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Improved hypoglycemic effect of anthocyanin extract combination from red rice and black soybean

Improved hypoglycemic effect of anthocyanin extract combination from red rice and black soybean

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Abstract. Red rice and black soybean, both contains anthocyanin, have long been part of diabetic patient diet. was investigated. This study aimed to investigate possible synergetic anti-hyperglycemic effect of crude anthocyanin extract from red rice and black soybean combination. Observation was conducted on glucose level, total plasma antioxidant capacity, and antioxidant enzyme superoxide dismutase (SOD) activity of STZ-NA induced hyperglycemic 2-months old male *Sprague Dawley* rats. Thirty rats were divided into five groups (n=6] of healthy group, hyperglycemic control, and three hyperglycemic treated groups. Treated groups were orally administered with daily dose of 100 mg/kg body weight of anthocyanin extract from red rice, black soybean, and their 1:1 (w/w) combination for 35 days. Anthocyanin extract consumption significantly reduced blood glucose by 45.3%-50.9%, prevented body weight loss by 36.42-51.52%, increased total antioxidant capacity by 15.84 – 25.30% and SOD activity than those of hyperglycemic groups. Consumption of anthocyanin from red rice and black soybean significantly improve hyperglycemic rats condition. Possible synergetic effect of extract combination was indicated by 8.2 % lower plasma glucose reduction and 40.06% higher SOD activity than those of each material effect.

1. Introduction

According to Indonesia Basic Health Survey, there was increasing in average prevalence of diabetic individuals in the country from 1.1 in 2007 to 2.1 in 2013 among a million people (1). Prolonged hyperglycemia, a condition by which blood glucose level is above 120 mg/dl during fasting and 100 – 180 mg/dl at post-prandial, triggers antioxidant imbalance due to lower antioxidant enzyme activity, such as superoxide dismutase (SOD), which lead to type 2 diabetes (2). Moreover, prolonged hyperglycemia trigger antioxidant imbalance due to lower antioxidant enzyme activity, such as superoxide dismutase (SOD), which is double jeopardy for the islet beta cell since the tissue has lowest SOD activity besides the primary target of hyperglycemic pathogenic condition (3). With many pathologic impacts and complication diseases, diabetes prevention becomes the main focus of many researches, particularly in the individuals with impaired glucose metabolism, a condition by which blood glucose level is above 120 mg/dl during fasting and 100 – 180 mg/dl at post-prandial (2).

Among antioxidant-rich food, red rice and black soybean have been widely consumed by diabetic patient as they're believed to possess anti-hyperglycemic effect (4,5). They contain anthocyanin whose

antioxidant activity lies in its ability to donate hydrogen atoms from hydroxyl group to stabilize free radical (6). Anthocyanin from various food sources was reportedly able to improve hyperglycemic condition, prevents pancreatic beta cell apoptotic pathway, improves insulin secretion and resistance, increase glucose uptake for glycogen synthesis in liver and muscle tissue, normalizes plasma blood glucose and improve plasma antioxidant capacity of type 2 diabetic animals (5,7,8). It was also reportedly has synergetic effect with other compounds, such as anti-platelet effect anthocyanin-rich grape skin extract with polygalloyl polyflavan-3-ol (PGPF)-rich grape seed extract (9) and synergetic effect of malvidin-3-glucoside and malvidin-3-galactoside combination against TNF-α (10).

Red rice and black soybean are Yogyakarta local commodities with increasing consumption, particularly in diabetes prevention diet. However, little is known on how anthocyanin co-consumption from different food source in diet, such as red rice with black soybean, might be beneficial. Therefore, this study was conducted to investigate anti-hyperglycemic activity of anthocyanin extract from those local sources on streptozotocin-nicotinamide hyperglycemic animals and its possible synergetic effect. Animal's feed efficiency ratio, blood glucose level, total plasma antioxidant capacity, and CuZn-superoxide dismutase (CuZn-SOD) enzyme activity were analyzed.

2. Materials and Method

2.1 Materials

Red rice var. Mandel Handayani was obtained from farmer in Gunung Kidul district, Yogyakarta Special Province. Black soybean var. Mallika was obtained from farmer in Malang district, Jawa Timur Province. Technical-grade 96% ethanol, citric acid powder, and distilled water were purchased from local chemical store. Gallic acid, sodium acetate (CH_3COONa), potassium chloride (KCl), sodium carbonate (Na_2CO_3, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Vitamin mix AIN 93, Mineral mix AIN 93, streptozotocine, and nicotinamide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol, Folin-Ciocalteu phenol reagent, iron (III) chloride hexahydrate ($FeCl_3.6H_2O$), $FeSO_4$, and hydrochloric acid (HCl) were from Merck (Germany). 2.4.6-tripyridyl-s-triazine (TPTZ) were from Across Organics (New Jersey, USA). Glucose Oxidase Phenol 4-Aminoantipirin (GOD-PAP) was from DiaSys (Diagnostic System, Germany). RanSOD kit was purchased from Randox Laboratories (Antrim, UK).

2.2 Sample preparation and extraction

Dehulled unpolished red rice grain was separated into two fractions of red aleurone and white endosperm using food processor for 60 seconds (Brabender Co., New Jersey). Black soybean seed coat was peeled from the grain manually and powdered using blender (Phillips 2L-350W, Indonesia). Each of red aleurone and seed coat powder was sifted on a Ro-Tap shaker equipped with 60 mesh sieves. The powder then stored in vacuum packaged at –20°C prior to extraction. For each batch of extraction, 50-gram sample in 500 ml of citric acid – ethanol (3% w/v) solution was stirred using magnetic stirrer at 600 rpm for 3 hours. After 14 hours maceration at 4°C, mixture was filtered through a Whatman filter paper no 42. Solvent was separated using vacuum rotary evaporator at temperature below 30°C for 3 hours.

2.3 Anthocyanin quantification

Anthocyanins was quantified using pH differential method of Giusti and Wrolstad (11) with slight modification. For red rice and black soybean seed coat extract, samples were diluted at 1:400 and 1:600, respectively, in 0.025 M KCl (pH 1) and 0.4 M Na-acetate (pH 4.5) buffer before absorbance measurements at 510 nm and 700 nm using spectrophotometer (Shimadzu UV-1601, Kyoto, Japan) with distilled water as blank. Data were reported as means ± SD from triplicate sampling in total milligram of anthocyanins per ml extract calculated using dilution factor (DF), molar extinction coefficient (ε) of 26.900, and molecular weight (MW) of cyanidin-3-glucoside of 449.2.

$$\text{Anthocyanins (mg/ml)} = \frac{\{(A_{510}-A_{700})_{pH1}-(A_{510}-A_{700})_{pH4.5}\} \times MW \times DF}{\varepsilon}$$

2.4 In vivo experiment

Thirty male 2 months-old *Sprague–Dawley* rats provided by Food and Nutrition Study Center (Universitas Gadjah Mada, Yogyakarta) weighing 150-250 gram with blood glucose < 120 mg/dl were used. After 1 week acclimated, each animal was housed in transparent single cage at room temperature (27 ± 1°C) with a 12 hours light-12 hours dark cycle, fed with AIN 93 feed contained vitamin-mineral mix, while water was provided *ad libitum*. All protocols were approved by the Pre-clinical Ethical Clearance Commission of Integrated Laboratory for Research and Experiment (Universitas Gadjah Mada, Yogyakarta).

Rats were randomly divided into five groups (n=6), consisted of C group of healthy control rats given only buffer, H (hyperglycemic controls, STZ-NA induced rats), and three treated groups of RR, BS, and RR-BS (hyperglycemic rats treated with anthocyanin extract from red rice, black soybean, and combination of red rice and black soybean at 1:1 w/w). Hyperglycemia was induced by single intraperitoneal injection of freshly-prepared streptozotocin (65 mg/kg rat body weight) in 0.1 M citrate buffer (pH 4.5) 15 minutes after intraperitoneal injection of nicotinamide (230 mg/kg rat body weight). The animals were considered as diabetic if their fasting blood glucose values were above 200 mg/dL on the third day after STZ injection. Treatments were started on the fourth day after induction, which considered as the first of 35 days. Daily dosage of 100 mg/kg rat body weight was administered through oral gavage to anthocyanin-treated groups. All rats were weighed weekly. Remaining feed was weighed daily to measure previous day's feed consumption.

2.5 Plasma glucose analysis

Glucose analysis was conducted using GOD-PAP enzymatic colorimetric assay (12) on plasma extracted from blood taken through orbital sinus of overnight fasting rats. Approximately 10 μl sample, or distilled water as blank, or glucose standard solution sample was mixed with 1000 μl GOD-PAP reagent (contained glucose oxidase + peroxidase + 4-aminoantipyrine), let stand for 10 minutes at 37°C. In aqueous medium with the presence of oxygen, glucose oxidase (GOD) catalyses glucose oxidation to gluconic acid and hydrogen peroxide (H_2O_2). In the presence of peroxidase (POD), H_2O_2 reacts with 4-chloro-phenol and 4-aminoantipyrine (PAP) which is colorless in the reduced state to form a pink color quinonimine whose intensity is proportional to the glucose concentration. The intensity was measured with a spectrophotometer at 500 nm, calculated using equation below.

$$\text{Glucose (mg/dl)} = \frac{(Abs_{sample} - Abs_{blank})}{(Abs_{standard} - Abs_{blank})} \times \text{standard concentration}$$

2.6 Ferric Reducing Ability of Plasma (FRAP)

The analysis was conducted once every two weeks (day 7, 21, 35] on plasma extracted from blood taken through orbital sinus of overnight fasting rats, according to the method by Benzie and Strain (13) with modifications. The fresh working FRAP solutions included 25 ml 0.3 M buffer sodium acetate (pH 3.6], 2.5 ml of 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 2.5 ml of 20 M $FeCl_3.6H_2O$ solution (ratio of 10:1:1 v/v/v) was warmed at 37°C before used. Three milliliters of working solution were allowed to react 100μl plasma for 5 minutes in the dark condition. Readings of the colored product (ferrous tripyridyltriazine complex) were then taken at 593 nm. The standard curve made using $FeSO_4$ between 100 – 1000 µmol/L.

2.7 CuZn-Superoxide Dismutase (CuZn-SOD) enzyme activity

SOD activity was analyzed using RanSOD® commercial kit. Superoxide (O_2•) generated from xanthine-xanthine oxidase reaction transformed I.N.T (2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride) into formazan, thus SOD activity was measured from formazan inhibition percentage. Erythrocyte was taken from serum by 4–cycle blood centrifugation of 0.5 ml at 3000 rpm for 10 minutes, added with 3 ml of 0.9% NaCl and centrifuged. Erythrocyte was added with distilled water up to total volume of 2 ml, homogenated, and let stand for 15 minutes at 4°C. Approximately 0.1 ml sample was taken, added with 9.9 ml of 0.01 mol/l phosphate buffer pH 7 (dilution factor = 100). Then 1 vial of xanthine oxidase was added with 10 ml of dd.H_2O. Meanwhile, 1 vial mixed substrate (contained xanthine and I.N.T.) was added with 20 ml buffer from RanSOD kit. Standard solution (S1) was phosphate buffer pH 7, measured as 100% formazan formation. Approximately 30 μl sample or standard solution (S1) was put into cuvette, added with 1000 μl mixed substrate then homogenized. After 5 minutes incubation at room temperature, mixture was added with 150 μl xanthine oxidase. Absorbance was measured at 505 nm during initial 30 seconds after enzyme addition (A1) and 3 minutes later (A2). SOD activity was calculated as follow:

$$\%\ \text{formazan inhibition} = 100 - \left\{ \frac{\frac{(A_1 - A_2)_{sample}}{3}}{\frac{(A_1 - A_2)_{standard}}{3}} \times 100 \right\}$$

2.8 Statistical analysis

All data are shown as the mean ± S.D., and the data were subjected to one-way analysis of variance followed by Duncan's multiple range tests to determine significant different. Statistical analysis was performed using SPSS software (Chicago, IL, USA). In all cases, P value of 0.05 was used to determine significance. Pearson correlation test was performed to measure parameters correlation rate among treatment groups.

3. Result and Discussion

3.1 Body weight and feed efficiency ratio (FER)

Three treated hyperglycemic group had different body weight pattern but shared similar pattern with healthy group during initial second week until the final period of observation (Figure 1). Treated groups showed increased body weight while hyperglycemic control group was otherwise. Statistical analysis showed significant different in all treated group during initial and final period of treatment. In treated group, highest body weight increase was obtained by RR group of 80.5 gram, followed by RR-BS of 64.5 gram and BS of 59.84 gram.

C group (healthy control rats given only buffer), H (hyperglycemic controls, STZ-induced rats), RR (hyperglycemic rats, treated with red rice anthocyanin extract), BS (hyperglycemic rats, treated with black soybean seed coat anthocyanin extract), RR-BS (hyperglycemic rats, treated with combination of red rice – black soybean anthocyanin extract, 1 : 1 w/w ratio). Daily dosage of anthocyanin extract was 100 mg/kg rat body weight.

Anthocyanin consumption was indicated to be related to hyperglycemic rat body weight change. During initial and final period of intervention, treated groups were significantly different compare to hyperglycemic control and health control (Table 1). During initial period, treated groups were lower that both control groups. But during final period, body weight of treated groups can be maintained and even increased. Different source of anthocyanin did not lead to any significant difference.

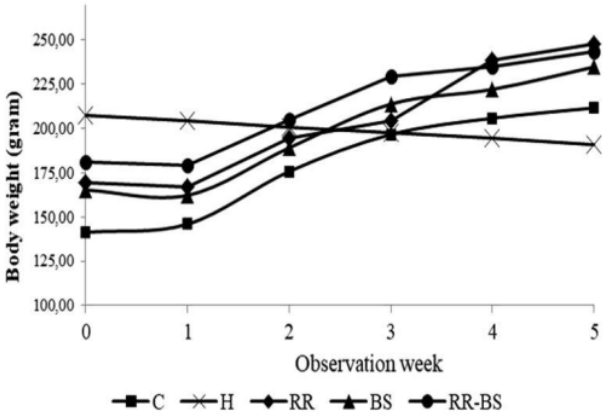

Figure 1. Rat body weight during 35 days treatment.

Table 1. Body weight, body weight change, daily feed consumption (DFC), and Feed Efficiency Ratio (FER) rats during 35 days treatment.

Groups	Body weight (gram)		Body weight change (BWC)		DFC	FER
	Initial	Final	(gram)	(%)	(gram/day)	
C	146.01± 7.85$^{a\,\#}$	211.67± 8.57$^{b\,\#}$	65.67±4.57^{b}	45.74±4.33^{b}	11.91±0.46^{a}	0.158±0.02^{bc}
H	204.33±16.20$^{c\,\#}$	190.67±14.80$^{a\,\#}$	13.66±1.91^{a}	6.66±0.57^{a}	13.61±0.42^{b}	-0.029±0.01^{a}
RR	167.17±12.52$^{b\,\#}$	247.67±15.73$^{c\,\#}$	80.51±4.19^{b}	51.52±8.79^{b}	13.55±1.11^{b}	0.171±0.02^{c}
BS	162.33±11.86$^{b\,\#}$	222.17± 3.97$^{c\,\#}$	59.84±8.64^{b}	39.26±6.72^{b}	13.59±0.29^{b}	0.126±0.05^{b}
RR-BS	179.33+ 8.75$^{b\,\#}$	243.83+10.14$^{c\,\#}$	64.51±5.62^{b}	36.46±3.90^{b}	11.47±0.61^{a}	0.136±0.03^{b}

Different superscript (a, b, c) in the same column showed significant different ($p < 0.05$)
$^{\#}$Significant different between initial and final period of treatment.
C (healthy control rats given only buffer), H (hyperglycemic controls, STZ-induced rats), RR (hyperglycemic rats, treated with red rice anthocyanin extract), BS (hyperglycemic rats, treated with black soybean seed coat anthocyanin extract), RR-BS (hyperglycemic rats, treated with combination of red rice – black soybean anthocyanin extract, 1:1 w/w ratio). FER was calculated from average body weight divided by daily feed consumption (BWC:DFC) during treatment.

Feed efficiency ratio (FER) reflected how efficient consumed feed (g) used by the body to maintain the weight (14). Higher FER indicated higher feed efficiency. Several researches on hyperglycemic or type 2 diabetic rats showed that decreased FER can be related to the condition (15,16). Based on statistical analysis, feed consumption of all rat during 5 weeks treatment increase but not significant, aside from RR group in the middle period of treatment. Similar results were also obtained by previous researches noted that hyperglycemia was able to increase rat feed consumption, whereas antioxidant was able to reduce feed consumption (16,17). It was also noted that acute hyperglycemia can lead to body inability to utilize blood glucose despite abundant supply, thus in order to maintain body energy balance, body uses deposit stored in adipose tissue as well as protein, resulted decreased body weight, as shown by hyperglycemia control group in this study.

3.2 Plasma blood glucose

During initial first week of treatment, plasma blood glucose of healthy rats was slightly increased, but remained in the normal range below 80 mg/dl. There was also no significant difference among treated groups and controls. However, significant difference occurred started during middle period. Consumption of anthocyanin extract from red rice, black soybean, and their combination were able to significantly reduce plasma blood glucose, so that in the final stage, there was 102.28 – 119.2 mg/dl decrease obtained by treated group.

In H group, plasma blood glucose of hyperglycemic rats was remained high at 220 mg/dl. In the other hand, significant decrease was seen every week in treated groups down to range around 120 mg/dl in the final period of treatment. The results showed that consumption of anthocyanin from red rice, black soybean, and their combination at 100 mg/kg body weight for 5 weeks was able to decrease hyperglycemic rat's plasma blood glucose, with the longer treatment lead to greater achievement close to normal range.

Table 2. Rat plasma blood glucose during 35 days treatment

Group	Blood glucose (mg/dl)				
	Pre-induction	Induction	Days-		
			7	21	35
C	$7718\pm1.14^{a}{}_{C}$	$77.54\pm1.06^{a}{}_{KL}$	$78.31\pm2.37^{a}{}_{K}$	$79.15\pm0.82^{ab}{}_{K}$	$81.57\pm0.99^{b}{}_{K}$
H	$76.04\pm0.82^{a}{}_{KL}$	$223.08\pm1.75^{b}{}_{L}$	$229.69\pm3.17^{cd}{}_{C}$	$231.28\pm1.24^{c}{}_{C}$	$233.33\pm1.10^{d}{}_{C}$
RR	$75.32\pm1.07^{a}{}_{KL}$	$225.73\pm1.49^{e}{}_{LM}$	$210.78\pm2.54^{e}{}_{L}$	$174.68\pm1.21^{d}{}_{L}$	$123.45\pm1.09^{b}{}_{L}$
BS	$73.25\pm0.72^{a}{}_{K}$	$229.15\pm2.14^{e}{}_{MN}$	$210.92\pm4.24^{e}{}_{L}$	$174.41\pm1.64^{d}{}_{L}$	117.37 ± 1.45^{b} RR-$_{L}$
BS	$75.75\pm0.97^{a}{}_{KL}$	$234.11\pm2.03^{e}{}_{N}$	$208.01\pm2.40^{e}{}_{L}$	171.49 ± 1.19^{d}	$114.91\pm1.17^{b}{}_{L}$

C (healthy control rats), H (hyperglycemic controls, STZ-induced rats), RR (hyperglycemic rats, treated with red rice anthocyanin extract), BS (hyperglycemic rats, treated with black soybean seed coat anthocyanin extract), RR-BS (hyperglycemic rats, treated with combination of red rice–black soybean anthocyanin extract, 1:1 w/w ratio). Different superscript (a, b, c, d, e) in a row and subscript (K, L, M, N) in a column showed significant difference ($p<0.05$).

Interaction was seen in combined anthocyanin extract to reduce plasma glucose level, in form of synergetic effect as the result from RR-BS group was 8.2 % higher than additive effect from single anthocyanin source (Figure 2). In this study, anthocyanin was able to decrease plasma glucose level. Previous research mentioned that the mechanism behind the occurrence was related to insulin-mediated increasing of glucose transport 4 (GLUT4). It was also noted that anthocyanin might improve hyperglycemia through reduction of insulin resistance by up-regulation of insulin receptor expression in muscle cell membrane, which lead to higher glucose uptake, thus reduce plasma glucose level (5).

3.3 Total plasma antioxidant capacity

Both healthy and hyperglycemic control groups shared FRAP reduction, though in different rate (Table 3), whereas all treated groups exhibited significant increase ($p<0.5$). Healthy group had 1.8% lower FRAP level than initial level, but still higher than those of hyperglycemic group of 5.1%. The highest result was obtained by BS group (25.3%) which had slightly higher result than those of RR-BS (24.1%). The results showed that anthocyanin from black soybean and combination had better ability to increase total plasma antioxidant capacity in hyperglycemic rats.

Hyperglycemia reduced total antioxidant capacity of plasma in rat and human (18) due to increasing rate of AGEs-induced free radical formation, oxidative stress triggered by various biological changes, such as mitochondria in particular, and glucose toxicity-induced polyol pathway

activation, although healthy individual can also suffered from such reduction due to lack of antioxidant diet (19). The result of this study confirmed that anthocyanin consumption can prevent antioxidant status decline.

FRAP value represents antioxidant status in plasma, either due to exogenous antioxidant compound consumption, vitamin, cellular antioxidant enzyme, or their interaction, thus can be regarded as total antioxidant capacity. The high value of FRAP obtained by treated groups compare to hyperglycemic group indicated improvement of plasma antioxidant capacity. Consumption of phenolic compounds, of which black soybean seed coat-extracted anthocyanin is among them, was able to reduce oxidative stress while increasing total antioxidant capacity of plasma (20). Anthocyanin ability to increase antioxidant status in hyperglycemic individual was reportedly correlated to inhibition of pathogenetic pathway of free radical (21).

Table 3. Total plasma antioxidant capacity expressed as Ferric Reducing Antioxidant of Plasma (FRAP) during 35 days treatments

Group	Day -		
	7	21	35
C	$324.14\pm1.53^{a}{}_{V}$	$314.34\pm1.48^{a}{}_{V}$	$318.31\pm1.50^{b}{}_{V}$
H	$347.86\pm1.09^{c}{}_{X}$	$335.80\pm1.05^{b}{}_{W}$	$330.12\pm1.04^{a}{}_{W}$
RR	$342.62\pm0.86^{a}{}_{W}$	$362.15\pm0.91^{b}{}_{X}$	$396.90\pm0.92^{c}{}_{X}$
BS	$344.52\pm1.13^{a}{}_{WX}$	$367.09\pm1.21^{b}{}_{Y}$	$431.69\pm1.42^{c}{}_{Y}$
RR-BS	$342.62\pm1.63^{a}{}_{W}$	$367.44\pm1.74^{b}{}_{Y}$	$425.19\pm2.02^{c}{}_{Z}$

C (healthy control rats), H (hyperglycemic controls, STZ-induced rats), RR (hyperglycemic rats, treated with red rice anthocyanin extract), BS (hyperglycemic rats, treated with black soybean seed coat anthocyanin extract), RR-BS (hyperglycemic rats, treated with red rice–black soybean seed coat anthocyanin combination extract, 1:1 w/w ratio). Different superscript (a, b, c) in a row and subscript (K, L, M, N) in a column showed significant difference ($p<0.05$).

3.4 Superoxide dismutase (SOD) enzyme activity

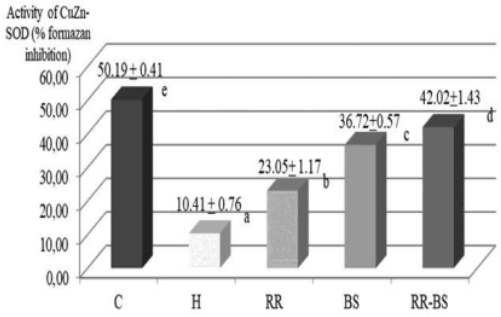

Figure 2. Superoxide dismutase (CuZn-SOD) enzyme activity of rat erythrocyte. C (healthy control rats), H (hyperglycemic controls, STZ-induced rats), RR (hyperglycemic rats, treated with red rice anthocyanin extract), BS (hyperglycemic rats, treated with black soybean seed coat anthocyanin extract), RR-BS (hyperglycemic rats, treated with red rice–black soybean seed coat anthocyanin

extract combination, 1 : 1 w/w ratio). Different superscript (a, b, c, d, e) showed significant difference ($p < 0.05$).

Activity of endogenous SOD enzyme has previously used in several researches as comparison for FRAP assay, particularly as biological marker in hyperglycemic individual, beside glutathione peroxidase and catalase (22). Data showed that anthocyanin consumption from red rice, black soybean seed coat, and their mixture for 5 weeks was able to inhibit SOD activity decline with significant margin. In the final period of treatment, healthy control showed highest SOD activity of 50.19%, while the lowest level of 10.41% was obtained by hyperglycemia control. These results indicated that hyperglycemia reduced SOD activity down to 21% from initial level, far lower than non-hyperglycemia individuals. The highest result of 42.02% was obtained by RR-BS, was only 16% lower than initial stage, followed by BS group of 36.72% with 27% reduction, whereas the lowest of the treated groups obtained by RR rats of 23.05% or half of those of healthy control.

The results showed that anthocyanin extract from black soybean seed coat was able to maintain SOD activity of hyperglycemic rats better than those of red rice. It was also indicated that combination of both extracts had synergetic effect to maintain SOD activity, as showed by anthocyanin combination-treated group result which 40.61% higher than those obtained from RR and BS effect.

Among 70-80% SOD is in form of CuZn-SOD found in cytoplasm, nucleus, and lysosome of mammalian cells. Besides, CuZn-SOD is also presents in tetramer form as extracellular EC-SOD (23). It was also noted that diabetogenic compound such as alloxan and streptozotocin are able to decrease blood SOD activity (24). Several researches reported that such decrease can be generated in hyperglycemia individuals. Improvement of SOD activity is among biological marker for improved antioxidant status in hyperglycemic individual through three ROS production pathway inhibition, which are inhibition of protein kinase C (PKC), decreased AGEs formation, and inactivation of glucose via aldose reductase pathway (25).

The results of this study also indicated synergetic effect of anthocyanin from different source, with better plasma glucose reduction and SOD activity improvement in hyperglycemic rats than those of single source of anthocyanin. The effect is probably due to higher absorption rate with the help of several protein transporters, such as natrium-dependent glucose transport (SGLT-1) or GLUT2, triggered by protein-bound anthocyanin of red rice (26,27) together with dominant high bioactivity effect of anthocyanin contained in black soybean seed coat (4,28).

4. Conclusions

Consumption of anthocyanin extracted from red rice var Mandel Handayani and seed coat of black soybean var. Mallika for 35 days significantly improve glycemic condition of STZ-NA induced hyperglycemic rats showed by plasma glucose reduction in the range of 45-51%, improvement of total antioxidant capacity of plasma by 15.84 – 24.10%, and improvement of superoxide dismutase enzime activity 1.2 -3 times than those of hyperglycemic rats. Synergetic effect of anthocyanin from different source was also found in the reduction of plasma glucose and higher SOD activity by 8.2 % and 40.06%, respectively, higher than addition of each source alone.

References

[1] Anonymous 2013 *Basic Health Research* 2013 [National Report] (Jakarta: Indonesia Ministry of Health)
[2] Defronzo R A 2009 From the triumvirate to the ominous octet: A new paradigm for the treatment of type 2 diabetes mellitus *Diabetes* **58** 773.
[3] Vossen E, Ntawubizi M, Raes K, Smet K, Huyghebaert G and Arnouts S 2011 *J Anim Physiol Anim Nutr* **95** 198
[4] Oki T, Masuda M, Kobayashi M, Nishiba Y, Furuta S, and Suda I 2002 *J Agric Food Chem*. **50**

7524
[5] Nizamutdinova I T, Jin Y C, Chung J, Shin S C, Lee S J and Seo H G 2009 s *Mol Nutr Food Res*. **53** 1419
[6] Rojo L E, Ribnicky D, Logendra S, Poulev A, Rojas-Silva P and Kuhn P 2012 *Food Chem* **131** 387
[7] Takikawa M, Inoue S, Horio F and Tsuda T 2010 *J Nutr* **140** 527
[8] Lila M A 2004 *J Biomed Biotechnol* **5** 306
[9] Shanmuganayagam D, Beahm M R, Osman H E, Krueger C G, Reed J D and Folts J D 2002 *J Nutr*. **132** 3592
[10] Huang W Y, Liu Y M, Wang J, Wang X N and Li C Y 2014 Anti-inflammatory effect of the blueberry anthocyanins malvidin-3-glucoside and malvidin-3-galactoside in endothelial cells. *Molecules* **19** 12827
[11] Lee J, Rennaker C and Wrolstad R E 2008 Correlation of two anthocyanin quantification methods: HPLC and spectrophotometric methods *Food Chem*. **110** 782
[12] Baroroh F, Aznam N and Susanti H. 2011 Anti-hyperglycemic effect measurement of Kacapiring (*Gardenia august*, Merr) leaves ethanolic extract on male Wistar mice. *Journal of Farmacy Science* **1** 43
[13] Benzie I F F and Strain J J 1999 Ferric reducing (antioxidant) power as a measure of antioxidant capacity: the FRAP assay *Methods Enzym* **299** 15
[14] Bethke E, Bernreuther M and Tallman R 2013 Feed efficiency versus feed conversion ratio - demonstrated on feeding experiments with juvenile cod (*Gadus morhua*). SSRN (2013)
[15] Zaki E A 2009 Physiological response to diets fortified with jerusalem artichoke tubers (*Helianthus tuberosus* L.) powder by diabetic rats *Environ Sci* **5** 682
[16] Khattab H A H, Al-Amoudi N S and Al-Faleh A A A 2013 Effect of ginger, curcumin and their mixture on blood glucose and lipids in diabetic rats *Life Sci J*. **10** 428
[17] El-Yamani M A S 2011 Cinnamon, cardamom and ginger impacts as evaluated on hyperglycemic rats *Research Journal Specific Education of College of Applied Medical Sciences of University Of Om El- Qura, K.S.A*. **20** 663
[18] Nakhaee A, Bokaeian M, Saravani M, Farhangi A and Akbarzadeh A 2009 Attenuation of Oxidative Stress in Streptozotocin-Induced Diabetic Rats By Eucalyptus Globulus *Indian J Clin Biochem*. **24** 419
[19] Pawar S M, Tolanur S I, Lakshmi T M and Vaithilingam A 2011 FRAP status in diabetic with coronary heart disease patient *J Pharm and Biomed Sci* **04** 10
[20] Jeng T L, Shih Y J, Wu M T and Sung J M 2010 Comparisons of flavonoids and anti-oxidative activities in seed coat, embryonic axis and cotyledon of black soybeans *Food Chem* **123** 1112
[21] Miguel MG 2011 Anthocyanins: Antioxidant and/or anti-inflammatory activities. *J Appl Pharm Sci*. **1** 7
[22] Tiwari B, Pandey K, Abidi A and Rizvi S 2013 Markers of oxidative stress during diabetes mellitus *J Biomarkers* (2013) 378790)
[23] Zelko I N, Mariani T J and Folz R J 2002 Superoxide dismutase multigene family: A comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression *Free Radic Biol Med*. **33** 337
[24] Crouch R K, Gandy S E, Kimsey G, Galbraith R A, Galbraith G M and Buse M G 1981 The inhibition of islet superoxide dismutase by diabetogenic drugs *Diabetes* **30** 235
[25] Brownlee M, Nishikawa T, Edelstein D, Du X L, Yamagishi S and Matsumura T 2000 Normalizing mitochondrial superoxide production blocks three pathways\nof hyperglycaemic damage. *Nature* **404** 787
[26] Perera A S and Jansz E R 2000 Preliminary investigation on the red pigment in rice and its effect on glucose release from rice starch *J Nat Sci Foundation Sri Lanka* **28** 185
[27] Fernandes I, Faria A, Calhau C, de Freitas V and Mateus N 2014 Bioavailability of anthocyanins and derivatives *J Funct Foods*. **7** 54

[28] Astadi I R, Astuti M, Santoso U and Nugraheni P S 2009 In vitro antioxidant activity of anthocyanins of black soybean seed coat in human low density lipoprotein (LDL) *Food Chem.* **112** 659

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