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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).