forming reactive compounds [31]. Vitamin E (TocOH) acts as a scavenger of free radical by donating a H atom in the peroxidation process of lipids radical

(PUFA-OO*) formed from PUFA peroxide which will produce a hydroperoxide PUFA (PUFA-OOH) and tocopheroxil radical (Toco*). Tocopheroxil radicals are stable and with the help of GHS and vitamin C will produce non-radical compounds such as tocopherol and vitamin C as well as oxidized glutathione [33].

Phenols, flavonoids and anthocyanins also have an indirect effect, specifically by increasing the gene expression of endogenous antioxidants. One mechanism is an increase in antioxidant gene expression via activation of nuclear factor erythroid 2 related factor 2 (Nrf2) resulting in an increase in genes involved in the synthesis of endogenous antioxidant enzymes, such as the SOD gene [34]. The content of phenols, flavonoids, anthocyanins and vitamin E in TTU is capable of scavenging free radicals or increasing the activation of Nrf2, thereby increasing the antioxidant activity of SOD, decreasing free radicals and increasing antioxidant activity of SOD that will prevent oxidative stress. This cycle will cause a decrease in lipid peroxidation that decreases levels of plasma MDA [35].

The higher the dose of TTU that were given to hyperglycemia rats, the higher the antioxidant activity of SOD and the lower the levels of plasma MDA than control hyperglycemia rats. The P3 group has antioxidant activity of SOD that were higher ($64.29 \pm 3.82\%$) and MDA levels were lower (1.89 ± 0.17 nmol/L) than in the group of P1 and P2. This result indicates that the higher the dose given, the greater the effect of antioxidant activity and plasma MDA concentration. The antioxidant activity of SOD and MDA plasma levels in group P3 were closer to the KN group. This result is because the group is the intervention group with the highest dose of TTU (7.07 g/day). The more TTU consumed, the higher the concentration of active compounds that can potentially prevent oxidative stress.

Based on these results, rats' intake of TTU that was 2.07-4.71 grams (equivalent to 115.92-263.76 grams for human requirement) can reduce levels of GDP and oxidative stress, which are characterized by increased antioxidant activity of SOD and decreased levels of plasma MDA in rat models of DM. To get the same effect with this research, we recommend that TTU be regularly consumed by people along with a healthy diet. Purple eggplant flour can be processed into foods, including snacks such as biscuits, cakes, and other types of food. The prevention plans of DM are intended for the people who have any risk factors for Type 2 DM including family history, overweight or obesity ($BMI \geq 25$ kg/m² or ≥ 23 kg/m²), central obesity (abdominal circumference ≥ 90 cm for men and ≥ 80 cm for women), and prediabetes (fasting plasma glucose is 100-125 mg/dL, or 2-hour plasma glucose during oral glucose tolerance test (OGTT) 140-199 mg/dL, or A1C from 5.7 to 6.4%). The purple eggplant flour can also be used in therapeutic efforts for DM patients' diets to prevent the progression of DM and prevent DM complications.

Conclusions

The result of this study demonstrated that administration of TTU significantly prevents oxidative stress in hyperglycemic rats as evidenced by improved antioxidant activity of SOD and reduced levels of plasma MDA.

Acknowledgments. The author would like to thank the Agency for *Badan Pemberdayaan dan Pengembangan Sumber Daya Manusia Kesehatan* (BPPSDMK) Ministry of Health for covering research costs.

Conflict of Interest. The authors declare that they have no conflicts of interest.

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