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Long-term supplementation of high pigmented rice bran oil (*Oryza sativa* L.) on amelioration of oxidative stress and histological changes in streptozotocin-induced diabetic rats fed a high fat diet; Riceberry bran oil

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ABSTRACT

Diabetes is a serious health problem. Searching for alternative natural antioxidants is considered important strategy to manage diabetes. This study evaluated the effect of Riceberry bran oil (RBBO) supplementation on oxidative stress and organ histology in streptozotocin-induced diabetic rats fed a high fat (HF) diet. Adult male Sprague-Dawley rats with hyperglycemia were divided into four groups: DM group fed a HF diet alone; DMRL group fed a HF diet and 5% RBBO; DMRM group fed a HF diet and 7.5% RBBO; DMRH group fed a HF diet and 15% RBBO. Normal rats were used as normal control and were divided into NC and NR group fed a normal diet containing either 5% corn oil or 5% RBBO, respectively. After 12 weeks, RBBO significantly decreased malondialdehyde and restored superoxide dismutase, catalase, glutathione peroxidase, coenzyme Q_{10} and ORAC levels in diabetic rats. RBBO additionally improved the regenerative changes of the pancreas, kidneys, heart and liver. These findings indicate that pigmented RBBO could provide beneficial effect on diabetes by decreasing oxidative stress and recovering organ histology.

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1. Introduction

The prevalence of diabetes among Thai adults between 2010 and 2030 is projected to increase from 7.7% to 9.8% (Shaw, Sicree, & Zimmet, 2010). Oxidative stress results from the overproduction and/or insufficient removal of free radicals that usually accompanies diabetes mellitus. Under chronic hyperglycemia, oxygen radical species (ROS) are accelerated through multiple sources, including enzymatic, non-enzymatic, and mitochondrial pathways, thus causing cellular damage through the oxidation of protein, lipid and DNA and leading to disease complications (Kaneto, Katakami, Matsuhisa, & Matsuoka, 2010). However, ROS can be initially eliminated by essential scavenger enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx).

Antidiabetic drugs, such as biguanides, sulfonylureas and α-glucosidase inhibitors, can control blood glucose levels, but they may also produce several undesirable side-effects. Natural antioxidants offer an alternative therapeutic means with which to counteract oxidative stress associated with an excess of ROS. Riceberry bran oil (RBBO), derived from pericarb and germ of Riceberry (*Oryza sativa* L.), a newly developed pigmented Thai rice cultivar, contains a number of antioxidant compounds, such as vitamin E (tocotrienols), polyphenols (ferulic acid, cyanidin-3-glucoside and peonidin-3-glucoside) and phytosterols (γ-oryzanol, β-sitosterol and triterpene alcohol) (Leardkamolkarn, Thongthep, Suttiarporn, Kongkachuichai, Wongpornchai, & Wanavijitr, 2011). Several studies have demonstrated that colourless rice bran oil has important bioactive capabilities that can play a role in hypoglycemic (Wilson, Nicolosi, Woolfrey, & Kritchevsky, 2007), hypolipidemic (Siddiqui, Khan, & Siddiqui, 2010) and immune function modulation (Sierra, Lara-Villoslada, Olivares, Jimenez, Boza, & Xaus, 2005). Tocotrienol and γ -oryzanol isolated from colourless rice bran oil were also reported to suppress oxidative stress in animal studies (Ghatak & Panchal, 2012; Siddiqui et al., 2010). In addition, RBBO contains a higher amount of vitamin E, γ-oryzanol and other bioactive compounds than those in non-pigmented rice bran oil (Hoed, Depaemelaere, Ayala, Santiwattana, Verhe, & Greyt, 2006; Yoshie, Kanda, Nakamura, Igusa, & Hara, 2009). RBBO also was an excellent source of other active compounds that were not reported in colourless rice bran oil. RBBO's distinctive characteristic is that it contains a high level of antioxidant properties that might be beneficial in managing diabetic complications. Consequently, the role of RBBO supplementation of streptozotocin (STZ)-induced diabetic rats fed a high fat diet in preventing oxidative stress and ameliorating histopathological changes was investigated in this study.

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2. Materials and methods

2.1. Riceberry bran oil preparation

RBBO was carefully extracted from the bran of black rice cultivar Riceberry, a new Thai rice cultivar developed by the Rice Research center:centre, Kasetsart University (Nakhon Pathom, Thailand), using the supercritical carbon dioxide fluid extraction (Model 7037, Serial 770000062760, Applied Separations, Inc., PA, USA) under high-pressure (300 bar) and low-temperature (45 °C) conditions (Balachandran, Mayamol, Thomas, Sukumar, Sundaresan, & Arumughan, 2008). The compositions of γ-oryzanol and 8 isoforms of vitamin E (tocopherols and tocotrienols) of RBBO and other vegetable oils commonly consumed in Thailand were analyzed using high performance liquid chromatography (HPLC) as described previously (Chen & Bergman, 2005) (Table 1).

2.2. Animals

Sixty male 5 weeks-old Sprague-Dawley rats with body weight (BW) from 145 to 154 g were provided by the National Animal Center, Mahidol University, Salaya, Thailand. All rats were individually housed in stainless steel cages maintained at 23 ± 2 °C with a 12 h light–dark cycle. After one week of acclimation, the rats were randomly divided into two groups: control group ($n = 20$) with free access to a basal diet (AIN-76) and experimental group ($n = 40$) with free access to a high fat (HF) diet (modified AIN-76 containing 200 g of casein, 30 g of corn starch, 108 g of sucrose, 50 g of cellulose, 280 g of corn oil per kilogram, with supplementation 3 g of DL-Methionine, 10 g of vitamins and 35 g of minerals) as shown in Table 2.

HF fed rats were intraperitoneally injected twice (once every other day) with freshly prepared STZ (Merck KGaA, Darmstadt, Germany) (20 and 30 mg/kg BW dissolved in 10 mM citrate buffer pH 4.5) in order to induce diabetes, while control rats were administered only citrate buffer solution. STZ-treated HF rats with non-fasting blood glucose (non-FBG) value ⩾16.65 mM and % glycated hemoglobin (HbA1c) ⩾6.5 were considered to be diabetes (Islam, Choi, & Loots, 2008), and randomly divided into 4 experimental groups of 10 rats each. DM group was fed a HF diet only; DMRL group fed HF diet supplemented with 5% RBBO; DMRM group fed a HF diet supplemented with 7.5% RBBO; and DMRH group fed a HF diet supplemented with 15% RBBO (Table 2). Normal rats were divided into 2 groups, NC group was fed a basal diet containing 5% corn oil and NR group was fed a basal diet containing 5% RBBO (Table 2).

After 12 weeks of experimental period, rats were anaesthetized by intraperitoneal injection of Xylazine® (5 mg/kg BW) and Zoletil® (20 mg/kg BW). The abdomen was opened and a blood sample was collected from abdominal vena cava into two tubes, one with and another without ethylenediamine tetra-acetic acid (EDTA) as an anticoagulant. Blood samples were centrifuged at 2000*g* for 10 min, 4 °C. Serum was used for measurements of vitamin E and plasma for malondialdehyde (MDA), oxygen radical absorbance capacity (ORAC) and coenzyme Q_{10} (CoQ_{10}) measurements. The pancreas, kidney, heart and liver were excised and flushed with ice-cold phosphate-buffered saline pH 7.4 (PBS) and then fixed in

Table 1
Tocopherols, tocotrienols and γ-oryzanol in Riceberry bran oil and other vegetable oils.

	Riceberry bran oil	Corn oil	Soy bean oil	Palm oil	Rice bran oil[a]
α-tocopherol (mg/100 g)	10.84	13.56	9.15	21.16	9.47
β-tocopherol (mg/100 g)	1.47	0.19	1.21	0.00	0.41
γ-tocopherol (mg/100 g)	27.27	14.07	68.18	0.00	6.79
δ-tocopherol (mg/100 g)	2.05	0.00	22.26	0.00	0.00
α -tocotrienol (mg/100 g)	2.39	0.00	0.00	21.08	1.68
β-tocotrienol (mg/100 g)	0.00	0.00	0.00	3.87	0.31
γ-tocotrienol (mg/100 g)	42.81	0.00	0.00	28.04	25.18
δ-tocotrienol (mg/100 g)	3.28	0.00	0.00	5.87	0.52
γ-oryzanol (g/100 g)	1.93	0.09	0.05	0.06	0.46

Values are shown as mean of duplicate analyzed by HPLC.
[a] The commercial rice bran oil (colourless) claims a high γ-oryzanol content.

Table 2
Composition of experimental diets.

Ingredient (g/100 g)	Gr I. NC	Gr II. NR	Gr III. DM	Gr IV. DMRL	Gr V. DMRM	Gr VI. DMRH
Casein[a]	20.0	20.0	20.0	20.0	20.0	20.0
Corn starch	15.0	15.0	3.0	3.0	3.0	3.0
Sucrose	50.0	50.0	10.8	10.8	10.8	10.8
Cellulose[b]	5.0	5.0	5.0	5.0	5.0	5.0
DL-Methionine[c]	0.3	0.3	0.3	0.3	0.3	0.3
AIN-76 mineral mix[d]	3.5	3.5	3.5	3.5	3.5	3.5
AIN-76 vitamin mix[d]	1.0	1.0	1.0	1.0	1.0	1.0
Choline–bitartrate[c]	0.2	0.2	0.2	0.2	0.2	0.2
Corn Oil[e]	5.0	–	28.0	23.0	20.5	13.0
Riceberry bran oil	–	5.0	–	5.0	7.5	15.0
Water	–	–	28.2	28.2	28.2	28.2
Energy (kcal)	371.0	371.0	373.0	373.0	373.0	373.0

Normal control (NC); 5% RBBO, normal (NR); untreated diabetic (DM); 5% RBBO (DMRL); 7.5% RBBO (DMRM); 15% RBBO (DMRH).
[a] Erie Food International, Illinois, USA.
[b] Solka Floc, New York, USA.
[c] Sigma–Aldrich, Missouri, USA.
[d] MP Biomedicals, Illkirch, France.
[e] Mazola, Bangkok, Thailand.

10% formalin for subsequent histopathological examination. Remaining liver was immersed in liquid nitrogen for subsequent determination of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), MDA and ORAC activities. All of the procedures in this animal study were approved by the Animal Care Ethical Committee of the Central Animal Facility Research Division, Faculty of Science, Mahidol University, Thailand (Approval No. MUSC53-014-187).

2.3. Determination of oxidative stress

Liver samples were homogenized in RIPA buffer pH 7.5 (containing 25 mM Tris HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate and 0.1% SDS) using a homogenizer (T25 digital Ultra-Turrax®, Ika Labortechnik, NC, USA), and centrifuged at 10,000*g* for 20 min at 4 °C. MDA contents of liver supernatant and plasma were measured using TBARS (thiobarbituric acid-reactive substances) assay kit (Cayman Chemical Company, Ann arbor, MI, USA). Absorbance at 532 nm was converted to MDA concentration as described by the manufacturer.

2.4. Determination of antioxidant enzyme activity

After homogenizing one g of liver with 2 ml of 20 mM 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) buffer pH 7.2 containing 1 mM ethylene glycol tetra-acetic acid (EGTA), 210 mM mannitol and 70 mM sucrose, and centrifuged at 10,000*g* for 20 min at 4 °C. Supernatant SOD activity was measured using a commercial kit (SOD Assay Kit; Cayman Chemical Company, Ann Arbor, MI, USA) according to the manufacturer's instructions.

For determination of CAT activity, one g of liver was homogenized with 2 ml of 50 mM potassium phosphate buffer pH 7.0 containing 1 mM EDTA and then centrifuged at 10,000*g* for 20 min at 4 °C. CAT activity was measured using a commercial CAT assay kit (Cayman Chemical Company, Ann Arbor, MI, USA). In brief, the reaction was initiated by incubating with hydrogen peroxide for 20 min and terminated with potassium hydroxide, followed by addition of 4-amino-3-hydrazine-5-mercapto-1,2,4-triazole and potassium periodate.

For measurement of GPx activity, one g of liver was homogenized with 2 ml of 50 mM Tris HCl pH 7.5 containing 5 mM EDTA and 1 mM dithiothreitol (DTT) and centrifuged at 10,000*g* for 20 min at 4 °C. A commercial GPx assay kit (Cayman Chemical Company, Ann Arbor, MI, USA) was employed according to the manufacturer's protocol. Briefly, GPx activity was initiated in the supernatant by adding cumene hydroperoxide and measuring absorbance at 340 nm over a period of 5 min.

2.5. Determination of vitamin E concentration

Serum vitamin E concentration was determined using a reverse-phase HPLC with slightly modified the method of Bieri, Tolliver, and Catignani (1979). A 100 μl aliquot of serum, 100 l of internal standard and 200 l of ethanol were vortex mixed for 30 s, and then the sample was extracted twice with 750 l hexane and subsequently evaporated under nitrogen gas. The residue was dissolved in 100 l of methanol and injected into HPLC equipped with a UV detector at wavelength of 295 nm.

2.6. Determination of coenzyme Q_{10} concentration

Plasma Coenzyme Q_{10} was measured according to the protocol described by Okamoto, Fukunaga, Ida, and Kishi (1988). Employing HPLC equipped with an electrochemical detector. Ubiquinone was used to generate a calibration curve.

2.7. Determination of antioxidant capacity

Antioxidant capacity in plasma and liver was measured in terms of ORAC in both hydrophilic and lipophilic phases as modified by the previous method (Prior et al., 2003). Briefly, 100 l of plasma was extracted with 10% ethanol, while 1 g of liver sample was homogenized with 4 ml of PBS and centrifuged at 10,000*g* for 20 min at 4 °C. Both plasma and liver supernatant samples were extracted with hexane and used 7% methylated -cyclodextrin (RMCD) in 50% acetone/50% water (v/v) as a dilution and to dissolve the standards for lipophilic assay. Remaining samples were extracted with 50% acetone and diluted with 75 mM phosphate buffer (pH 7.2) for hydrophilic assay. The 2,2′-azobis (2-amino-propane) dihydrochloride (AAPH) was used as the generator of peroxyl radical, while 6-hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was used as standard antioxidant. Three ml of fluorescein solution (1.95 ml/100 ml phosphate buffer) and 500 μl of sample were mixed together, followed by 500 μl of AAPH (0.414 g/10 ml phosphate buffer). Fluorescence (emission of 515 nm and excitation of 493 nm) was immediately recorded using luminescence spectrophotometer (PerkinElmer, MA, USA).

2.8. Histopathological study

The pancreas, kidney, heart and liver biopsies were processed for histology examination. After fixing with 4% paraformaldehyde in PBS, samples were dehydrated, embedded in paraffin, and sliced into 5 μm thickness sections, and stained with hematoxylin and eosin. Slides were examined under using a light microscopy (40–100× magnification) equipped with a digital camera system (DXIT 1200, Nikon, Japan).

2.9. Statistical analysis

Data analysis was done using the Statistical Package for the Social Science (SPSS, version 13.0, Chicago, IL). Results of variables were expressed as mean ± standard error of mean (SEM). Data of all biochemical parameters were analyzed using one-way ANOVA, followed by Tukey's test, depending on the normality of variables. Significant difference was considered when $p < 0.05$.

3. Results

3.1. Effect of Riceberry bran oil (RBBO) on oxidative stress in diabetic rats

Elevation in MDA levels were observed in both plasma and livers of untreated diabetic rats when compared to normal control rats. In contrast, dietary supplementation with RBBO in DMRL group (5% RBBO supplementation) significantly decreased plasma MDA levels, while hepatic MDA levels significantly decreased in all RBBO supplemented groups when compared to the DM group ($p < 0.01$) (Table 3).

3.2. Effect of Riceberry bran oil (RBBO) on enzymatic and non-enzymatic antioxidants in diabetic rats

The activities of enzyme SOD and GPx in the liver of untreated diabetic rats (DM) significantly decreased compared to the normal rats. Administration of RBBO to STZ-induced diabetic rats fed a HF diet significantly increased or restored those enzyme activities close to normal values ($p < 0.01$). Hepatic CAT activity significantly increased in DM group, while RBBO supplemented diabetic rats

Table 3
Effect of Riceberry bran oil (RBBO) on oxidative stress and antioxidant levels of rats fed the experimental diets for 12 weeks.

	NC	NR	DM	DMRL	DMRM	DMRH
Oxidative stress levels						
Plasma MDA (μM)	23.30 ± 2.01[a]	23.28 ± 1.09[a]	45.86 ± 2.58[c]	31.35 ± 3.61[ab†]	39.87 ± 0.93[bc]	42.09 ± 4.86[bc]
Liver MDA (μM/mg protein)	1.12 ± 0.08[a]	1.09 ± 0.10[a]	1.77 ± 0.11[b]	1.19 ± 0.05[a†]	1.19 ± 0.08[a†]	1.18 ± 0.03[a†]
Antioxidant levels						
Liver SOD activity (U/mg protein)	0.64 ± 0.07[a]	0.71 ± 0.06[a]	0.23 ± 0.07[b]	0.62 ± 0.06[a†]	0.52 ± 0.05[a]	0.48 ± 0.05[ab]
Liver CAT activity (nmol/min/mg protein)	5.32 ± 0.38[a]	5.58 ± 0.43[a]	8.44 ± 0.60[b]	5.63 ± 0.54[a†]	6.15 ± 0.43[a]	7.05 ± 0.59[ab]
Liver GPx activity (nmol/min/mg protein)	10.66 ± 1.18[a]	11.16 ± 0.90[a]	4.01 ± 0.76[b]	10.64 ± 1.31[a†]	12.79 ± 1.08[a†]	12.92 ± 1.28[a†]
Serum vitamin E (μmol/L)	200.50 ± 4.50	188.72 ± 3.02	211.33 ± 5.34	209.97 ± 7.58	207.42 ± 4.31	201.69 ± 8.73
Plasma coenzyme Q_{10} (μg/L)	201.69 ± 8.73	22.20 ± 0.89[a]	12.90 ± 0.59[c]	17.00 ± 0.52[b†]	17.82 ± 0.85[b†]	18.36 ± 0.39[b†]

Data are expressed as mean ± SEM. Each group contained 10 rats.
Values in the same row with different superscript are significantly different at $p < 0.05$ by ANOVA coupled with Tukey's test, † are significantly different compared to untreated diabetic group at $p < 0.01$. Normal control (NC); 5% RBBO, normal (NR); untreated diabetic (DM); 5% RBBO (DMRL); 7.5% RBBO (DMRM); 15% RBBO (DMRH); Malondialdehyde (MDA); Superoxide dismutase (SOD); Catalase (CAT); Glutathione peroxidase (GPx). One unit of SOD = the amount of enzyme required to exhibit 50% dismutation of superoxide radical.

showed a reduction of CAT activity, wherein a significant difference in the concentration of CAT activity was observed in DMRL ($p < 0.01$) and DMRM ($p < 0.05$) groups (Table 3).

Deleterious plasma CoQ_{10} levels were observed in the DM group compared to the NC group ($p < 0.05$). A significant elevation in the plasma CoQ_{10} was observed in RBBO supplementation (DMRL, DMRM and DMRH groups) compared to the DM group ($p < 0.01$). No significant difference in serum vitamin E levels was observed in all normal and supplemented groups (Table 3).

3.3. Effect of Riceberry bran oil (RBBO) on antioxidant capacity in diabetic rats

Reductions in lipophilic and hydrophilic ORAC levels were observed in the plasma and livers of the DM group. Plasma lipophilic and hydrophilic levels in the supplemented groups, particularly those DMRM and DMRH groups, increased when compared to those of the DM group ($p < 0.01$) (Fig. 1A). Similarly, liver lipophilic and hydrophilic levels significantly increased in all RBBO

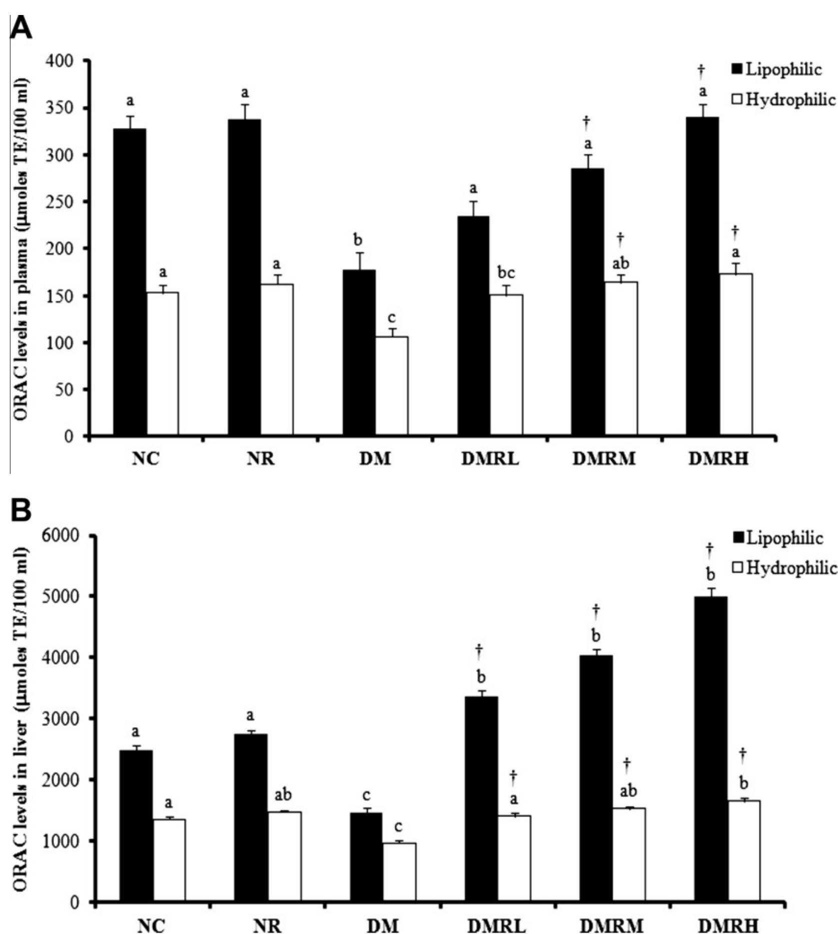

Fig. 1. Riceberry bran oil (RBBO) on oxygen reactive absorbance capacity (ORAC) in the plasma (A) and livers (B) of rats fed experimental diets for 12 weeks. Data are expressed as mean ± SEM. Each group contained 10 rats. Values with different superscript are significantly different at $p < 0.05$ by ANOVA coupled with Tukey's test, † are significantly different compared to untreated diabetic group at $p < 0.01$. Normal control (NC); 5% RBBO, normal (NR); untreated diabetic (DM); 5% RBBO (DMRL); 7.5% RBBO (DMRM); 15% RBBO (DMRH).

supplemented groups ($p < 0.05$; $p < 0.01$), which was more pronounced in dose-dependent RBBO (Fig. 1B).

3.4. Effect of Riceberry bran oil (RBBO) on histopathology of pancreas, kidney, heart and liver

Pancreatic cells of normal groups (NC and NR) showed normal proportions of islets of Langerhans (Fig. 2A and B). Acinar cells were arranged in lobules with prominent nuclei and surrounding a healthy islet cells. Cellular damage to islets of Langerhans was evident in DM group, which exhibited pancreatic β-cell necrosis and degeneration with irregular vacuoles together with atrophy (reduction of cell size and number), but acinar cells surrounding the islets appeared normal (Fig. 2C). DM rats treated with RBBO (DMRL, DMRM and DMRH groups) showed improvement of cell damage, as characterized by partial restoration of islet cells, reduced β-cell necrosis and vacuolization and an increase in numbers of islets (Fig. 2D and F).

Similarly, normal morphology and structure kidney of NC and NR rats (collecting ducts with descending and ascending loops

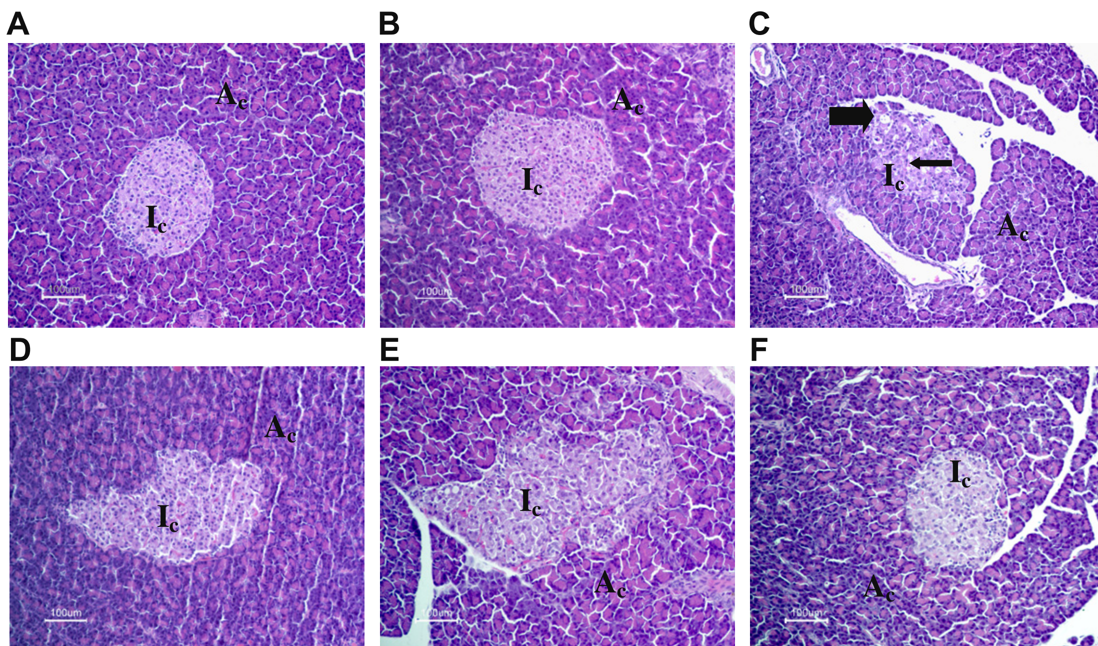

Fig. 2. Hematoxylin and eosin stained sections of the pancreases of normal and diabetic rats fed basal and experimental diets for 12 weeks. Normal control (NC) (A); 5% RBBO, normal (NR) (B); untreated diabetic (DM) (C); 5% RBBO (DMRL) (D); 7.5% RBBO (DMRM) (E); 15% RBBO (DMRH) (F); Acinar cells (A_c); Islets of Langerhans (I_c). Pancreatic necrosis (thick arrow) and vacuolization (thin arrow) are presented, Magnification 100× (DXIT 1200, Nikon, Japan).

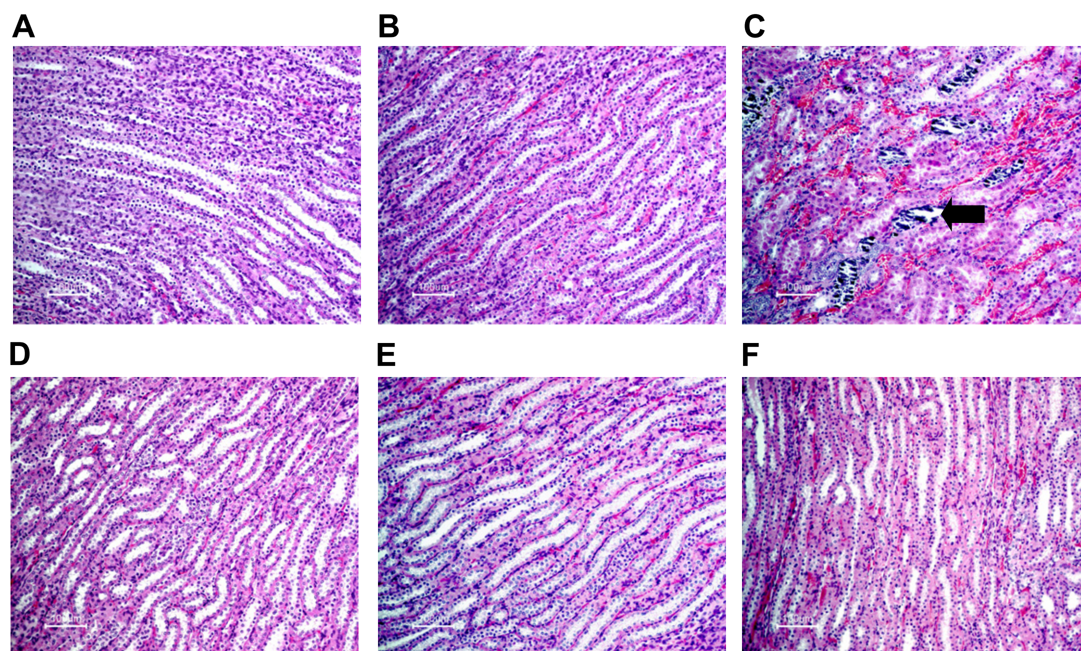

Fig. 3. Hematoxylin and eosin stained sections of kidneys of normal and diabetic rats fed basal and experimental diets for 12 weeks. Normal control (NC) (A); 5% RBBO, normal (NR) (B); untreated diabetic (DM) (C); 5% RBBO (DMRL) (D); 7.5% RBBO (DMRM) (E); 15% RBBO (DMRH) (F). Crystal deposition (thick arrow) is presented, Magnification 100× (DXIT 1200, Nikon, Japan).

arranged in cubic capsules with prominent nuclei) (Fig. 3A and B), was affected in DM animals, with presence of crystal deposition in swollen lobular lumens and infiltration of red blood cells (Fig. 3C). These pathological appearances were reversed in all RBBO supplemented groups (DMRL, DMRM and DMRH), as shown by cell regeneration and the disappearance of crystal deposition in the kidney (Fig. 3D and F).

As for the heart, sections (oblique cut) in NC and NR rats showed cardiac myocytes arranged regularly with prominent nuclei and normal architecture (Fig. 4A and B). DM rats exhibited infarct areas with lymphocyte infiltration and fibrosis (Fig. 3C). RBBO supplementation (DMRL, DMRM and DMRH groups) improved cardiac myocytes pathology (Fig. 4D and F).

The cell architectures of liver in normal rats (NC and NR) showed normal hepatocytes with a central vein (Fig. 5A and B). Hepatocytes were arranged with well distinct cytoplasm and nuclei, in which sinusoids radiated from the central vein. In DM group, cell degeneration was presented with foamy macrophage

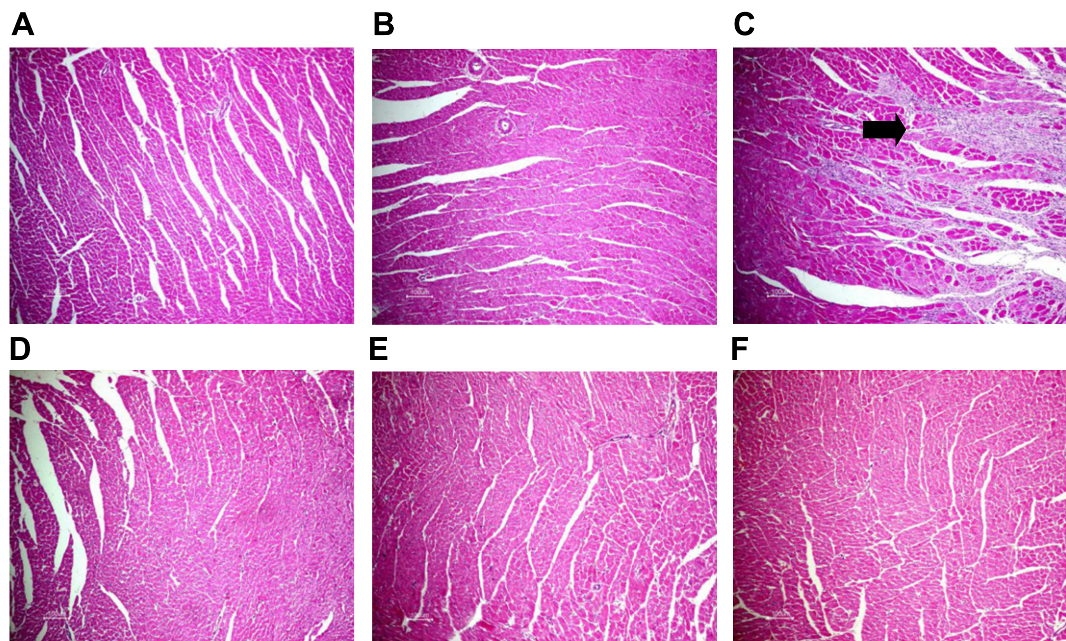

Fig. 4. Hematoxylin and eosin stained sections of hearts of normal and diabetic rats fed basal and experimental diets for 12 weeks. Normal control (NC) (A); 5% RBBO, normal (NR) (B); untreated diabetic (DM) (C); 5% RBBO (DMRL) (D); 7.5% RBBO (DMRM) (E); 15% RBBO (DMRH) (F). Myocardial infarction (thick arrow) is presented, Magnification 100× (DXIT 1200, Nikon, Japan).

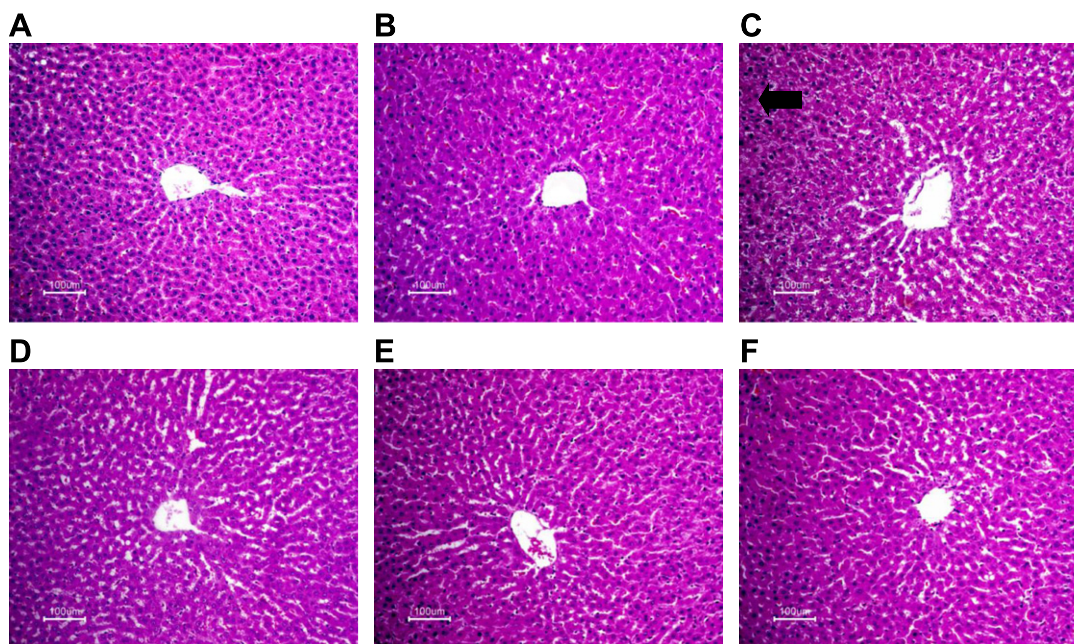

Fig. 5. Hematoxylin and eosin stained sections of livers of normal and diabetic rats fed basal and experimental diets for 12 weeks. Normal control (NC) (A); 5% RBBO, normal (NR) (B); untreated diabetic (DM) (C); 5% RBBO (DMRL) (D); 7.5% RBBO (DMRM) (E); 15% RBBO (DMRH) (F). Foamy macrophage (thick arrow) is presented, Magnification 100× (DXIT 1200, Nikon, Japan).

and sinusoid dilation (Fig. 5C). These damages were reversed in rats supplemented with RBBO (Fig. 5D and F).

4. Discussion

In worst cases of DM there is destruction of pancreatic β-islet cells of Langerhans, resulting in reduction of insulin secretion or degranulation. Diabetic rats in the present study was induced by a HF diet and low dose of STZ resulting in destruction of pancreatic β-cells (Fig. 2), which causes hyperglycemia (non-FBG 17.80 ± 1.98 mM and 6.50 ± 0.37% HbA1c). This finding is in agreement with previous studies that demonstrated that an STZ-induced diabetic rat was a good experimental diabetes model with regard to raising free radical production (Islam et al., 2008; Srinivasan, Viswanad, Asrat, Kaul, & Ramarao, 2005). In addition, ROS in diabetes could damage inner endothelial tissues, which this phenomenon would be directly responsible for elevating high blood glucose in diabetic rats (Tas, Celikler, Ziyanok-Ayvalik, Sarandol, & Dirican, 2011).

Our study found that untreated STZ-induced diabetic rats had significantly increased MDA concentration in both the plasma and liver. Elevation in MDA indicated that there was greater production of ROS that increased the rate of lipid peroxidation. These might be due to an increase in high blood glucose levels and glucose autoxidation. This agrees with results of previous studies demonstrating increase of lipid peroxidation in liver and kidney in clinical and experimental diabetes (Ou, Jackson, Jiao, Chen, Wu, & Huang, 2007; Tas et al., 2011). Our results also showed that supplementation of RBBO could reduce oxidative stress by decreasing MDA levels in plasma and in livers of diabetic rats. Previous research has shown that MDA is a reliable indicator to detect lipid peroxidation product and it was widely used as indicator of cell injury from excess ROS production (Maritim, Sanders, & Watkins, 2003).

Antioxidant enzymes, including SOD, CAT and GPx, play an important role in scavenging the toxic intermediate of incomplete oxidation. A reduction in these antioxidant enzymes activities can lead to an excess availability of the superoxide anion O_2^- and hydrogen peroxide (H_2O_2) in biological systems, which in turn generates more hydroxyl radicals as well as the regeneration of propagation of lipid peroxidation. A review study, however, has shown diminished hepatic SOD and GPx activities as a result of increasing ROS generation (Maritim et al., 2003), which were confirmed by our results. Generally, the diabetic tissue showed decreased CAT activity, but some reports have noted an increase in CAT activity in different tissues of diabetic rats, especially in the liver and kidney (Dias, Porawski, Alonso, Marroni, Collado, & Lez-Gallego, 2005; Aliciguzel, Ozen, Aslan, & Karayalcin, 2003), which are in agreement with our study. Elevation of CAT activity found in diabetic tissue may indicate an adaptive response to overcome the overproduction of H_2O_2 (Hunkar, Aktan, Ceylan, & Karasu, 2002). These elevation was reversed by supplementation with RBBO (5% and 7.5%), which indicates that the bioactive substances contained in RBBO had a high ability to scavenge H_2O_2. Furthermore, CoQ_{10} could act together with vitamin E to suppress lipid peroxidation and also indirectly recover tocopherol from tocopheroxyl radical. Our study has found that plasma CoQ_{10} levels significantly declined in DM rats. This finding confirms those of earlier study (Lim et al., 2006). The significant increase in plasma CoQ_{10} levels was observed after 12 weeks in DM rats treated with RBBO.

Antioxidant capacity (ORAC) in both lipophilic and hydrophilic phases significantly decreased in the plasma and livers of untreated diabetic rats due to an increase in antioxidant requirements for ROS homeostasis regulation. There also reported a positive correlation between total antioxidant capacity and antioxidant enzymes (Ou et al., 2007). In our study, lipophilic and hydrophilic ORAC levels markedly increased in the plasma and livers of RBBO supplementation groups. This result implies that oxidative stress was reduced by the actions of lipid and water soluble antioxidants and their bioavailability in RBBO.

We speculate that tocotrienols and γ-oryzanol, which are two major antioxidants found in RBBO of this study, played important role in against oxidative stress. Tocotrienols has abilities to suppress lipid peroxidation via donation of a hydrogen atom to lipid radicals (Siddiqui et al., 2010). It was shown that tocotrienols are more effective than -tocopherol in increasing activities of SOD and CAT levels and decreasing lipid peroxide and nitric oxide levels (Kuhad & Chopra, 2009). Siddiqui, Khan, and Siddiqui reported that a tocotrienol-rich fraction isolated from rice bran oil improves the levels of MDA, SOD and CAT in the kidneys of diabetic rats closely to control values. Tocotrienol was able to reduce plasma and aorta oxidative damage in diabetic rats (Budin, Othman, Louis, Bakar, Das, & Mohamed, 2009). While, γ-oryzanol has antioxidant properties to increase hepatic SOD, GPx and CAT activities and to decrease plasma MDA levels in mice (Son, Rico, Nam, & Kang, 2010). Ferulic acid, a one component of γ-oryzanol, has functional property of free radical scavenger via electron donation (Srinivasan, Sudheer, & Menon, 2007). From our preliminary study, ferulic acid (3.02 μg/g) was analyzed (personal communication). In addition, other biological active ingredients in RBBO, including phenolic acid (*p*-coumaric acid and gentisic acid), flavonoids (cyanidin-3-glucoside and peonidin-3-glucoside) and phytosterols (β-sitosterol and triterpene alcohol), might synergistically work to improve plasma and hepatic MDA levels and hepatic SOD, CAT and GPx activities. Phenolic acid, such as *p*-coumaric acid and gentisic acid, have been reported in rats in which these compounds stimulate mRNA expression of SOD, GPx and CAT in liver (Yeh & Yen, 2006). Increased SOD and CAT activities in the livers of rats fed black rice bran possibly result from upregulation of antioxidant enzymes, while cyanidin-3-glucoside and peonidin-3-glucoside attenuated oxidative stress by reducing ROS level (Chiang, Wu, Yeh, Chu, Lin, & Lee, 2006).

Regarding the histological changes after RBBO administration, it was found the restoration of the organ histopathology, particularly in pancreas. RBBO could enhance pancreatic activity in regenerating β-cells by reducing β-cells necrosis and vacuolization. In addition, RBBO supplementation could alleviate renal, heart, and liver injuries in DM rats close to normal. At present, no other studies have shown the effect of other rice bran oil supplementation on histology, except for a few studies showing that rice bran oil compositions, such as tocotrienols and ferulic acid could protect pancreatic β-cells through alleviating oxidative stress and antioxidant enzymes (Balasubashini, Rukkumani, Viswanathan, & Menon, 2004; Budin, Yusof, Idris, Hamid, & Mohamed, 2011). Therefore, the histopathological examination in this study supported our biological markers which reveal the beneficial effect of tocotrienols and γ-oryzanol presented in RBBO on the improvement of oxidative stress and antioxidant enzymatic and non-enzymatic scavengers.

5. Conclusion

This study has shown that Riceberry bran oil (RBBO) supplementation could have beneficial health effects in diabetic rats by enhancing the amount of hepatic antioxidant defense mechanism and reducing oxidative stress, thereby causing regenerating cells of the pancreas, kidney, heart and liver to return to normal. Further studies are required to investigate the major bioactive ingredients of RBBO and their antioxidant mechanism at cellular level.

6. Conflict of interest

The authors certify that there is no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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