

The Effect of Anthocyanin of Whole-Grain Pigmented Rice Attenuated Visceral Fat, Cholesterol, LDL and PPAR γ Gene Cascade in Dyslipidemia Rat

Fatchiyah Fatchiyah^{1,2}, Anna Safitri^{1,3}, Rista Nikmatu Rohmah^{1,4}, Lidwina Faraline Triprisila^{1,4}, Nia Kurnianingsih^{1,5}, Yudhistira Nugraha⁶, Sisca Fajriani^{1,7}, Hazna Noor Meidinna^{1,8}, James Ketudat Robert-Cairns^{1,9}

¹Research Center of Smart Molecule of Natural Genetics Resource, Brawijaya University, Malang, East Java, Indonesia

²Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang, East Java, Indonesia

³Department of Chemistry, Faculty of Mathematics and Natural Sciences, Brawijaya

⁴Biosains Institute, Brawijaya University, Malang, East Java, Indonesia

⁵Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia

⁶Indonesian Center for Rice Research, Subang, West Java, Indonesia

⁷Faculty of Agriculture, Brawijaya University, Malang, East Java, Indonesia

⁸DBT-AIST International Laboratory for Advanced Biomedicine (DAILAB), National Institute of Advanced Industrial Science & Technology (AIST), and Tsukuba Life Science Innovation, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan

⁹School of Chemistry, Institute of Science, & Center for Biomolecular Structure, Function and Application, Suranaree University of Technology, Thailand

Corresponding Author: Fatchiyah Fatchiyah

Email: fatchiya@ub.ac.id

ABSTRACT

Pigmented rice is the nutritional food contributing to cholesterol biosynthesis through the activity of its bioactive compounds. The aim of this study is to investigate the effects of anthocyanins from whole grain pigmented rice to the visceral fats, triglycerides, LDL and total cholesterol level, also PPAR γ gene cascade in dyslipidemia rat. Sixty-six Sprague-Dawley female rats were randomly assigned into two groups: control and dyslipidemia. These groups were received two doses of black and rice extracts, 125 and 250 mg/kg BW. We measured total cholesterol, triglyceride, HDL & LDL by the photometer and MDA level in sera rats using ELISA, respectively. The activity of antioxidant was analyzed using FRAP and mRNA levels of PPAR γ , C/EBP α , and FABP4 were measured using qPCR. All samples were performed using One Way ANOVA in SPSS. The visceral fat weights in control and dyslipidemia rats were markedly decreased with oral administration of all cultivars black rice (BRE, BRC, and BRW). All treatments to dyslipidemia rats caused decreases in total cholesterol, triglyceride, HDL, and LDL levels. In addition, serum MDA levels in rats, both in control and in dyslipidemia group lowered after receiving BRW in 125mg/kg BW dose. Finally, the mRNA level of C/EBP α and PPAR γ genes expression decreased in groups of WREJ125, BREJ250, BRWJ250 treatments with the lowest level was shown in group given BRWJ125mg/kg BW. Conclusions: our results highlight potentially relevant beneficial effects of anthocyanin from Indonesian local black rice as anti-oxidants anti-obesity and anti-adipogenesis.

Keywords: Anthocyanin, anti-oxidative, nutritional food, phytochemical, pigmented rice.

Correspondence:

Fatchiyah Fatchiyah

Research Center of Smart Molecule of Natural Genetics Resource, Brawijaya University, Malang, East Java, Indonesia

Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang, East Java, Indonesia

Email: fatchiya@ub.ac.id

INTRODUCTION

Metabolic syndrome (MetS) is one of major public health issues in the world connected to life style, environmental, genetics and metabolic factors. The factors of metabolic abnormalities, such as high blood pressure, hyperglycemia, central adiposity, and dyslipidemia, characterized in metabolic syndrome that related with obesity, high risk of cardiovascular disease, diabetes mellitus, and stroke. Several studies proved that there is a positive communication between bioactive compounds of dietary intake and MetS in body weight, blood pressure, blood glucose, insulin resistance, cholesterol, and oxidative stress markers [1–4].

The pathogenesis of MetS influenced by the increasing of insulin resistance-mediated in free fat in circulating free fatty acids (FFA). Visceral fat deposits contribute to insulin resistance more than subcutaneous fat, as visceral lipolysis leads to an up-regulated FFAs supply to the liver. Rise in FFAs leads to activate the triglyceride synthesis, elevate low-density lipoprotein (LDL) cholesterol and reduce in high-density lipoprotein (HDL) cholesterol. Visceral adipose tissue is also considered metabolically

active and synthesizes significantly higher amounts of bioactive secretory proteins, which promotes smooth muscle cell proliferation and vascular remodeling [5,6]. The abnormality of lipid metabolism with removal from optimum vascular cholesterol and triglyceride levels caused dyslipidemia status that leads to atherosclerosis and generates the FFA plaque disposition in aorta blood vessel [7].

The AMP-activated protein kinase (AMPK) is not only as a key factor for balancing cellular energy but also for regulating SREBP-1c in adipogenesis that can stimulate lipogenic enzymes and adipogenic-specific factors [8,9]. Adipogenic transcription factors such as CCAAT-enhancer-binding protein- α (C/EBP α), peroxisome proliferator-activated receptor- γ (PPAR γ), and adipocyte binding protein 2 (aP2)/fatty acid-binding protein 4 (aP2/FABP4) provide the differentiation from pre-adipocytes to adipocytes [9–11]. To maintain the cellular cholesterol homeostasis, nutrition contents that can stimulate the regulation of transcription factors directly activates expression cascade genes susceptibility with

cholesterol synthesis and PPAR γ gene through MAPK pathway are required.

Bioactive compounds of natural nutrition in crop plant can promote personal health beyond its nutritional value is gaining acceptance in linked with nutrition components to disease prevention and treatment. Rice is one important crop food in Asia. There are three subspecies of rice dominated in Asia, including japonica, indica, and javanica. Indonesia has rice varieties contain high quality nutrients, rich minerals and bioactive compounds. These bioactive compounds are found in red, brown and black rice, which are now most popular as Indonesia's healthy food. Many local black rice growths in different region such as Waja Laka and Laka from NTT region, cempo ireng & Toraja from Java Island, Black Borneo from Kalimantan and Black lampung from Sumatra [12,13].

Bran and whole grains rice-pigmented are rich in nutrients and phytochemicals with known health benefits that have high concentrations of dietary fiber, antioxidants, trace minerals, vitamins, phenolic compounds, and oligosaccharides. They have many potential biological functions such as antioxidant, anti-inflammatory, anti-hypertensive, cardio-protective, lowering total cholesterol, and anti-obesity [4]. Anthocyanins-rich in rice, many fruits, flowers, grains, and other plant-derived foods are present to display the red-orange to blue-violet pigments [14]. Recently our group studies reported that total anthocyanin in black rice have potential activity as anti-apoptosis through the caspase-3 interaction in specific region [15]. Moreover, delphinidin-3-O-glucoside and peonidin-3-O-glucose in black rice might have function as anti-inflammatory factor through the TNF- α signaling pathway [16]. Delphinidin-3-O- β -Glucoside had affected as anti-adipogenic in 3T3-L1 cell lines of Pre-adipocytes [17]. In animal studies, anthocyanins inhibit the absorption of lipids and glucose in the intestine & significantly triglyceride reduction, and in CVD patient decrease in systolic and diastolic blood pressure [14]. Therefore, in this study, we investigated the total anthocyanin in whole grain of rice-pigmented that attenuated the level of visceral fat, triglycerides, LDL- and total cholesterol, and PPAR γ gene cascade in dyslipidemia rat.

MATERIAL AND METHODS

Sample Collection

Five varieties of rice were collected from three provinces in Java Island, Indonesia. They were Mentik Wangi white rice (WREJ) from East Java, Mentik red rice (RREJ) from East Java, NTT local (N790) black rice (BREJ) from East Java, Mentik black rice (BRCJ) from Central Java, and Toraja local black rice (BRWJ) from West Java. The rice samples were stored at room temperature until used for further analysis.

Pigmented Rice Extraction for in Vivo Analysis

Twenty-five gram pigmented rice samples were added 250ml 1% HCl in 85% methanol then homogenate was filtered with Whatman filter papers (0.45 μ m). Homogenate was added 250ml 1% HCl in 85% methanol for overnight then filtered again and condensed by rotary evaporator 70 $^{\circ}$ C, 700 rpm for 4 hours[18]. The obtained total anthocyanin extracts were used for oral administration of control and dyslipidemia rat.

Experimental Animal

Sixty-six Sprague-Dawley female rats (8 weeks old, 125–185g) were acquired from Integrated Research and Testing Laboratory, Gadjah Mada University, Yogyakarta,

Indonesia. They were acclimatized for 1 week to the laboratory conditions (Animal Laboratory, Biosains Institute, Brawijaya University, Malang, Indonesia). Rats were randomly assigned into the two groups: control group and dyslipidemia group. Rats in control group ($n = 33$) were fed a standard food contained cornstarch, wheat bran, palm oil, essential amino acid, mineral, vitamin (Comfeed PARS, JAPFA Comfeed Indonesia, Tbk.). Those in dyslipidemia group were fed a high cholesterol composition food (45-50% standard diet, 20-30% wheat flour, 3-6% duck egg yolk, 10-20% goat fat, 2-3% coconut oil, 0.1-0.5% Cholic acid) for 7 weeks until the cholesterol levels exceeding 200mg/dl. Rats were given free access to food and water. The body weight, food intake, water intake, fecal weight, and urine volume of each rat were recorded during the experimental period.

The Treatment of Pigmented Rice Extracts in the Experimental Rat

We treated the control and dyslipidemia rats with the total anthocyanin extract from WREJ, RREJ, BREJ, BRCJ, and BRWJ. We used two doses of each extracts, 125 and 250mg/kg BW. There were twenty-two groups of experiment: control rat without rice extract treatment (C), control rat + 125mg/kg BW of WREJ extract (WREJ125), control rat + 250mg/kg BW of WREJ extract (WREJ250), control rat + 125mg/kg BW of RREJ extract (RREJ125), control rat + 250mg/kg BW of RREJ extract (RREJ250), control rat + 125mg/kg BW of BREJ extract (BREJ125), control rat + 250mg/kg BW of BREJ extract (BREJ250), control rat + 125mg/kg BW of BRCJ extract (BRCJ125), control rat + 250mg/kg BW of BRCJ extract (BRCJ250), control rat + 125mg/kg BW of BRWJ extract (BRWJ125), control rat + 250mg/kg BW of BRWJ extract (BRWJ250), dyslipidemia rat without rice extract treatment (D), dyslipidemia rat + 125mg/kg BW of WREJ extract (DWREJ125), dyslipidemia rat + 250mg/kg BW of WREJ extract (DWREJ250), dyslipidemia rat + 125mg/kg BW of RREJ extract (DRREJ125), dyslipidemia rat + 250mg/kg BW of RREJ extract (DRREJ250), dyslipidemia rat + 125mg/kg BW of BREJ extract (DBREJ125), dyslipidemia rat + 250mg/kg BW of BREJ extract (DBREJ250), dyslipidemia rat + 125mg/kg BW of BRCJ extract (DBRCJ125), dyslipidemia rat + 250mg/kg BW of BRCJ extract (DBRCJ250), dyslipidemia rat + 125mg/kg BW of BRWJ extract (DBRWJ125), and dyslipidemia rat + 250mg/kg BW of BRWJ extract (DBRWJ250). The rice extracts treatment into the control rats was carried out orally for 28 days, while the treatment for dyslipidemia rats was conducted for 3 months. This study had been evaluated and approved by the Research Ethics Committee of Brawijaya University, Malang, and East Java, Indonesia (Certificate number, 896-KEP-UB).

Blood Samples and Visceral Fat Collection

All rats were dissected at the end of experiment. The blood samples were collected by cardiac puncture, and then maintained at room temperature for coagulation. The serum was obtained by centrifugation at 3000 rpm at 25 $^{\circ}$ C for 10 min. The serum was stored at -80 $^{\circ}$ C until used. Visceral fats were excised, weighed, and washed in phosphate buffered saline (PBS)-diethylpyrocarbonate (DEPC) for total RNA isolation preparation [11].

Measurement of Total Cholesterol, Triglyceride, HDL and LDL

Serum levels of total cholesterol, triglyceride, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were measured enzymatically using commercial DiaSys Kit (Diagnostic System GmbH, Germany), according to the

manufacturer's instructions. Instrument and test strips using an enzymatic reflectance photometric assay based on the glycerol-phosphate oxidase (GPO) and cholesterol oxidase methods respectively following manufacturer's instructions. Briefly, one drop of fresh venous blood was applied to the reagent area of a test strip and, when prompted, inserted into the test chamber of the instrument that directs light onto the test area. The triglycerides/total cholesterol in the sample reacts with the reagents in the strip pad causing a color change. The amount of light reflected from the colored test area is proportional to the concentration of triglycerides/total cholesterol measured by the photometer and is converted into a digital readout.

Serum HDL-cholesterol was estimated using an enzymatic colorimetric assay based on the cholesterol oxidase method after removal of the other lipoproteins by precipitation with phosphotungstate-magnesium, following the manufacturer's instructions. The absorbance was measured at 600 nm using an Ultrospec 2000 UV/VIS spectrophotometer (Biochrom Ltd, Cambridge, UK). Serum LDL-cholesterol was calculated indirectly by the Friedewald's equations: VLDL = Triglycerides/5 and LDL = Total-cholesterol- [HDL + VLDL] [19].

Measurement of Malondialdehyde Level

Serum malondialdehyde (MDA) levels were estimated using the method described by [20]. The serum was added with 15% trichloroacetic acid followed by the addition of 0.67% thiobarbituric acid. The mixture was placed in a boiling water bath (95°C) for 10 minutes. The mixture was

cooled to room temperature then its absorbance was measured at 532 nm.

Antioxidant activity determination using Ferric-reducing power (FRAP)

A hundred microliter of total anthocyanins of pigmented rice extract (0, 2, 4, 6, 8, 10 μ g/mL) was reacted by adding the 2.5mL 200mmol/L phosphate buffer pH 6.6 and 2.5mL 1% potassium ferricyanide. The solutions were incubated on 50°C for 20 minutes. The reactions was stopped with 2.5mL 10% TCA and centrifuged 10000 rpm for 10 minutes. Five milliliters supernatant was diluted with 5mL water and 1mL 0.1% ferric chloride and analyzed by spectrophotometer at 700nm [21].

Reverse-transcription and quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from the visceral fat of rats using PureZOL, according to the manufacturer's instructions (Bio-Rad Laboratories, USA). Total RNA concentration and purity were determined using the NanoDrop 1000 Spectrophotometer (Thermo Scientific, USA). The RNA was reverse transcribed into cDNA using an iScriptTM cDNA Synthesis kit (Bio-Rad Laboratories, USA) according to the manufacturer's instruction. The quantitative PCR was performed using the SSOFast EvaGreen Super mix reagent kit and CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, USA). The relative mRNA levels of PPAR γ , C/EBP α , and FABP4 were analyzed by the 2- $\Delta\Delta$ CT method. The expression levels of the target genes were normalized against that of GAPDH. The specific primers were used in this study (Table 1).

Table 1. Specific Primer Sequences for RT-qPCR

Genes	Sequence
PPAR γ	PPAR γ -F-5'-TCTGGGAGATCCTCCTGT T-3'
	PPAR γ -R-5'-CAATCGGATGGTTCTTCGGA-3'
C/EBP α	C/EBP α -F-5'-CGACTTCTACGAGGCGGAG -3'
	C/EBP α -R-5'-TGGCTTTATCTCGGCTCTTG-3'
FABP4	FABP4-F-5'-GGACCTGGAAACTCGTCTCC-3'
	FABP4-R-5'-GGACCTGGAAACTCGTCTCC-3'
GAPDH	GAPDH-F-5'-ACCACAGTCCATGCCATCAC-3'
	GAPDH-R-5'-TCCACCACCCTGTTGCTGTA-3'

Statistical Analysis

The analyses were performed using One Way ANOVA in SPSS 20.0 software (IBM Corp, Armonk, NY, USA) for windows. The results were expressed by means \pm standard deviation. Statistical significant differences were considered at $p < 0.05$ and markedly significant differences were considered at $p < 0.01$.

RESULT

The body weight, BW gaining and visceral fat weight

To investigate the affect of total anthocyanin in rice-pigmented, the body weight and BW gaining and also visceral fat weight of all rat groups was determined. In Figure 1 A and D, the control rat groups treated with total anthocyanin of RREJ (125mg/kg BW), BREJ (250mg/kg BW), BREJ (125mg/kg BW), and BRWJ (125 & 250mg/kg BW) were significantly decreased than others control rat groups. In dyslipidemia rat groups did not show a significant different. According to BW gaining data (Fig. 1B and E), the BW gradually reduced in all of dyslipidemia rat groups after total anthocyanin of rice-pigmented treatment, in which the lowest level was shown in BRWJ (125 and 250mg/kg BW) treatments, Fig. 1E. Moreover, the visceral fat weights in control

(Fig. 1C) and dyslipidemia (Fig. 1F) rat groups were significantly decreased after rats fed with black rice cultivars from East, Central and West Java Island ($P < 0.05$).

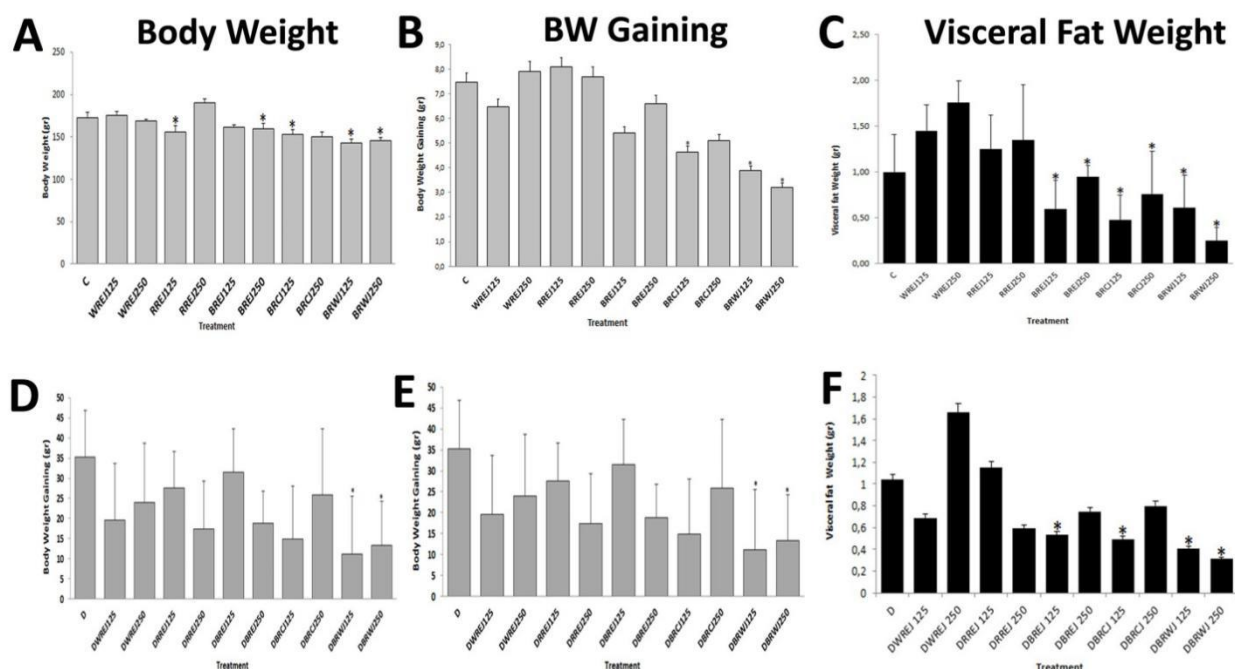


Figure 1. Characterization of body weight, body weight gaining and visceral fat weight in control, dyslipidemia, pigmented rice-treated control and pigmented rice-treated dyslipidemia rats. A, B, C: in control rat groups. D, E, F: in Dyslipidemia rat groups. *: Significantly lower than other groups ($P < 0.05$).

Serum lipid profile in control and dyslipidemia rats

In Table 1 shown that normal level of total cholesterol in control mice was 39mg/dl and there was almost no change after being given the total anthocyanins of all rice variants. The highest of total cholesterol levels was in dyslipidemia rat without any treatment (98mg/dl) and the total cholesterol levels were reduced in all treatment groups. The normal triglycerides level in control mice was 112mg/dl, and the level was declined in treatment groups except in control rat-treated BREJ at 250mg/kg BW dose that increased significantly (118mg/dl). The highest level of triglyceride in dyslipidemia rats was 226mg/dl and there was a decrease in levels in all treatment groups.

The HDL level in control rat group was at 8.2mg/dl and after received total anthocyanin from rice, these groups showed differential level. The highest level of HDL level in control rat was treated with BREJ at 250mg/kg BW dose (9.7mg/dl). In dyslipidemia rat, HDL level was 24.6mg/dl and the highest is in dyslipidemia rat treated with BREJ at 250mg/kg BW dose at 32.4mg/dl. The LDL level in control rat was 8.5mg/dl and after treated with total anthocyanin of rice grain demonstrated various level. In normal rat the lowest LDL level was 8.1mg/dl after being given WREJ 250mg/kg BW and the highest LDL level was 27.5mg/dl after added BREJ 250mg/kg BW, definitely. The highest level of LDL found in dyslipidemia treated with BRCJ at 250mg/kg BW dose at 35.6mg/dl and the lowest level of LDL showed significantly at 6.7mg/dl after treated with BRCJ at 125mg/kg BW dose. The grade of total cholesterol, triglyceride, HDL, and LDL level in both rat groups in giving WREJ (125mg/kg BW), RREJ (125 and 250mg/kg BW), BREJ (125mg/kg BW), and BRWJ (125 and 250mg/kg BW) were in normal state.

Malondialdehyde (MDA) level in control and dyslipidemia rats

To examine the oxidative defect, the Malondialdehyde (MDA) level of serum in this was also measured. The serum MDA level in dyslipidemia rat 75% higher than in control rat without.

Table 2. The level of total cholesterol, triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) from Serum of Dyslipidemia and control rats treated orally by total anthocyanin of pigmented rice.

Treatment in Experimental Group	Total Cholesterol Level (mg/dl)		Triglyceride Level (mg/dl)		HDL Cholesterol Level (mg/dl)		LDL Cholesterol Level (mg/dl)	
	Control Rat	Dyslipidemia Rat	Control Rat	Dyslipidemia Rat	Control Rat	Dyslipidemia Rat	Control Rat	Dyslipidemia Rat
Without Treatment	39 \pm 0.04	98 \pm 2.91	112 \pm 3.78	226 \pm 11.15	8.2 \pm 0.13	24.6 \pm 0.06	8.5 \pm 1.02	28.3 \pm 0.62

WREJ 125	29 \pm 1.04	74 \pm 0.51	39 \pm 3.52**	128 \pm 1.35	5.5 \pm 0.14	26.1 \pm 0.21	25.8 \pm 0.71**	22.3 \pm 0.02
WREJ 250	28 \pm 1.14**	51 \pm 1.79**	71 \pm 0.32	60 \pm 5.45**	5.8 \pm 0.11	15.6 \pm 0.84**	8.1 \pm 1.06	23.5 \pm 0.14
RREJ 125	31 \pm 0.84**	73 \pm 0.41	50 \pm 2.42*	90 \pm 2.45*	3.4 \pm 0.35**	24.2 \pm 0.02	17.7 \pm 0.10	30.9 \pm 0.88
RREJ 250	47 \pm 0.76	69 \pm 0.01	58 \pm 1.62*	112 \pm 0.25	8.8 \pm 0.19	22.9 \pm 0.11	26.7 \pm 0.80*	23.7 \pm 0.16
BREJ 125	43 \pm 0.36	68 \pm 0.09	77 \pm 0.28	95 \pm 1.95	7.1 \pm 0.02	26.5 \pm 0.25	20.5 \pm 0.18	22.5 \pm 0.04
BREJ 250	49 \pm 0.96	73 \pm 0.41	118 \pm 4.38	96 \pm 1.85	9.7 \pm 0.28	32.4 \pm 0.84*	15.8 \pm 0.29	21.4 \pm 0.07
BRCJ 125	37 \pm 0.24*	68 \pm 0.09	99 \pm 2.48	158 \pm 4.35	6.8 \pm 0.01	29.8 \pm 0.58*	10.5 \pm 0.82	6.7 \pm 1.54**
BRCJ 250	44 \pm 0.46	70 \pm 0.11	56 \pm 1.82*	87 \pm 2.75*	5.4 \pm 0.15	17.0 \pm 0.70	27.5 \pm 0.88*	35.6 \pm 1.35
BRWJ 125	39 \pm 0.04*	53 \pm 1.59**	50 \pm 2.42*	94 \pm 2.05	8.6 \pm 0.17	19.8 \pm 0.42	20.5 \pm 0.18	14.5 \pm 0.76*
BRWJ 250	47 \pm 0.76	61 \pm 0.79	86 \pm 1.18	113 \pm 0.15	6.2 \pm 0.07	25.0 \pm 0.10	23.7 \pm 0.50	13.4 \pm 0.87*

Note: * The highest significant, and ** the lowest significant (P<0.05).

Any treatment. After giving the anthocyanin from whole grain of rice-pigmented, control rat displayed the differential MDA expression levels and in all dyslipidemia rat group showed.

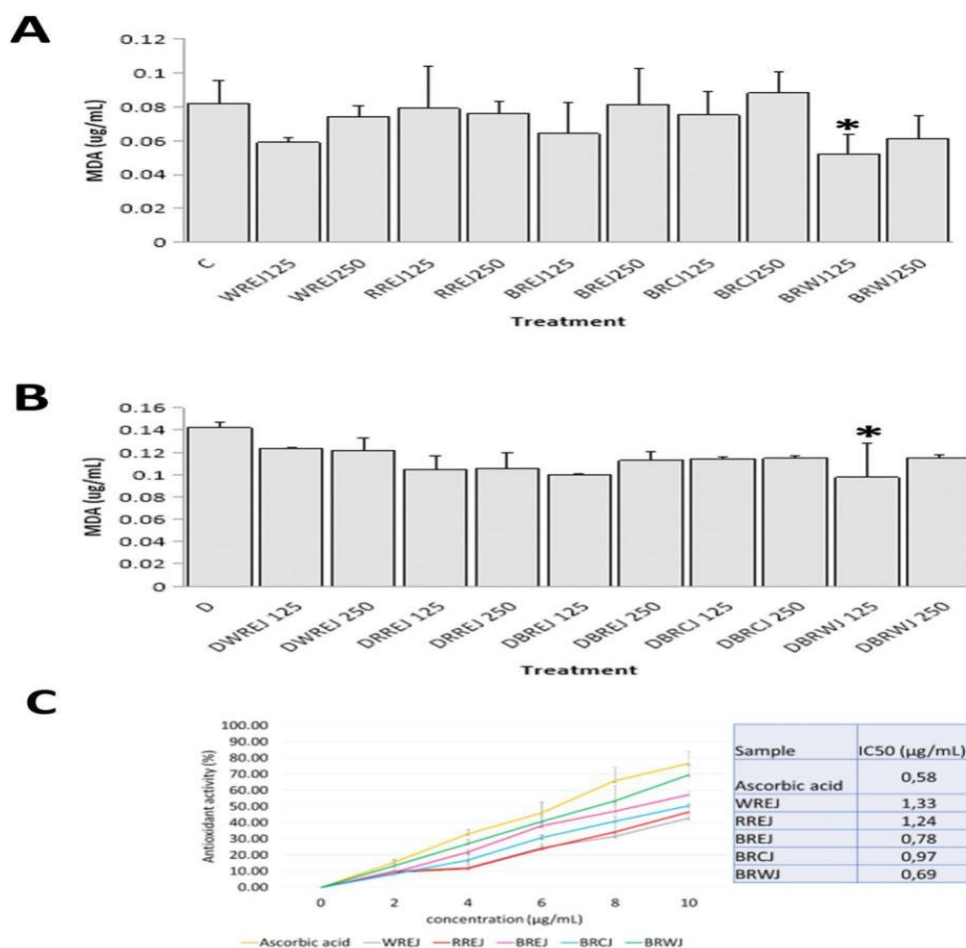


Figure 2. The Malondialdehyde (MDA) Level of Serum rats and Antioxidant activity of rice-pigmented. A. MDA level of Control rat group, B. MDA level of Dyslipidemia rat group. C. Total antioxidant activity of colored rice anthocyanin based on Ferric reducing antioxidant power (FRAP) analysis. *: Significantly lower than other groups (P<0.05).

Declining MDA levels. The lowest MDA level was in rat treated with BRWJ 125mg/kg BW dose, this was not only in control rat but also in dyslipidemia rat groups (Fig. 2A – B). The percentage of antioxidant and IC50 activity of total rice anthocyanin extract showed in black rice included BREJ (0.78 μ g/mL), BRCJ (0.97 μ g/mL) and BRWJ (0.69 μ g/mL) closed with IC50 of ascorbic acid (0.58 μ g/mL) as control. These results displayed that the all black rice have high antioxidant activities.

The level of mRNA C/EBP α , FABP4 & PPAR γ genes susceptibility with obesity

The mRNA level of C/EBP α , FABP4 & PPAR γ genes susceptibility with obesity was also determined in this study. In Figure 3A shows that in rat control group mRNA level of C/EBP α gene expression decreased after given the total anthocyanins WREJ125 and 250, RREJ250, BREJ250, BRCJ250 (low), and BRWJ 125 and 250. The lowest of C/EBP α and FABP4 gene expression were shown in rat group added BRCJ 125mg/kg BW doses. In some control

rat group after being given RREJ125 and 125mg/kg BW the mRNA level of C/EBP α gene expression increased slightly. Interestingly, the mRNA level of FABP4 gene expression was decreased in all treatment rat groups. Whereas the mRNA level of PPAR γ genes expression decreased in the treatment of WREJ 250, RREJ250, and BRCJ 125-250mg/kg BM doses. And the lowest of PPAR γ genes expression was in control rat treated with BREJ 250mg/kg BM. While in some the control rat group treated with WREJ125, RREJ125, BRWJ 125-250 groups had no effects except in rat with BREJ 250mg/kg BW administration that increased significantly.

In the dyslipidemia rat group (Fig. 3B), the mRNA level of C/EBP α and PPAR γ genes expression decreased in WREJ125, BREJ250, BRWJ250 treatments, and the lowest after being given BRWJ125mg/kg BW. Although the other dyslipidemia rat group did not show expression changes. Similar with control rat, in dyslipidemia rat the mRNA level of FABP4 gene expression.

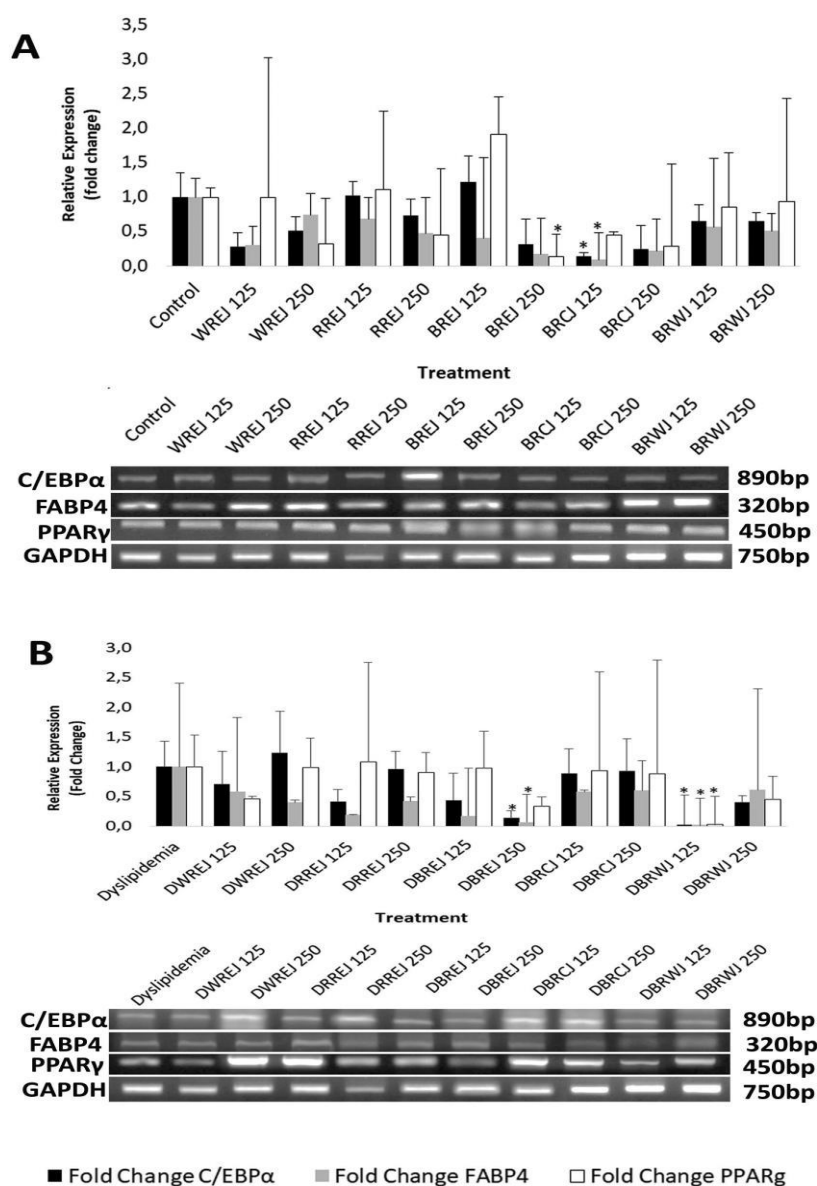


Figure 3. The mRNA level C/EBP α , FABP4 & PPAR γ genes in control, dyslipidemia, pigmented rice-treated control and pigmented rice-treated dyslipidemia rats. A. Control rat group, B. Dyslipidemia rat group *: Significantly lower than other groups (P<0.05).

Decreased almost in all treatment groups, and the lowest was in the administration of BREJ125 and BRWJ125mg / kg BW.

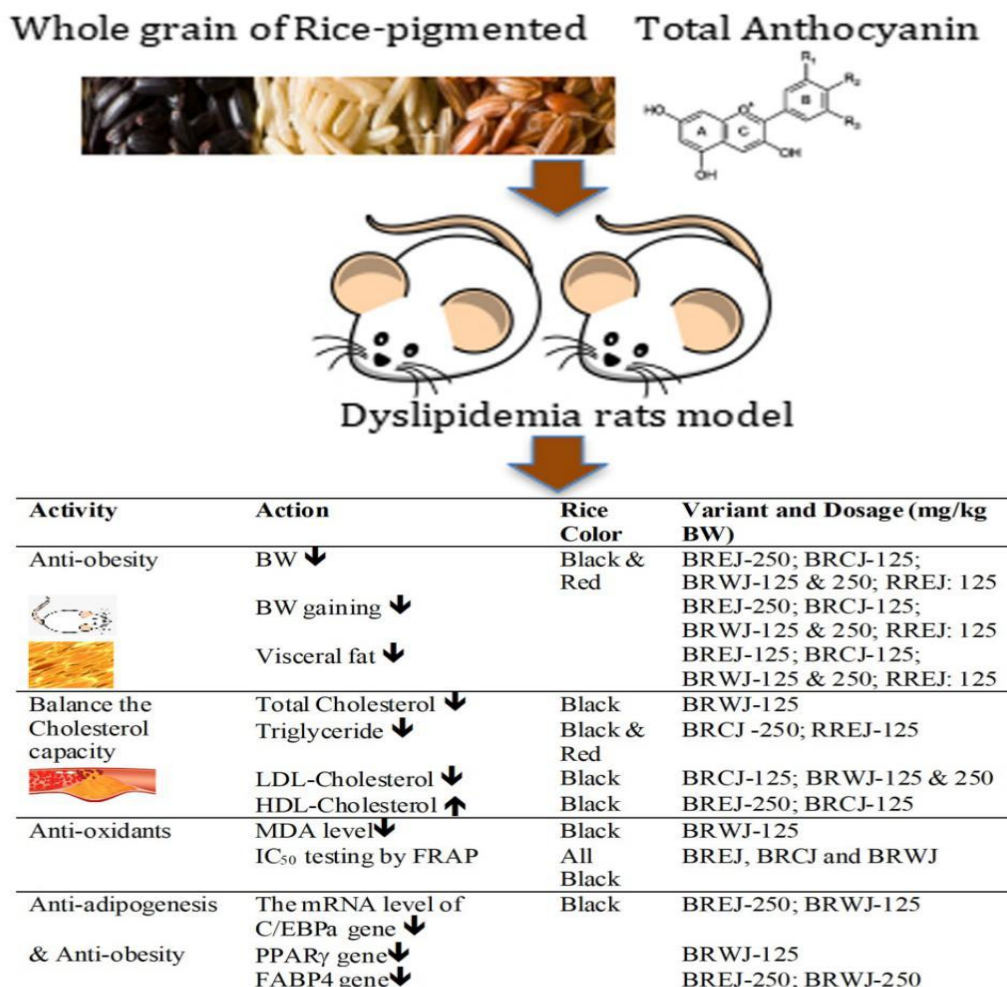


Figure 4. Summary of the activities of total anthocyanin of rice-pigmented whole grains attenuated visceral fat, cholesterol, triglyceride, LDL, MDA, and adipogenesis gene cascade in dyslipidemia rat model.

DISCUSSION

Food consumptions imbalance is one of the factors contributed to obesity that related to impairing health quality life. The high fat cholesterol food consumption has stimulated the body and visceral fat weight and influences the occurrence of overweight and obesity. The body weight and visceral adipocyte can be controlled by bioactive compounds of food such as total anthocyanin in rice pigmented [22]. Food therapy may use dietary fiber crop, fruit and vegetables plant of phenolic compounds such as anthocyanin and flavonoid improved the obesity status of people [23]. Moreover anthocyanin has value to control the health high risk in non-communicable diseases such as diabetes, obesity and cardiovascular disease [24]. In this study, we found that the total anthocyanin of red and black rice succeeds decreasing the body weight and also BW gaining attenuated in control rat groups. Meanwhile the body weights in dyslipidemia rat group were not significantly reduced, although overall of body weigh gaining rats were declined after giving the total anthocyanin of red and black rice.

The animal model fed the high-energy food consumption induced the fat weight in adipocyte tissues. Fat accumulation in visceral adipocyte tissue is harmful sign to overweight or obesity [25]. In our study the visceral fat

weight between control and dyslipidemia rat without any treatment were not different. The total anthocyanin of black rice reduced the visceral fat in control and dyslipidemia rats, definitely. However after giving the total anthocyanin of white rice (250mg/kg BW) and red rice (125mg/kg BW) the visceral fat in both rat groups was increased compared with others. Recently cohort study in woman consumed the daily median intake of total flavonoids include anthocyanin could improve the decreasing of weight maintenance [26]. Daily intake of anthocyanins from several of plants might have benefit to fat adipocyte weight maintenance due to the metabolic disorder for better healthy life, individually [23].

The risk factors for the development of adipogenesis development has respectively promoted by level of LDL and HDL-cholesterol through the AMPK/MAPK mechanism pathway. Anthocyanin is one of bioactive compounds in several plants were reduced the triglyceride, total cholesterol and LDL level [22]. Dyslipidemia is high risk factor contributing to the development of atherosclerosis in T2D, in T2D animal model that provide to combine the HFD-feeding and low-dose STZ injection markedly up-regulated the serum triglycerides, total, VLDL- and LDL-cholesterol with a tendency to decrease HDL-cholesterol [19]. In this study,

the level of total cholesterol and triglyceride in dyslipidemia rat reduced after given the total anthocyanin of whole grain of rice-pigmented. The HDL-cholesterol level increased in dyslipidemia rat treated with anthocyanin of rice-pigmented, definitely. Exception was shown in the dyslipidemia rat with BRCJ 250mg/kg BW and RREJ 125mg/kg BW, the level of LDL-Cholesterol in dyslipidemia rat-treated anthocyanin of rice-pigmented were almost declined. The cholesterol reducing mechanism was affected by anthocyanins for inhibiting of cholesterol synthesis. The energy homeostasis regulation is involved the cholesterol-synthesis through the activation of AMP-activated protein kinase (AMPK) and the inhibition of 3-hydroxy-3-methylglutaryl-coenzyme-A (HMG-CoA)-reductase. When the AMPK activity increased, the cholesterol synthesis inhibited and lead the cholesterol level declined [8,27,28]. The anthocyanins have the potency of a unique pharmacological function in adipocytes-isolated rat to controlling the regulation of adipogenesis through AMPK activation [29]. One of the anthocyanins in pigmented rice, Cyanidin-O-glucose, acts as the inhibitor of HMG-CoA reductase interaction that may lead to a decrease of cholesterol biosynthesis[30]. Dietary black rice extract decreased the serum triglyceride and total cholesterol level [31].

The high contents of anthocyanins in colored grains such as blue-purple maize, purple wheat, and black soybean has potential function as antioxidants and anti-inflammatory because they have a lot of free radical scavenging capacity [32]. The six-anthocyanin glucosides in purple maize were detected higher than cyanidin 3-glucoside that has antioxidants activities [33]. The high level of MDA in dyslipidemia rat group is one biomarker for imbalance between high level of oxidants and low levels of antioxidants that can induce the oxidative stress condition. Our result displayed after giving the total anthocyanins of rice-pigmented, the MDA level of dyslipidemia rat was improved properly and these all black rice of local Indonesia has high potential as anti-oxidants based on Ferric reducing antioxidant power (FRAP) analysis. Moreover, our previous study reported that anthocyanins in pigmented rice had a role antioxidants showed in α , α -diphenyl- β -picrylhydrazyl (DPPH) radical scavenging assay [18]. Oxidants are reactive compounds that can move electrons from other molecules and produce oxidation in these molecules [34,35]. Lipids that have a double carbon chain can react with oxidants. This process is called lipid peroxidation. Lipid hydroperoxide is the main product of the lipid peroxidation process. The structure of lipid hydroperoxides is very unstable and can easily turn into malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE), and several other forms of aldehydes. MDA is the main secondary product in the lipid peroxidation process because it is more mutagenic than other aldehydes [34,36]. Lipid oxidants that are formed can stimulate the production of pro-inflammatory cytokines and activation of Peroxisome proliferator-activated receptors (PPAR) [36].

High cholesterol diets play a role in molecular mechanism associated with obesity stimulation. In adipogenesis, extracellular factors stimulate the activation of cascade transcriptional regulation that controls the differentiation of all adipocytes type. Key regulators of adipogenesis are peroxisome proliferator-activated receptor- γ (PPAR- γ) and CCAAT/enhancer binding protein- α (C/EBP- α) that directly provides the transcriptional cascade genes susceptibility with adipocyte program [37,38]. The

C/EBP α and PPAR γ genes expression promote downstream genes such as the fatty acid binding protein 4 (FABP4), 1-acylglycerol-3-phosphate O-acyltransferase and lipoprotein lipase that stimulates the pre-adipocytes maturation, respectively [11,39].

The mRNA level of C/EBP α , PPAR γ and FABP4 gene in control rat were showed differential expression. The lowest of mRNA level of C/EBP α and FABP4 genes were in control rat added BREJ 125mg/kg BW doses, and the lowest of mRNA level of PPAR γ genes expression is in control rat added BREJ 250mg/kg BW doses. Whereas in the dyslipidemia rat group showed the lowest mRNA levels of C/EBP α , FABP4 and PPAR γ genes were expressed in dyslipidemia rat after being given BRWJ125mg/kg BW. Gene expression in all dyslipidemia rat treatment groups decreased gene expression, except in the C/EBP α gene in rat-treated, which was increased slightly compared with others. The black rice extract significantly improved the mRNA levels of fatty acid metabolism-related genes in liver [31].

Recently study of my research group reported that the elagic acid as bioactive compounds in local rambutan (*Nephelium lappaceum* L.) peel extract (RPE) was reduced significantly the mRNA level of C/EBP α and FABP4 gene expression. That elagic acid in RPE definitely stimulated the declining of PPAR γ protein levels, however the PPAR γ gene expression did not change [11]. In PPAR γ (-/-) mouse embryonic fibroblasts (MEFs) provided the downstream of C/EBP α and PPAR γ target genes and the endogenous of C/EBP α and PPAR γ were extremely low [37]. The PPAR γ and C/EBP α gene expression levels in 3T3-L1 adipocytes treated with soy genistin and genistein were reduced dose-dependently [19]. Adipocytes was treated with anthocyanins enhanced the secretion of adiponectin and leptin (adipocytokine) and provided the increasing of adipocyte specific gene expression in adipocytes-isolated rat to preventing obesity [29]. Other research reported that anthocyanin had a pivotal role to inhibit the adipogenesis through MAPK pathway and promote lipid metabolism by activation of AMK-mediated signaling [17]. According to summary figure of anthocyanin biological function activities (Fig. 4), these studies conclude that the anthocyanin of whole grains of local black rice from Indonesia has functional capability as antioxidants, anti-obesity and anti-adipogenesis.

The present study has shown diminish levels to the visceral fats, triglycerides, LDL, and total cholesterol level, also PPAR γ gene cascade in dyslipidemia rats after administration of anthocyanin from whole grain rice-pigmented. Therefore, we conclude that the anthocyanin of local black rice from Indonesia has potential function as anti-oxidants, anti-obesity and anti-adipogenesis.

CONFLICT OF INTEREST

Authors declare no conflict of interests.

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AUTHOR'S CONTRIBUTIONS

FF, AS, JKRC was design concept the idea and the study. FF, AS, RNR, LFT, HNM provides the in vivo assays and

interpreted the data. FF, AS, JKRC, NK, YN, SF and JKRC wrote the manuscript. FF, AS, JKRC performed the critical revisions. FF and JKRC provided the resources and supervised the study

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