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Amelioration of hyperglycemia, hyperlipidemia, oxidative stress and inflammation in steptozotocin-induced diabetic rats fed a high fat diet by riceberry supplement

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ABSTRACT

Dark purple riceberry bran contains a higher dietary fiber and antioxidant compounds than unpigmented rice bran. Riceberry supplement (RB) was used to evaluate the effects on biochemical parameters, skeletal muscle glucose transporter 4 (GLUT4), oxidative stress and inflammation in a streptozotocin (STZ)-induced diabetes rat. To elucidate the effects were due to dietary fiber supplementation and/or bioactive components, equivalent amounts of dietary fiber present in RB were also fed to STZ-induced diabetic rats. Diabetes Sprague–Dawley rats (non-FBG $\geqslant$ 16.65 mM) were randomly divided into five groups: DM fed a high fat (HF) diet, DM-RB1 fed 5% RB, DM-RB2 fed 41% RB, DM-F1 fed 0.6% fiber and DM-F2 fed 5% fiber. After 12 weeks, significant improvement of BG, insulin, $HbA1_C$, IPGTT and GLUT4 levels were observed in DM-RB1 and DM-RB2 groups. Hyperlipidemia was significantly improved in DM-RB2 and DM-F2 groups. Oxidative stress (TBARS), antioxidant enzymes (SOD, CAT, and GPx), antioxidant capacity (ORAC), pro-inflammation cytokine (TNF-α and IL-6) were improved in DM-RB1 and DM-RB2 groups. Improvement of pancreas and spleen histology was found in DM-RB1 and DM-RB2 groups. These indicate the potential of RB to improve hyperglycemia and hyperlipidemia conditions as well as alleviate oxidative stress and inflammation.

1. Introduction

According to the World Health Organization, approximately 171 million people (2.8% of the total world population) suffer from some form of diabetes. Among the diabetic subjects, 95% are type II diabetic mellitus (T2DM) or non-insulin dependent diabetic mellitus (NIDDM) (World Health Organization, 1999). Hyperglycemia occurring in diabetes does not only damage cellular proteins, membrane lipids and nucleic acids but also increase the rate of onset of disease complications. Treatment and control of diabetes are costly and require long periods of time (Songer & Ettaro, 1998). As treatment with medication in type II DM patients could cause undesirable side-effect, therefore, much interest is directed to the

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supplementation of natural compounds in dietary food, which may provide benefit in terms of the minimization of adverse side effects (Apostolidis, Li, Lee, & Seeram, 2011; Khanal, Howard, Wilkes, Rogers, & Prior, 2010; Qureshi, Sami, & Khan, 2002).

Several studies have shown that rice bran is a rich source of fiber, tocotrienols, γ-oryzanols and ferulic acid, all of which can act as hyperglycemia and hyperlipidemia lowering agents (Qureshi et al., 2002; Zawistowski, Kopec, & Kitts, 2009). Dark purple rice contains considerably more anthocyanin (flavonoid) than unpigmented rice in the aleurone layer of its grain. This compound exerts a strong antioxidant effect (Yodmanee, Karrila, & Pakdeechanuan, 2011) and reduces oxidative stress by enhancing activity of antioxidant enzymes (Chiang et al., 2006). Riceberry, a black purple rice variety (*Oryza Sativa* L.), is a new breeding line developed by the Rice Research Center, Kasetsart University, Kamphaeng Saen, Nakhon Pathom, Thailand. Recently, Leardkamolkarn et al. (2011) reported that a crude extract of riceberry bran contains two major bioactive compounds (trimethylapigenin and triterpenes) and two major anthocyanins (cyanidin-3-glucoside, and peonidin-3-glucoside) that are able to reduce inflammation (Adriano et al., 2006). Therefore, riceberry bran was used in this study as a major ingredient to formulate into dietary riceberry supplement (RB). High fat (HF)-fed rat treated with steptozotocin (STZ) is a widely used animal model of T2DM as it produces many of the characteristics of T2DM, such as insulin resistance or reduced insulin secretion, abnormal lipid profiles as well as β-cell dysfunction (Srinivasan & Ramarao, 2007).

The objective of this study was to demonstrate the effect of varying levels of RB supplement on changes in biochemical parameters, muscle glucose transporter 4 (GLUT4) protein expression level, inflammation, oxidative stress and histology in STZ-induced diabetic HF-fed rat model. Additionally, dietary fiber content was added to the diet of HF-fed diabetic rat in order to determine whether the effects of RB supplement was due to its fiber content and/or from the bioactive components.

2. Materials and methods

2.1. *Riceberry supplement and diets*

RB was kindly provided by the Rice Research Center, Kasetsart University, Kamphaeng Saen, Nakhon Pathom, Thailand. RB contained 60% defatted-black purple riceberry bran, 5% black purple riceberry bran oil and 35% other ingredients for improving texture and flavor of riceberry product. Basal and experimental diets were freshly prepared every 2 weeks in a clean room according to American Institute of Nutrition-76 (AIN-76) and stored in an air-tight dark plastic bag at −20 °C and used within 2 weeks. Food and water were provided *ad libitum* and changed at 10.00 a.m. every day.

2.2. *The analysis of nutrient composition, bioactive compounds and antioxidant capacity of riceberry supplement*

The proximate and mineral compositions were verified according to the standard Association of Analytical Communities methods (Association of Analytical Communities, 2005). γ-Oryzanol and vitamin E were determined by the method of Zawistowski et al. (2009) and Amaral, Casal, Torres, Seabra, and Oliveira (2005), respectively, using high performance liquid chromatography (HPLC) with UV detector at 530 nm (Jasco, Great Dunmow, Essex, UK).

Polyphenol was determined as gallic acid equivalents (mg GAE/g) by Folin-Ciocalteu assay (Madrigal-Carballo, Rodriguez, Krueger, Dreher, & Reed, 2009) using spectrophotometry at 750 nm (Tecan, Männedorf, Zurich, Switzerland). Total flavonoid contents were determined as catechin equivalents (mg CE/g) by the aluminum chloride colourimetric assay (Marinova, Ribarova, & Atanassaova, 2005) using a spectrophotometer at 510 nm (Unicam, Cambridge, UK). Phytochemicals were analyzed as described by Merken and Beecher (2000). Briefly, RB was extracted with a mixture of 30 ml of 0.05% tertiary-butylhydroquinone in 65% methanol and 10 ml of 6 M hydrocholic acid at 90 °C for 2 h. The sample was added with 100 μl of 1% ascorbic acid and 50 ml of absolute methanol then sonicated and filtered prior to inject in HPLC for auto-injection coupled with UV-detector at 210 nm (Agilent, Boeblingen, BW, Germany). Phytochemical standards (ferulic acid, catechin, cyaniding-3-glucoside and peonidin-3-glucoside) used were HPLC grade (Sigma–Aldrich, St. Louis, MO, USA). Oxygen radical absorbance capacity (ORAC) was measured as Trolox equivalents (μmol TE/g) using the method of Prior et al. (2003) and a luminescence spectrometer with the excitation at 493 nm and emission 515 nm (Perkin Elmer, Boston, MA, USA). All analyses were carried out in triplicates (Table 1).

2.3. *Animals and feeding regimens*

Five-weeks-old male Sprague–Dawley rats (147.49 ± 8.45 g) were obtained from the National Animal Center, Salaya campus, Mahidol University, Thailand. All rats were maintained in accordance with the guidelines of the Animal Care Ethical Committee of Central Animal Facility Research Division, Faculty of Science, Mahidol University (Animal Protocol Approval Number: MUSC53-014-187, Validity Dates: March 2, 2010–December 31, 2011). Rats were individually housed in stainless cages at ambient humidity (60 ± 5%), temperature

Table 1 – Active constituents and antioxidant capacity of riceberry supplement.

Compounds	Values (per gram dry matter)
α-Tocopherol (μg)	11.61 ± 0.50
γ-Oryzanol (mg)	1.80 ± 0.20
Ferulic acid (μg)	176.80 ± 5.56
Cyanidin-3-glucoside (μg)	431.50 ± 11.10
Peonidin-3-glucoside (μg)	141.90 ± 5.50
Catechin (mg)	4.39 ± 0.10
β-Carotene (μg)	1.86 ± 0.10
CoQ10 (μg)	2.33 ± 0.10
Polyphenol (mg GAE)	12.37 ± 1.99
Total flavonoids (mg CE)	8.26 ± 0.31
Total ORAC (μmol TE)	317.64 ± 14.07

Values are shown as mean ± SD of sampling (n = 5).

(22 ± 2 °C) and light–dark (12:12) standard environmentally controlled room. Rats were allowed to be accustomed to the new environment for 1 week with free access to rat chow diet and water.

Rats were randomly assigned into two groups: control ($n = 10$), provided with basal diet and water; HF group ($n = 50$), fed a diet containing 28% corn oil (providing 67% of total energy from fat). After 2 weeks, all rats were starved for 12–16 h. Then, control rats received an intraperitroneal (i.p.) injection of 3 ml/kg body weight (BW) of citrate buffer (0.01 M, pH 4.5) and were allowed access to basal diet and water. Rats in HF group were subjected to i.p. injection of 20 and 30 mg/kg BW of STZ (Merck KGaA, Darmstadt, HE, Germany) in citrate buffer within a 1 week period. One week following the second STZ dosage, non-fasting blood glucose (5–10 μl from tail tip) was measured using a portable glucometer (Accu-Chek Performa®, Roche Diagnosis Ltd., Bangkok, Thailand), and treated rats were considered hyperglyceamic when the non-fasting blood glucose value is ⩾16.65 mM (Islam & Choi, 2008). Diabetic rats then were randomly divided into five groups (10 per group) as follows: DM, diabetic rats fed HF diet (28% fat); DM-RB1, diabetic rats fed HF diet and 5% (w/w) RB (containing 0.6% (w/w) fiber); DM-RB2, diabetic rats fed HF diet and 41% (w/w) RB (containing 5% (w/w) fiber); DM-F1, diabetic rats fed HF diet and 0.6% (w/w) fiber; and DM-F2, diabetic rats fed HF diet and 5% (w/w) fiber (Table 2). All rats in the six groups were allowed free access to basal or experimental diets for 12 weeks. Body weight and food consumption (HF and control rats) were measured three times every week, and non-fasting blood glucose levels of STZ-treated rats were measured every week at 10.00 a.m., as chemical-induced diabetes can at times be unstable and reversible due to spontaneous regeneration of β-islet cells especially in long term experiments (Srinivasan & Ramarao, 2007).

2.4. *Intraperitoneal glucose tolerance test*

At week 11 of the experimental period, 12-h fasted rats (test and control) were i.p. injected with 2 g/kg BW glucose solution (50% (w/v) in normal saline (Islam & Choi, 2008). Blood glucose concentration was measured just before injection ($t = 0$), and subsequently at 15, 30, 60, 120, and 180 min. Blood glucose levels were plotted against time.

2.5. *Blood & tissue sampling and preparation*

At the end of the 12-weeks experimental period, each rat was anesthetized with i.p. injection of 5 mg of Xylazine®/kg BW and 20 mg of Zoletil®/kg BW. During anesthesia, 10 ml aliquot of blood was immediately drawn from the abdominal aorta, 1 ml of which was immediately placed in a heparin-coated tube and kept at 4 °C for analysis of glycated hemoglobin (HbA1c), and the remaining was placed into a heparin-coated and non-heparin-coated tubes and centrifuged at 2000*g* at 4 °C for 10 min in order to obtain plasma and serum respectively. Whole blood and 3 ml of plasma were subjected to standard biochemical analysis (total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triacylglycerol (TG), liver enzymes (aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP)), blood urea nitrogen (BUN), and creatinine (National Healthcare Systems, Bangkok, Thailand)), whilst the remaining plasma and serum samples were stored at −80 °C until analyzed.

The right hind limb muscles (gastrocnemous and soleus), liver, pancreas and spleen were excised, cleaned with ice-cold phosphate-buffered saline, pH 7.4 (PBS), and blot dried with filter paper. The right hind limb muscles and liver were separately homogenized in appropriated buffer for individual assay for 30 s (T25 digital Ultra-Turrax® homogenizer, Ika

Table 2 – Diet composition.

Ingredient (g/100 g)	Control (NC)	DM	DM-RB1	DM-RB2	DM-F1	DM-F2
Casein[a]	20.00	20.00	20.00	15.60	20.54	20.00
Corn starch	15.00	3.00	3.00	3.00	3.70	8.74
Sucrose	50.00	10.75	10.75	10.75	13.26	31.33
Cellulose[b]	5.00	5.00	–	–	0.60	5.00
DL-Methionine[c]	0.30	0.30	0.30	0.30	0.30	0.30
AIN-76 Mineral mix[d]	3.50	3.50	3.50	3.50	3.50	3.50
AIN-76 Vitamin mix[d]	1.00	1.00	1.00	1.00	1.00	1.00
Choline-bitartrate[c]	0.20	0.20	0.20	0.20	0.20	0.20
Corn Oil[e]	5.00	28.00	28.00	24.64	28.41	28.00
Riceberry supplement	–	–	5.00	41.00	–	–
Water	–	28.25	28.25	0.00	28.25	2.25
Energy (kcal)	371.0	373.0	392.0	479.0	392.0	479.0

NC: normal rat fed a normal diet (AIN 76A); DM: diabetic rat fed a high fat diet (28% fat).
DM-RB1: diabetic rats fed a high fat diet and 5% (w/w) riceberry supplement.
DM-RB2: diabetic rat fed a high fat diet and 41% (w/w) riceberry supplement.
DM-F1: diabetic rat fed a high fat diet and 0.6% (w/w) fiber (0.39% (w/w) cellulose + 0.21% (w/w) pectin);
DM-F2: diabetic rat fed a high fat diet and 5% (w/w) fiber (3.26% (w/w) cellulose + 1.74% (w/w) pectin).
[a] Erie Food International, Illinois, USA.
[b] Cellulose from Solka Floc, New York, USA, pectin from J.F. Hydrocolloids, Chicago USA.
[c] Sigma–Aldrich, Missouri, USA.
[d] MP Biomedicals, Illkirch, France.
[e] Mazola, Bangkok, Thailand.

Labortechnik, Wilmington, NC, USA) and centrifuged at 10,000*g* for 20 min at 4 °C. The supernatants were stored at −80 °C until analysis. Pancreas and spleen were immersed in 4% paraformaldehyde in PBS for histological examination.

2.6. Serum insulin determination

Serum insulin concentration was measured using an enzyme-linked immunosorbent (ELISA) rat insulin assay kit (Shibayagi Co. Ltd., Shibukawa, Gunma, Japan) in a microplate reader (Molecular Device Inc, Sunnyvale, CA, USA).

2.7. GLUT4 protein determination

GLUT4 protein levels in hind limb muscles (gastrocnemous and soleus) were determined by western blot analysis according to Tremblay, Lavigne, and Jacques (2001). Muscle was homogenized in five volumes of ice-cold RIPA buffer (25 mM Tris HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, and 0.1% SDS). Supernatant protein concentration was determined using bicinchoninic acid (BCA) protein assay kit (Novagen, Madison, WI, USA). Fifty micrograms of protein were separated by 12% SDS-PAGE and transferred to nitrocellulose membrane (GE Healthcare, Piscataway, NJ, USA), which was treated with mouse anti-rat GLUT4 monoclonal antibodies (1:1000 dilution) (Cell Signaling Technology, Boston, MA, USA) and horseradish peroxidase-conjugated rabbit anti-mouse secondary antibodies (1:1500 dilution) (Invitrogen, Grand Island, NY, USA). Immunochemical-stained bands were detected on hyperfilm using ECL Prime Western Blotting Detection Reagent (GE Healthcare, Piscataway, NJ, USA), quantified using National Institute of Health (NIH) software program (http://www.rsbweb.nih.gov/ij/download.html), and reported relative to β-actin (using antibodies from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA).

2.8. Inflammation indices

Tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) were measured in plasma using rat TNF-α and rat IL-6 ELISA kits (eBioscience, Inc., San Diego, CA, USA) according to manufacturer's instructions.

2.9. Oxidative stress, antioxidant enzymes and oxygen radical absorbance capacity (ORAC)

Thiobarbituric acid reactive substances (TBARS) of plasma and liver supernatant were measured and expressed as malondialdehyde (MDA) equivalents using TBAR assay kit (Cayman Chemical Company, Ann Arbor, MI, USA). Liver supernatants were prepared by homogenization in 10 volumes of ice-cold RIPA buffer. Supernatant protein concentration was determined using BCA protein assay kit (Novagen, Madison, WI, USA). Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) was measured in liver supernatants using SOD, CAT and GPx assay kit, respectively (Cayman Chemical Company, Ann Arbor, MI, USA). Plasma oxygen radical absorbance capacity (ORAC) was measured using the method of Prior et al. (2003).

2.10. Histological examination

Pancreas and spleen were prepared for histological examination using standard methods (Karthikesan, Pari, & Menon, 2010). In brief, tissues were fixed in 4% paraformaldehyde solution in PBS, and then embedded in paraffin, sectioned and stained with hematoxylin and eosin. Microscopic examination was conducted under a light microscope equipped with a digital camera (DXIT 1200, Nikon, Japan).

2.11. Statistical analysis

Data were analyzed using SPSS 13.0 for Windows and presented as mean ± SEM. One-way analysis of variance (ANOVA) with Tukey range test was used for statistical analysis of mean difference among group. Unpaired Student's *t*-test was used for statistical analysis of percentage difference (%) between the mean of treated and non-treated diabetic rats. Significance is when p-value < 0.05 at 95% confidence.

3. Results

3.1. The analysis of nutrient composition, bioactive compounds and antioxidant capacity of riceberry supplement

RB 100 g contained total energy 366.2 kcal, protein 8.9 g, fat 8.2 g, carbohydrate 64.2 g, insoluble fiber 8.37 g, soluble fiber 4.47 g, calcium 123 mg, magnesium 393 mg, sodium 307 mg, potassium 726 mg, iron 10.8 mg, zinc 1.9 mg, and copper 0.87 mg. The result of bioactive compounds and antioxidant capacity of RB was shown in Table 1. It was indicated that RB contained the greatest amount of γ-oryzanol 2.0 mg/g, ferulic acid 176.8 μg/g, cyanidin-3-glucoside 437.0 μg/g, peonidin-3-glucoside 130.80 μg/g, polyphenol 14.36 mg GAE/g, total flavanoids 8.25 mg CE/g and other known bioactive compounds.

3.2. Body weight and food consumption

Feeding a HF diet that provided 67% of total energy from fat for 2 weeks was able to significantly increase body weight of rats (from 223.35 ± 1.97 g to 294.37 ± 1.2 g) compared to controls on a basal diet (from 225.11 ± 4.1 g to 277.12 ± 5.50 g), an increase of 36%. One week following STZ treatment, all diabetic HF-fed (DM) rats were able to maintain a constant blood glucose level (21.37 ± 0.52 mM) and body weight (331.57 ± 2.17 g). After 12 weeks of experiment, body weights of all STZ-induced DM rats, both on supplemented and non supplemented diets were not significantly different from that of control group (Table 3).

3.3. Intraperitoneal glucose tolerance test and blood biochemical parameters

The areas under the blood glucose curves (0–180 min) after glucose injection were significantly greater in all DM group than in control ($p < 0.05$) (Table 3). However, feeding with RB (DM-RB1 and DM-RB2 groups) significantly improved glucose tolerance compared to untreated (DM) and fiber-supplemented (DM-F1 and DM-F2) rats ($p < 0.05$). After 12 weeks on the experimental and basal diets, DM rats had significantly higher

Table 3 – Effects of riceberry supplement on body weight (BW), food consumption, energy provide, intraperitoneal glucose tolerance test, blood glucose (BG), HbA1C, insulin and lipid profiles of STZ-induced diabetic (DM) rats on experimental and basal diets for 12 weeks.

	Control (NC)	DM	DM-RB1	DM-RB2	DM-F1	DM-F2
Initial BW (g)	323.08 ± 7.75	332.35 ± 6.14	332.46 ± 6.09	334.18 ± 7.22	332.27 ± 5.52	332.46 ± 2.05
BW gain (g)	155.68 ± 7.54	126.11 ± 8.73	140.06 ± 8.55	142.94 ± 13.78	134.13 ± 12.28	131.80 ± 10.29
Food consumption (g/d)	17.98 ± 0.33[b]	21.79 ± 0.40[a]	20.29 ± 0.23[a]	17.31 ± 0.70[b]	20.35 ± 1.17[a]	16.91 ± 0.92[b]
Energy provide (kcal)	66.74 ± 1.22[b]	81.26 ± 1.49[a]	79.53 ± 0.93[a]	82.92 ± 3.36[a]	79.79 ± 4.56[a]	81.01 ± 4.42[a]
Glucose AUC (mmol l^{-1}. 3 h^{-1})	597.93 ± 34.91[b]	2470.34 ± 254.6[a]	1877.52 ± 65.54[a,b]	1842.63 ± 151.13[a,b]	2291.12 ± 169.33[a]	2168.27 ± 176.10[a]
% AUC dif.	–	–	24.00 ± 2.65[A]	25.41 ± 6.12[A]	−7.26 ± 10.30	12.23 ± 9.37
Final BG (mmol/L)	6.32 ± 0.18[b]	21.82 ± 0.54[a]	13.93 ± 1.75[a,b]	16.03 ± 2.09[a]	20.42 ± 2.51[a]	19.65 ± 2.39[a]
% BG dif.	–	–	−36.15 ± 8.04[A]	−26.52 ± 9.60[A]	−6.34 ± 11.50	9.96 ± 10.97
HbA1c (%)	4.00 ± 0.05[b]	6.20 ± 0.17[a]	4.94 ± 0.25[a,b]	5.77 ± 0.26[a]	5.96 ± 0.32[a]	6.00 ± 0.32[a]
% HbA1c dif.	–	–	−20.36 ± 4.02[A]	−6.95 ± 4.15	−3.18 ± 5.14	−3.23 ± 5.10
Insulin (pmol/L)	97.29 ± 3.91[b]	26.54 ± 3.30[a]	34.02 ± 0.74[a]	31.98 ± 2.28[a]	26.73 ± 3.73[a]	27.90 ± 3.87[a]
% Insulin dif.	–	–	28.17 ± 2.79[A]	20.49 ± 8.60[A]	0.69 ± 14.07	5.10 ± 14.58
Cholesterol (mmol/L)	2.82 ± 0.05[b]	3.68 ± 0.05[a]	3.42 ± 0.02[a]	2.84 ± 0.03[b]	3.45 ± 0.21[a]	2.83 ± 0.02[b]
% Cholesterol dif.	–	–	−7.01 ± 0.67[A]	−22.86 ± 0.83[A]	−6.33 ± 5.60[A]	−23.21 ± 0.56[A]
Triglyceride (mmol/L)	0.98 ± 0.03[b]	1.30 ± 0.06[a]	1.26 ± 0.03[a]	1.02 ± 0.02[b]	1.28 ± 0.04[a]	0.95 ± 0.01[b]
% Triglyceride dif.	–	–	−2.66 ± 2.51	−21.67 ± 1.65[A]	−1.57 ± 3.00	−26.48 ± 1.16[A]
LDL (mmol/L)	0.55 ± 0.02[b]	0.70 ± 0.04[a]	0.67 ± 0.01[a]	0.50 ± 0.02[b]	0.68 ± 0.02[a]	0.46 ± 0.01[b]
% LDL dif.	–	–	−4.37 ± 2.02[A]	28.01 ± 2.54[A]	−2.70 ± 2.46	33.89 ± 0.74[A]

Dietary regimens are described in footnote of Table 2. Values are shown as mean ± SEM of 10 rats per group.
[a] $p < 0.05$ compared to NC by Tukey test;
[b] $p < 0.05$ compared to DM by Tukey test.
[A] $p < 0.05$ compared to DM by unpaired Student's *t*-test.

blood glucose, and HbA1c levels, but lower serum insulin concentration than control rats. On the other hand, blood glucose, and HbA1c levels were significantly lower in DM-RB1 compared to DM group. There were no significant changes in blood glucose, HbA1c and serum insulin concentrations between DM and DM-F1 and DM-F2 rats. TC, TG and LDL-C levels in DM group were significantly higher than control group ($p < 0.05$), whereas those of DM-RB2 and DM-F2 groups were significantly lower than DM group ($p < 0.05$).

3.4. *Skeletal GLUT4 protein*

Western blot analysis of muscle GLUT4 levels (relative to β-actin) revealed the greatest amounts of GLUT4 protein expression level in control rats. GLUT4 levels of DM-RB1 and DM-RB2 group were significantly increased (159% and 150% respectively) as compared to DM group (Fig. 1). No significant difference in GLUT4 level was observed among DM, DM-F1 and DM-F2 rats.

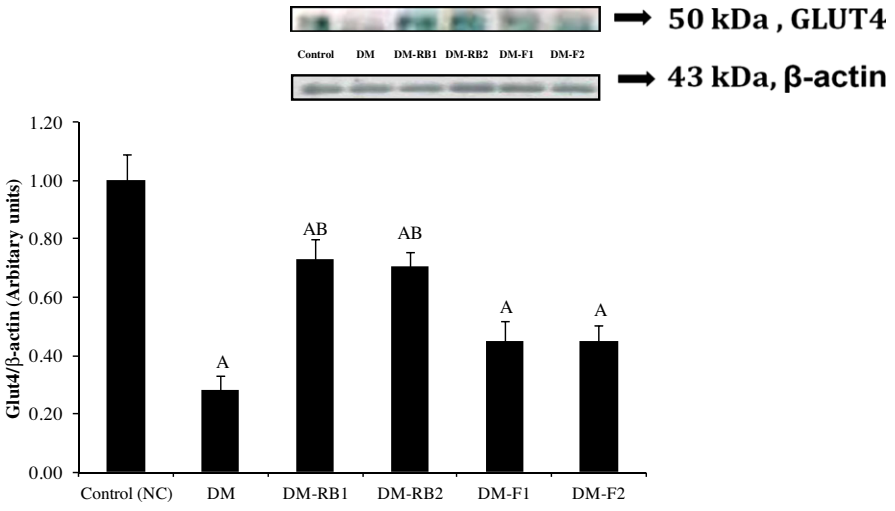

Fig. 1 – GLUT4 protein expression levels in skeleton muscle of STZ-induced diabetes (DM) rats after 12 weeks on experimental and basal diets. Dietary regimens are described in footnote of Table 2. The upper panel depicts representative western blot of skeletal muscle GLUT4 and β-actin (for normalization of gel loading). The relative band intensity is reported in Arbitrary unit, with that of NC set at one. (A) $p < 0.05$ compared to NC by unpaired Student's *t*-test; (B) $p < 0.05$compared to DM by unpaired Student's *t*-test.

Table 4 – Effects of riceberry supplement on pro-inflammation cytokines (TNF-α and IL-6), thiobarbituric acid reactive substances (TBARS), antioxidant enzymes (CAT, SOD & GPx) and oxygen radical absorbance capacity (ORAC) of STZ-induced diabetic (DM) rats on experimental and basal diets for 12 weeks.

	Control (NC)	DM	DM-RB1	DM-RB2	DM-F1	DM-F2
Plasma TBARS (μM)	22.17 ± 1.42[b]	48.80 ± 1.65[a]	31.00 ± 2.86[b]	39.69 ± 3.88[a]	44.40 ± 1.29[a]	44.00 ± 0.81[a]
Liver TBARS (μM/mg protein)	0.70 ± 0.17[b]	2.18 ± 0.38[a]	1.25 ± 0.31	1.77 ± 0.44	1.90 ± 0.33	1.80 ± 0.37
CAT (nmol/min/mg protein)	5.05 ± 1.30	8.16 ± 2.67	6.81 ± 0.86	7.82 ± 1.36	8.62 ± 0.46	7.98 ± 2.07
SOD (U/mg protein	0.63 ± 0.15[b]	0.27 ± 0.04[a]	0.42 ± 0.03	0.37 ± 0.02	0.27 ± 0.07	0.28 ± 3.44
GPx (nmol/min/mg protein)	10.67 ± 4.33	4.80 ± 1.41	8.32 ± 1.55	8.05 ± 2.70	3.60 ± 1.26	5.30 ± 1.18
IL-6 (pg/ml)	62.43 ± 13.33	104.13 ± 12.12	63.07 ± 13.87	67.74 ± 10.34	85.51 ± 9.57	86.70 ± 16.15
TNF (pg/ml)	34.88 ± 4.69	57.53 ± 18.97	35.38 ± 7.12	36.15 ± 5.65	50.22 ± 2.28	48.14 ± 13.28
Total ORAC (μmol TE/100 ml)	402.61 ± 40.72	309.77 ± 29.10	385.58 ± 34.29	451.80 ± 31.78[b]	340.08 ± 5.49	348.26 ± 3.42

Dietary regimens are described in footnote of Table 2. Values are shown as mean ± SEM of 10 rats per group.
One unit of SOD = the amount of enzyme required to exhibit 50% dismutation of superoxide radical.
[a] $p < 0.05$ compared to NC by Tukey test;
[b] $p < 0.05$ compared to DM by Tukey test.

3.5. *Inflammation indices*

IL-6 and TNF-α levels were higher in DM rats than that in controls (Table 4). There was an improvement in these biomarkers only in DM-RB1 and DM-RB2 rats, but not in DM-F1 and DM-F2 rats.

3.6. *Oxidative stress, antioxidant enzymes and oxygen radical absorbance capacity (ORAC)*

Levels of TBARS in both liver supernatant and plasma were significantly higher in DM rats than controls ($p < 0.05$) (Table 4). However, DM-RB1 rats show significantly lower plasma TBARS levels than DM rats ($p < 0.05$). SOD activity of DM group was significantly lower than control group ($p \leqslant 0.05$). No significant differences in CAT, GPx and ORAC values were observed among controls; and all DM groups, excepting DM-RB2 rats showed significantly higher plasma ORAC than DM rats ($p < 0.05$).

3.7. *Histology*

Histological examination of the pancreas in control group showed regular architecture of acinar cells in the exocrine region and intact appearance of islets of Langerhans in the endocrine region, with consistent distribution of small dark purple and a few pink granules within the islets (Fig. 2A). The DM pancreases had numerous irregular acinar cells and smaller islets containing vacuolization and necrotic cells (dense purple granules) (Fig. 2B). Whereas, the pancreas of DM-RB1 and DM-RB2 had greater regular pyramidal acinar cells and their islets were intact with greater number of dark

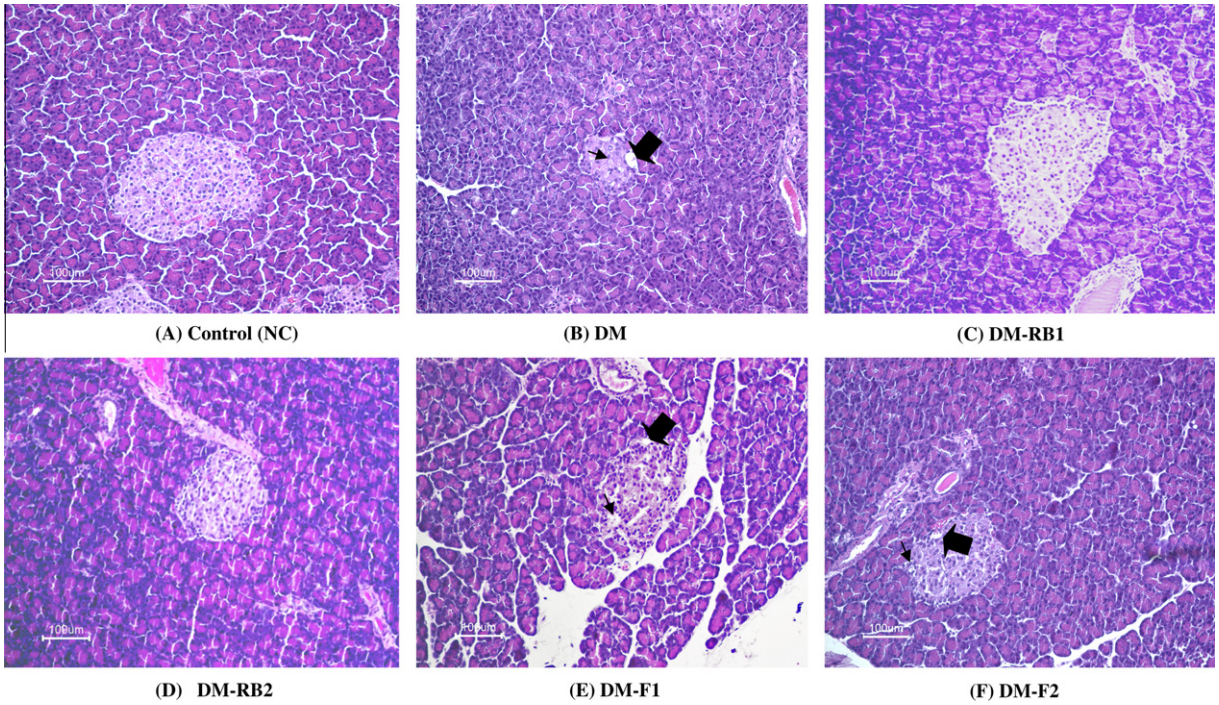

Fig. 2 – Histological appearance of pancreas of STZ-induced diabetic rats after 12 weeks on experimental and basal diets. Dietary regimens are described in footnote of Table 2. Large arrow head indicates vacuolization and small arrow cell necrosis. Magnification 20×. Scale bar = 100 μm (hematoxylin and eosin).

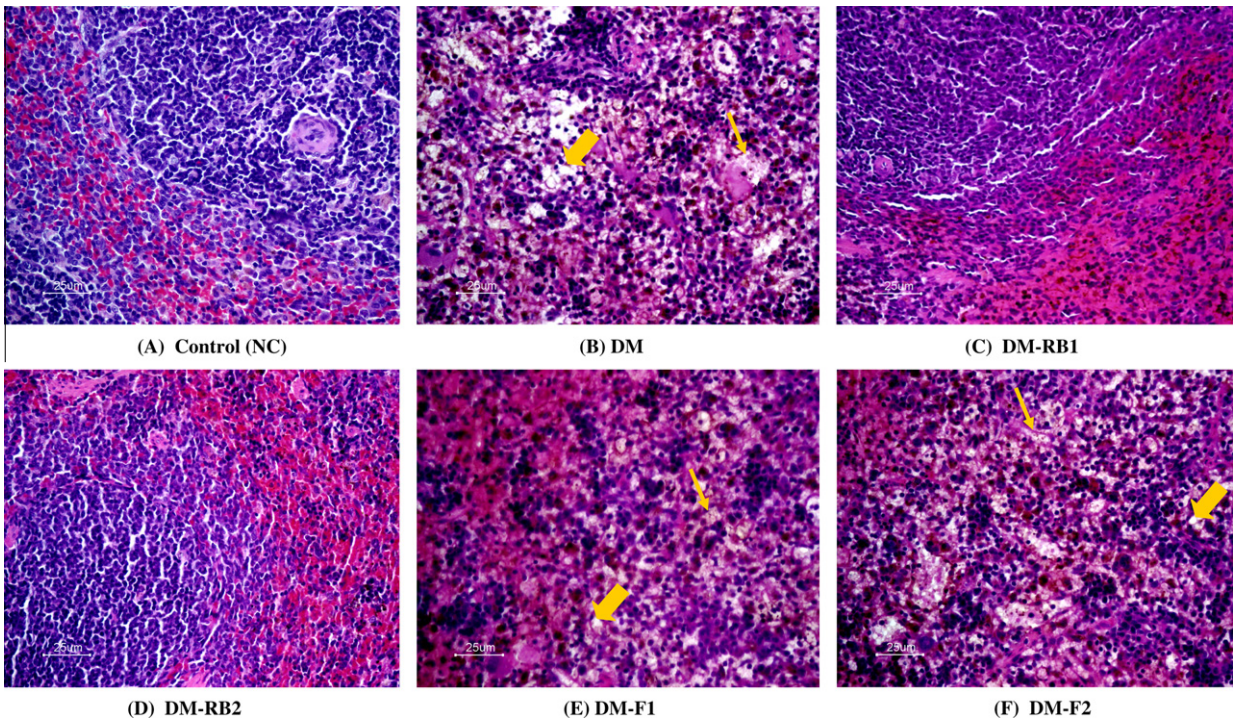

Fig. 3 – Histological appearance of spleen of STZ-induced diabetic rats after 12 weeks on experimental and basal diets. Dietary regimens are described in footnote of Table 2. Large arrow head indicates vacuolization and small arrow cell necrosis. Magnification 40×, Scale bar = 25 μm (hematoxylin and eosin).

purple granules with no appearance of vacuolization and cell necrosis (Fig. 2C and D). No improvement was found in DM-F1 and DM-F2 rats (Fig. 2E and F).

Spleen histology of control rats showed two distinct regions, namely, red pulp consisting of splenic cords and venous sinuses, and white pulp consisting of lymphoid tissue (Fig. 3A). In contrast, DM spleen appeared depleted of white pulps and activation of red pulp, with marked presence of foamy macrophages and vacuolization in red pulp (Fig. 3B). Spleen of DM-RB1 and DM-RB2 rats exhibited greater distinct areas of white and red pulps with lower numbers of foamy macrophages and vacuolization (Fig. 3C and D). No improvement was found in DM-F1 and DM-F2 rats (Fig. 3E and F).

4. Discussion

The results of the present study revealed that following 12 weeks of feeding with HF diet (supplying 67% fat of total energy), body weights of all DM rats were not significantly different from control group, even though DM rats had more energy intake than control group. This might be due to insulin deficiency and/or insulin resistance causing aberrant metabolism of macro- and/or micronutrients, as have been reported in diabetic patients (Qureshi et al., 2002). DM-F1 and DM-F2 rats, which were used as comparison groups with DM-RB1 and DM-RB2 in terms of the dietary fiber content, had body weights similar to the untreated DM group, whereas the body weights of DM-RB1 and DM-RB2 group increased nearly to those of the control group. This finding suggests that RB's bioactive compounds and dietary fiber were beneficial in controlling body weight among DM rats. Zawistowski et al. (2009) found a similar body weight pattern when feeding black rice bran to hyperlipidaemic rat.

As expected, significantly higher glucose tolerance (IPGTT), blood glucose and HbA1c levels and lower serum insulin concentration were present in DM rats than control rats (Gibson, 2005) as shown in Table 3. DM-RB1 and DM-RB2, but not DM-F1 and DM-F2 rats showed hyperglycemia amelioration and pathology improvement of pancreas islets of langerhans, although not complete recovery. This is supported the view that bioactive ingredients, γ-oryzanol, α-tocopherol, cyanidin-3-glucoside, peonidin-3-glucoside, ferulic acid, and other active compounds which contain the greatest amount in RB as shown in Table 1 contributed to these phenomena. Flavonoid extracts have been reported to lower blood glucose levels in IPGTT test by improving insulin sensitivity and enhancing glucose utilization and uptake in db/db mice (Jung et al., 2008). Phenolic compounds (viz. trans-cinnamic, feruric and *p*-coumaric acids) extracted from rice bran can reduce high blood glucose levels by restoring hepatic glycogen and increasing insulin and glucokinase levels (Jung, Kim, Hwang, & Youl, 2007). Tocotrienols have shown to improve body glucose utilization and insulin sensitivity among diabetic db/db mice by regulating genes of the peroxisome proliferator-activated receptors (Fang, Kang, & Wong, 2010). In addition, cyanidin-3-glucoside improved hyperglycemia and insulin sensitivity by reducing retinol binding protein 4 (RBP4) gene expression in T2DM mice. An increase in serum RBP4 expression adversely affects diabetes (Sasaki et al., 2007).

GLUT4 is mainly present in skeletal muscle. Insulin resistance or insulin deficiency causes a low response in signaling GLUT4 to take up glucose. An increase in the GLUT4 protein

level results in enhanced cellular insulin-dependent glucose uptake (Gropper, Smith, & Groff, 2004). Only DM-RB1 and DM-RB2 significantly increased muscle GLUT4 levels compared to DM supporting the role of bioactive compounds rich in RB. A few studies have demonstrated the beneficial effects of bioactive compounds in rice bran on GLUT4 levels. For example, tocotrienols and cyanidin-3-glucoside could upregulate GLUT4 protein expression levels in skeletal muscle of diabetic mice (Fang et al., 2010; Sasaki et al., 2007). Flavonoid extracts could upregulate GLUT4 content in adipose tissue of C57BL/KsJ-db/db mice, possibly through the activation of PPARα gene, which is related to glucose metabolism (Jung, Lee, Park, Kang, & Choi, 2006).

Improvement of lipid profile was found only in DM-RB2 and DM-F2 groups compared to DM group, suggesting a role of high dietary fiber feed content. Ohara, Tabuchi, Onai, and Econ (2000) have demonstrated that administration of arabinoxylane, a soluble fiber extracted from rice bran, for 60 days could improve TC and TG levels in STZ-induced diabetic rats. Lee et al. (2003) also reported that supplement of high fiber rice to diabetic KK mice could decrease TC and TG status significantly. In addition, Zawistowski et al. (2009) demonstrated that supplement of black rice bran to hyperlipidaemic rats for 10 weeks could reduce TC, TG and LDL-C concentrations. Soluble fiber available in rice bran is probably one of the active ingredients responsible for the beneficial effect on lipid profiles by binding to bile acids and inhibiting cholesterol absorption (Gropper et al., 2004).

As regards to the indices of liver and kidney function, plasma ALT, AST, ALP and BUN values were elevated significantly in DM rats compared with controls. The elevation of ALT and AST result from liver injury and elevation of ALP results from bile duct obstruction. While, increasing BUN is a sign of kidney damage (Gibson, 2005). These were ameliorated in DM-RB1 and DM-RB2, but not in DM-F1 and DM-F2 groups (data not shown). Again, these results point to the beneficial role of bioactive compounds present in RB in protecting against liver and kidney damage brought upon by T2DM.

In diabetes, oxidative stress with aberrant levels of antioxidant systems is mainly due to hyperglycemia (Maritim, Sanders, & Watkins, 2003). Our results indicated that RB has antioxidant properties as evidenced by the lower levels of TBARS (a marker of lipid peroxidation) in DM-RB1 and DM-RB2 rats compared to DM-F1 and DM-F2 rats. There was a study that the crude lipophilic extract of rice bran, which provides a rich source of α-tocopherol, tocotrienol and γ-oryzanol could significantly reduce markers of oxidative stress (MDA, urine 8-Isoprostane and 8-OHdG) by increase antioxidant enzyme levels in diabetic KKAy mice (Kanaya et al., 2004). Antioxidant enzymes (SOD, Cat and GPx) also tend to ameliorate in DM-RB1 and DM-RB2 compared to DM group. As oxidative stress is related to inflammation (Maritim et al., 2003), high levels of TNF-α and IL-6, the inflammation cytokines were present in DM rats. The increase of TNF-α and IL-6 might interfere the insulin action by suppressing insulin signal transduction. (Dandona, Aljada, & Bandyopadhyay, 2004). Supplementation of RB resulted in lower plasma TNF-α and IL-6 levels of DM-RB1 and DM-RB2 compared to DM-F1 and DM-F2 rat, which was supported by the improvement of histopathology of spleens and pancreas. Thus, RB may increase insulin signal transduction in DM rats by decreasing TNF-α and IL-6. Many bioactive components abundant in RB were reported to correct oxidative damage and inflammation. For example, feruloyl esters, a component of γ-oryzanol, extracted from rice bran reduced nitric oxide that is produced in macrophage cell by inhibiting NF-kB activation (Nagasaka et al., 2007). In addition, 22-, 23-dihydrostigmasterol and a cycloartane-type triterpene, the plant sterol extracts from riceberry, could inhibit cyclin-dependent kinase, (Leardkamolkarn et al., 2011), the enzyme that promotes inflammatory cell apoptosis in human blood neutrophils (Adriano et al., 2006). Anthocyanin, mainly cyanidin-3-glucoside and peonidin-3-glucoside in black rice, can enhance antioxidant and anti-inflammation activity by protecting against oxidative damage and suppressing nitric oxide synthase in mouse macrophage cell linings (Hu, Zawistowski, Ling, & Kitts, 2003).

Consequently, our study has indicated that RB can improve hyperglycemia and alleviate oxidative stress and inflammation mainly by the bioactive compounds in RB, while, lower hyperlipidemia mainly by dietary fiber in RB.

5. Conclusion

The present study has clearly demonstrated that the deleterious clinical manifestations accompanying STZ-induced hyperglycemia in rat on a long term (12 weeks) high fat diet can be ameliorated by dietary riceberry supplement (up to 41%, w/w). These beneficial hypoglycemic, hypolipidemic, antioxidant, and anti-inflammation effects could be attributed in part to the unique bioactive ingredients, rather than fiber content, present in riceberry bran. Although the results in the present study were obtained from a chemical-induced diabetes animal model, they are encouraging enough to warrant evaluation in human subjects. There is also a need for further studies in order to clarify the specific roles of the bioactive constituents in ameliorating type 2 diabetes mellitus in humans.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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