

The Role of Black Soybean and Purple Sweet Potato Active Compound on Advanced Glycation End-Product in Streptozotocin-Induced Type 2 Diabetes Mellitus Rat

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ABSTRACT

Diabetic nephropathy is one of the diabetes complications attacking kidney leading to kidney damage. Hyperglycemia accompanying DM causes the increase of Advanced Glycation End-Product (AGE) and Receptor Advanced Glycation End-Product (RAGE) activity, then develop kidney damage and other diabetes complications. The study aimed to investigate the effect of black soybean, purple sweet potato, or their combination on the expression of AGE, RAGE, and kidney necrosis T2DM model rats. The rats were given with high-calorie diet for five weeks and then injected with a low dose of streptozotocin (30 m/kg Body Weight) in intraperitoneal. DM rats were divided into: normal, K- (T2DM), K+ (T2DM + glibenclamide 0.6 mg/kg body weight), P1 (T2DM + black soybean), P2 (T2DM + purple sweet potato), and P3-5 Combination 1-3 (T2DM + combination of black soybean and purple sweet potato in ratio of 1 : 3, 2 : 2, and 3 : 1). DM rats were then given the treatments for thirty days. The effect of black soybean, purple sweet potato, or the combination of both was evaluated through the expression of AGE, RAGE, and necrosis of renal tubules. The changes in renal tubules histological characteristics were evaluated using hematoxylin-eosin (HE) staining. Immunohistochemistry analysis of renal tubules was to evaluate AGE-RAGE expression after the treatments. The research results indicated that there was a significant difference from the combination of black soybean and purple sweet potato in reducing AGE, RAGE, and renal tubules necrosis. The BSB and PSP combination ratio of 1:1 was able to improve renal tubules, decrease the expression of AGE and RAGE towards near normal. The combination of black soybean and purple sweet potato could be used as one of the alternatives to improve kidney damage in diabetic nephropathy.

Keywords: Black soybean, purple sweet potato, RAGE, AGE, renal tubules, T2DM

Introduction

Diabetes mellitus (DM) is a metabolic disorder which characterized by impaired of regulation in glucose, lipid, and protein metabolism [1]. DM is marker by high glucose level in blood stream [2]. Diabetic nephropathy (DN) is one of the most critical complications caused by DM with a consequence of kidney damage [3, 4]. DN is chronic kidney disease and has a high value of mortality among people with diabetes. DN has the most pa-

tient, which is 30 – 40% of all T1DM and T2DM patients [5, 6]. The increase in AGE-RAGE levels causes a histological change in the kidney [7]. DN marks with the occurrence of glomerular basement membrane thickening, the enlargement of extracellular matrix of mesangial cells, atrophy in tubules, the damage in microvascular vessels, and the presence of necrosis to fibrosis in the interstitial tubules [3, 6, 8, 9]. The hyperglycemic condition that accompanies DM could cause an increase

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in the number of Advanced Glycation End-Product (AGE) [5, 8, 9]. AGE is formed due to the interaction between glucose and amino acid group (especially lysine and arginine residue) and fat in non-enzymatic [5, 9, 10]. AGE will be recognized by a specific receptor called receptor of AGE (RAGE). When the AGE level increases, RAGE will increase. During the hyperglycemic condition, AGE accumulation occurs causing damage in kidney and other diabetic complications [10].

The current diabetes mellitus treatment is less optimal, especially to cure kidney damage due to DM complications. Herbal medicine is an alternative supported by the WHO, especially to cure degenerative diseases, such as diabetes [11]. Soybean (*Glycine max*) is a plant from Fabaceae family (legume) and epidemiologically, it has various benefits for health. Black soybean is one of soybean (*G. max*) varieties. The plant contains isoflavones and anthocyanin that has anti-oxidant, anti-cancer, anti-diabetes, and anti-inflammation activities [12, 13]. Purple sweet potato (*Ipomoea batatas*) is generally used by the traditional community to consume its roots and leaves. *I. batatas* also contain anthocyanin that functions as alternative treatment for various diseases, especially degenerative diseases [14, 15]. The combination of black soybean and purple sweet potato reported have ameliorate effect to suppressing reactive oxygen species (ROS) to improve sperm quality [16]. ROS is free radicals that can cause various disease by increasing oxidative stress in the body [17].

However, the combination of black soybean and purple sweet potato as T2DM alternative treatment, primarily to treat kidney damage (diabetic nephropathy) is never done therefore. This research aimed to evaluate the combination of black soybean and purple sweet potato as an anti-diabetic nephropathy agent.

Material and Methods

Black soybean and purple sweet potato preparation

Purple sweet potato (were harvested at ± 4 months) obtained from purple sweet potato plantation in Kawi Mountain, Malang Regency. Black soybean (var. Detam-1, were harvested at ± 3 months) purchased Indonesian Legumes and Tuber Crop Research Institute (ILETRI), Malang Regency. Purple sweet potato was washed, cut into small pieces, and air-dried for 4 days, then ground

into mild. The black soybeans were also clean washed and dried for 1 day and ground into mild. The process was done at the Balai Materia Medika, Batu City.

Experimental animals

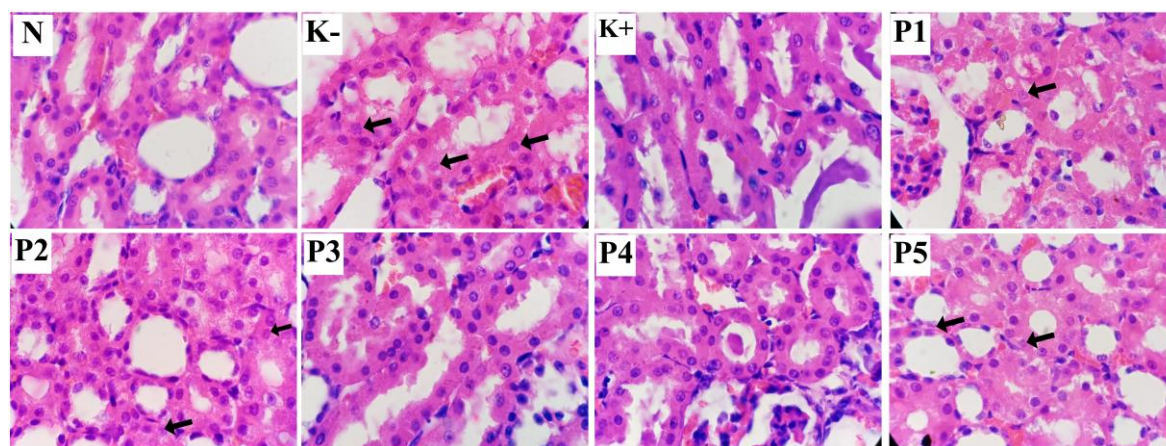
Twenty-four rats (*Rattus norvegicus*) from Wistar strain (age of 10 – 12 weeks, weight 85 ± 10 g) obtained from CV. Karunia Jasa Pratama, Malang, Indonesia. The rats placed in a plastic cage with free access to food and drink. Rats were acclimated for a week. After the acclimation, the rats were divided into two groups: normal diet and high-calorie diet (HCD). The normal diet (PT. Comfeed Indonesia) contains 63% carbohydrate, 3% fat, 13% protein, 21% vitamin and mineral. HCD feed used Hi-Gro Medicated 551 (produced by PT Pokhpand, Indonesia) containing 74% carbohydrate, 6% fat, 20% protein, vitamin, mineral, and 1% fiber (18). The water in HCD groups was placed with 10% sucrose.

Induction of diabetes and treatment

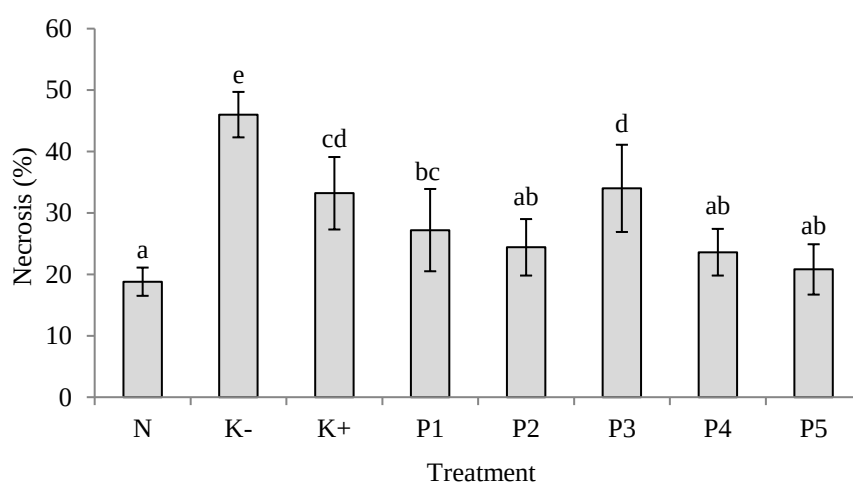
After 30 days of diet manipulation, rats were injected with streptozotocin (STZ) (41910012-3, Bioworld) multiple low doses of 30 mg/kgBW intraperitoneally [19]. The rats were considered DM if the blood glucose level ≥ 200 mg/dl. DM rats then divided into 8 groups randomly: normal, K- (T2DM), K+ (T2DM + glibenclamide 0.6 mg/kg BW), P1 (T2DM + black soybean), P2 (T2DM + purple sweet potato), and P3-5 Combination 1-3 (T2DM + combination of black soybean and purple sweet potato in ratio of 1:3, 2:2, and 3:1), respectively. Treatments were given for 30 days. The rats then sacrificed with euthanization (4% isofluorane), dissected, and the kidney organ was taken and washed using PBS three times. The kidney organ was fixed in 10% formalin. All research procedures had been approved by the Research Ethics Commission of the University of Brawijaya, Malang with No. App. 878-KEP-UB.

Histological analysis

Kidney organ fixed in 10% buffer formalin, dried, and embedded in paraffin. Kidney tissues cut into 5 μm thick pieces and stained using hematoxylin-eosin (HE). Kidney slides were observed using a light microscope (CX23, Olympus, Japan). Renal tubular necrosis observation based on count cell in ten different field with 1000 \times magnifica-



(a)



(b)

Figure 1. (a) HE staining in the renal tubular section on normal and experimental groups with 100 \times . (b) Necrosis percentage on normal and experimental groups. N, normal diet group; K-, T2DM group; K+, T2DM treated with glibenclamide 0,6 mg/kg BW; P1, T2DM treated with black soybean; P2, T2DM treated with purple sweet potato; P3-P5, T2DM treated with combination of black soybean and purple sweet potato 3 : 1, 2 : 2, 1 : 3 respectively. Black arrow showed necrosis of renal tubules cells. The different alphabetic indicated significance ($P < 0.05$) between groups based on DMRT as a post hoc test.

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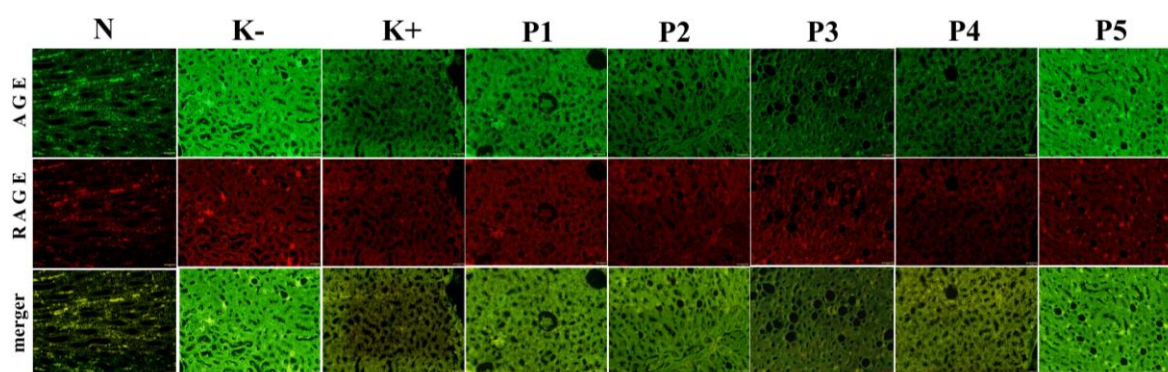
Immunohistochemistry analysis

The expression of Advanced Glycation End Product (AGE) and receptors of AGE-modified proteins (RAGE) in kidney tissues was tested using staining method of immunohistochemistry-fluorescence (IHC-F). The primary antibody of rat anti-AGE (No. cat. SC 365154, Abcam, USA), mice anti-RAGE (No. cat. SC-65154, Santa Cruz Biotechnology) and 2% BSA in the ratio of 1:1:1000, incubated for an hour. Preparations of secondary antibody staining conducted by mixing secondary antibody goat anti-rat IgG Fluorescein Isothiocyanate (FITC) (No. cat. 02-16-06, KPL,

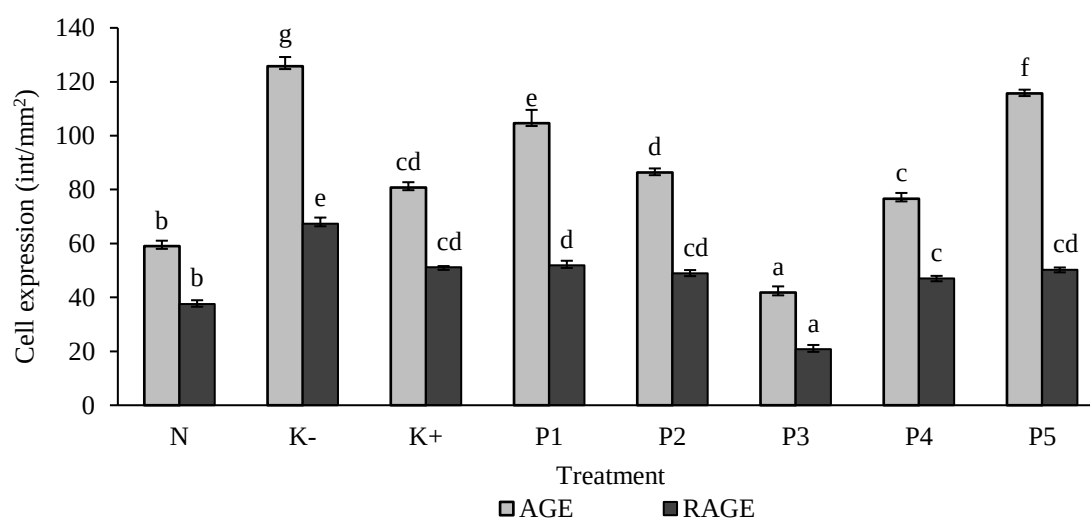
USA), secondary antibody goat anti-mouse IgG Tetramethylrhodamine isothiocyanate (No. cat. Ab6768, Abcam, USA), and 2% BSA in ratio of 1 : 1 : 1500 and incubated for an hour. The preparations then washed using PBS pH 7.4 three times, dried with tissue paper on the edge, and covered with cover glass. The double staining IHC-F method was observed with a fluorescent microscope (FSX 100, Olympus, Japan) to measure their intensity (intensity/mm).

Statistical analysis

Data from the observation result of prepara-



(a)



(b)

Figure 1. AGE-RAGE expression in rat after treatment with black soybean, purple sweet potato, and their combination. (a) IHC-F double staining in the renal tubular section on normal and experimental group (observed using a fluorescence microscope, 16 \times in magnification). (b) AGE-RAGE expression represents as mean \pm standard deviation. N, normal diet group; K-, T2DM group; K+, T2DM + glibenclamide 0.6 mg/kg BW; P1, T2DM treated with black soybean; P2, T2DM treated with purple sweet potato; P3-P5, T2DM treated with combination of black soybean and purple sweet potato 3 : 1, 2 : 2, 1 : 3 respectively. The different alphabetic indicated significance ($P < 0.05$) between groups based on DMRT as a post hoc test.

tions stained with HE were analyzed using descriptive qualitative analysis. Data of intensity measurement from IHC-F were analyzed using one-way ANOVA, if significant ($p < 0.05$), the analysis continued with a post hoc test of Duncan Multiple Range Test (DMRT).

Results and Discussion

Comparison of renal tubules necrosis in rats given with black soybean, purple sweet potato, and the combination of both

Kidney preparations stained with HE were used to observe the occurrence of tubules cell ne-

crosis in the kidney. Our result indicated that necrosis in tubules of DM group was an increase compared to normal group (Figure 1). Treatment using black soybean, purple sweet potato, or all of the combination indicated that those treatments can decrease necrosis in renal tubules compared to DM groups. The combination of black soybean and purple sweet potato in the ratio of 1 : 3 and 1 : 1 indicated an improvement in tubules condition closer to the normal groups.

Our result suggested that treatments with black soybean, purple sweet potato, or the combination of both reduce tubules cells necrosis on kid-

ney compared to DM group. Black soybean contains isoflavones genistein and daidzein [20, 21]. Jia *et al.* reported that the rats were given with genistein can attenuate renal fibrosis in STZ-induced diabetic rats. Supplementation with genistein for 8 weeks also increased endogenous antioxidant superoxide dismutase and decreased lipid peroxidase and malondialdehyde (MDA) [22]. Kim and Lim (2013) also reported that supplementation on early stage of diabetic-induced renal damage can decrease level of inflammation markers such as nuclear factor kappa B (NF κ B), tumor necrosis factor α (TNF- α), dan cyclooxygenase-1 (COX-1) [23]. Genistein is a group of phytoestrogens that plays a role as a specific inhibitor for α -glucosidase. α -glucosidase is an enzyme which has the function in carbohydrate digestion. The inhibition of the enzyme causes a delay in the termination of disaccharide chain into monosaccharide (glucose) thus reducing the absorption of glucose in the small intestine [20, 21, 24].

Comparison of AGE and RAGE expression on rats given with black soybean, purple, or the combination of both

Our results indicated that AGE and RAGE expression in DM was significantly increased compared to normal group (Figure 2a and b). Black soybean, purple sweet potato, or the combination of both were able to significantly decrease the intensity of AGE and RAGE expression compared to those in the DM group (Figure 2a). The combination of black soybean and purple sweet potato in the ratio of 2 : 2 decrease AGE and RAGE intensity closer to normal group. Interestingly, the combination of black soybean and purple sweet potato in the ratio of 1 : 3 decreased the expression of AGE and RAGE lower than the normal group (Figure 2b). Our result suggested that the combination of black soybean and purple sweet potato could be used as a candidate of alternative treatment for T2DM through the decrease of AGE and RAGE expression in kidney.

Type 2 diabetes mellitus is a progressive and degenerative disease marked with a blood glucose level of more than ≥ 200 mg/dL and caused by insulin resistance. Hyperglycemia causes an increase of AGE-RAGE, then affecting the development of kidney damage formation [10]. Our finding showed DM rats had the highest AGE-RAGE

expression in kidney tubules compared to treatment groups. The high expression of AGE-RAGE indicated that there was damage in the kidney tubules. The result was supported by observation on kidney histological changed. The histology of kidney showed there were many cells under necrosis condition.

AGE-RAGE accumulation mediated diabetic nephropathy since the binding between AGE and RAGE increase reactive oxygen species production lead to oxidative stress status [5]. ROS stimulate the overexpression of pro-sclerotic growth factors such as tumor growth factor β (TGF β) and connective tissue growth factor (CTGF). In line with our finding, the percentage of necrosis on the proximal tubule of DM rats was higher than non-diabetes rats [25]. Damage on tubules characterizes pyknosis, karyolysis, and necrosis on the brush border. The binding of AGE-RAGE also induces the activation of the transcription factor of NF κ B, and mitogen-activated protein kinase played a role in the change of phenotype and cell function [7].

Our research result indicated that glibenclamide decreased the AGE-RAGE expression compared to the DM group. Glibenclamide is one of synthesis drug includes in sulphonylurea group. Glibenclamide works by inhibiting ATP-sensitive potassium canal in pancreatic β -cells. The inhibition causes the occurrence of calcium canal depolarization thus the intracellular calcium level of pancreatic β -cells increases and stimulates insulin production [26]. However, prolonged use of synthesis drug could have side effects for the body. Continuous use of glibenclamide could cause damage in pancreatic-cells due to overstimulation for insulin production [24]; therefore, an alternative treatment from herbal is needed that to reduce the side effects of treatment.

Soybean is also contained protein derivatives, such as glycine and arginine that play an essential role in the secretion of glucagon and insulin by the pancreas [20]. Genistein reported to be able to decrease malondialdehyde levels in the kidney, which is the end product of oxidative stress [21]. Diet using genistein supplement in a dose of 600 mg/kg BW also indicated a decrease in glucose level [21]. The improvement in glucose absorption in the intestines and free radical have ability to alleviate AGE-RAGE expression in kidney cells.

Both black soybean and purple sweet potato

contain anthocyanin. Anthocyanin is a derivative compound of flavonoid that water soluble and has reddish or purple color pigment [13]. Anthocyanin plays an essential role as an inhibitor of α -amylase, maltase, and sucrose [27], thus it reduces the glucose level. Anthocyanin also has activities as an antioxidant since one of its function is as a ROS free radical scavenger [28], leading to the decrease of AGE and RAGE. Anthocyanin was reported to lowering blood glucose level by improving the activity of glucose transporter 4 (GLUT4) in muscles and adipose tissues [28].

Conclusion

The combination of black soybean and purple sweet potato was able to reduce the AGE, RAGE, and renal necrosis in DM rats. The combination suggested to improve AGE, RAGE, and necrosis was 2 : 2. Consumption of black soybean and purple sweet potato is expected to become a simple and cheap functional food to be used as alternative therapy for DM. However, further research is required before the combination of both can be applied to human by giving appropriate dose recommendation.

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