Research Article

Antidiabetic mechanism of ethanol extract of black rice bran on diabetic rats

Arifah Sri Wahyuni, Rima Munawaroh, Muhammad Da'i

Faculty of Pharmacy, Universitas Muhammadiyah Surakarta, Pabelan Kartasura, Jawa Tengah, Indonesia.

Correspondence to: Arifah Sri Wahyuni, E-mail: arifah.wahyuni@ums.ac.id

Received November 11, 2015. Accepted November 19, 2015

Abstract

Background: Black rice bran contains bioactive compounds; previous studies have shown the ethanol extract of black rice bran (EEBRB) can decrease levels in diabetic rats. **Aims and Objective:** To elucidate the mechanism of antidiabetic effect of EEBRB. **Materials and Methods:** This research was carried out by setting a few parameters for antidiabetic. The effects of inhibition of the enzyme α-glucosidase performed with in vitro studies using the extracted enzyme of intestines of mice. A total of 5 mL α-glucosidase enzyme was incubated for 5 min with EEBRB 1 mL with a serial concentration in phosphate buffer pH 7.2. Enzyme inhibitory effect was determined by measuring the concentration of formed glucose with Lane–Eynon method. The insulin level and glucose-lowering activity were determined on rats induced by alloxan 150 mg/kg and treated with EEBRB. Treated rats for 28 days were sacrificed and were prepared for histopathological observation of pancreas. **Result:** EEBRB was able to inhibit the activity of the enzyme α-glucosidase on glucose with IC_{50} values of 121 + 12.3 mg%. The EEBRB extract dose of 100 mg/kg was able to decrease blood sugar levels to 151 ± 38.58 mg/dL. The level of insulin in diabetic rats administered by EEBRB dose of 50, 100, and 200 mg/kg was obtained 6.52 ± 5.94, 11.5 ± 3.5, and 15.20 ± 9.5 ng/mL, respectively. Meanwhile, histopathological observations showed an improvement on the pancreatic beta cells. **Conclusion:** Antidiabetic mechanism of EEBRB is associated with increased insulin levels because of regeneration of pancreatic beta cells in diabetic rats.

KEY WORDS: Black Rice Bran; Antidiabetic; Insulin Level; Pancreatic Beta Cells

Introduction

Black rice (*Oryza sativa* L. *indica*) is one of the main rice crops in South Asia and Mainland China. Rice bran is a by-product of the process of grinding or comminution of paddy into rice. Black rice bran (BRB) is rich in fiber contains bioactive compounds including tocopherols, tocotrienols, oryzanols, vitamin B complex, and phenolic compounds.[1] Among the phenolic compounds, cyanidin-3-glucoside (C3G) is the major anthocyanins in black rice. This compound is 93% of total anthocyanins in black rice.[2] Anthocyanin is a member of flavonoid soluble in water and role as a natural pigment. These compounds produce red, purple, and blue colors in plants.[3] Total anthocyanin levels in EEBRB was 3.28 ± 0.34 mg/100 g.[7] The C3G is known as a natural anthocyanin with strong antioxidative and radical scavenging activities against hydroxyl and superoxide radicals,[4] reduce inflammation of adipose and liver steatosis in rats with high fat diet, and decreases hyperglycemia in diabetic mice.[5,6] Our previous studies showed that EEBRB able to decrease blood sugar levels of rats to levels of 130.50 ± 31.86 mg/dL, and to increase the levels of blood urea nitrogen to 32.25 ± 3.5 mg/dL on nephropathic diabetic rats.[7]

Flavonoids have been reported for its antidiabetic activity through beta-cell regeneration mechanism, making it possible to increase the release of insulin. Mechanisms of flavonoids in stimulating the release of insulin are increasing Ca^{2+} in Langerhans islet cells.[8] BRB has potential as a natural medicine

Access this article online	
Website: http://www.njppp.com	Quick Response Code:
DOI: 10.5455/njppp.2015.5.1111201590	

for lowering blood sugar. This study was conducted to determine the mechanism of EEBRB to decrease blood sugar levels on alloxan-induced mice. This experiment was conducted to observe the effect of EEBRB to inhibit enzyme α-glucosidase, increasing levels of insulin and glycogen levels, and histopathology of the pancreas cells.

Materials and Methods

Materials

BRB, 70% ethanol extract, alloxan monohydrate (Sigma-Aldrich, NSW, Australia), Glucose GOD FS (DiaSys, Holzheim, Germany), Insulin Elisa Kit (Sigma-Aldrich, St. Louis, MO, USA). Phosphate buffer pH 7.4, *n*-butanol, sucrose, fructose, glucose, methylene blue, $CuSO_4$, and Insulin kit for Rat (Sigma-Aldrich).

Experimental Animal

Twenty five male Sprague Dawley (SD) rats weighing 200–300 g were used into this study. Animals were maintained with standard environmental condition and fed with standard diet pellet. All studies were conducted in accordance with the animal ethical committee of the Sebelas Maret University, Indonesia.

Methods

α-Glucosidase inhibition activity assay

Isolation of crude enzymes from the intestine of mice. Male rats fasted for 24 hours were killed, dissected, and part of the small intestine was taken. The intestine was cleaned with a solution of NaCl and the layers of the epithelium (mucous tissue) were collected by scraping the surface with a spatula. Mucosal tissue that had been etched were homogenized in pH 7.4 phosphate buffer solution of 10 mL, then centrifuged at 6000 rpm for 15 min. Butanol was added on resulting supernatant to dissolve the lipids (1:1). Crude α-glucosidase enzyme was resulted as clear yellow solution by centrifuging at 6000 rpm for 15 min.[9]

α-Glucosidase inhibition activity assay. A total of 5 mL α-glucosidase enzyme was incubated for 5 min with 1 mL of EEBRB with a series of different concentrations (6.125, 12.5, 25, 50, and 100 mg%) in phosphate buffer pH 7.2, then added sucrose (90 mM) as substrate and incubated at 37°C for 120 min. The result was diluted with phosphate buffer pH 7.2 to 50 mL. And blank solution was carried out with same procedure without EEBRB. Resulting glucose was measured by Lane–Eynon method for reduced sugar, using methylene blue as indicator, titrated by using $CuSO_4$ solution until a brick red color as the end point of the titration.

The percentage of α-glucosidase inhibition activity was calculated by using the following formula:

$$\% \text{ inhibitory} : \frac{GK - GE}{GK} \; x \; 100\%$$

where, Gk is the level of reduced sugar without EEBRB (enzyme + substrate) and GE level of reduced sugar treated with EEBRB (enzyme + substrate + extract).[9]

Determination of blood sugar levels: Male rats were 2–3 months old, weighing 200–300 g, adapted for 7 days, and divided into five groups. The treatments were administered orally.

- Group I: normal controls, and were administered distilled water
- Group II: diabetic rats (induced by alloxan) were administered distilled water
- Group III: diabetic rats were treated with EEBRB dose of 50 g/kg
- Group IV: diabetic rats treated with EEBRB dose of 100 mg/kg
- Group V: diabetic rats treated with EEBRB dose of 200 mg/kg

The blood was obtained for day 0 (as baseline), and after diabetic rats was resulted (blood glucose levels >200mg/dL), the blood was obtained for days 4, 7 and 10, and were measured of glucose and insulin levels. A volume of 10 μL was added to 1000 mL of a mixture of monoreagent GOD Glucose Assay KIT (HK), incubated for 10 min at 37°C, and read at visible spectrophotometer.

Insulin level in serum. Series standard insulin solution concentration 0, 1.563, 3.125, 6.25, 12.5, 25, and 50 ng/mL dissolved and put into wells of 100 μL, as well as the serum of the isolated samples, incubated for 90 min at a temperature of 37°C. The wells were added with 100 μL biotinylated detection antibody and incubated for 1 h and washed 3 times using washing buffer solution, and added 100 μL horseradish peroxidise-conjugated secondary antibody and incubated for 30 min at a temperature of 37°C and washed 5 times with washing buffer, 90 μL substrate was added and incubated for 30 min at a temperature of 37°C. The last stage was the addition of 50 mL stop solution and measured levels of serum insulin at a wavelength of 450 nm in units of ng/mL (the procedure was adapted from manual of the product Insulin Kit for Rat with modification).

Histopathology of the pancreas. Pancreas in mice, which had been treated for 28 days, removed and preserved with formalin 10% and observed macroscopically. Histopathological preparations were carried out by soaking in formalin buffered solution, dehydrated, fixed in paraffin, and microscopic preparations were made with hematoxylin and eosin staining. Histopathological experiment was carried out in Anatomical Pathology Laboratory of Veterinary Medicine Universitas Gadjah Mada, Indonesia.

Result

α-Glucosidase Inhibitory Effect of EEBRB

α-Glucosidase enzyme is an enzyme located on the surface of the intestine, it plays a role in the metabolism of carbohydrates into monosaccharides[10] and hydrolyzes class of cereals (Poaceae or Gramineae). Inhibition of α-glucosidase in the intestine can inhibit the digestion and absorption of complex carbohydrates.[11]

Table 1: Mean percentage of α-glucosidase inhibition by serial concentration of EEBRB

EEBRB concentration (mg%)	% Inhibition
6.125	9.70 ± 3.97
12.5	16.94 ± 3.12
25	23.33 ± 2.88
50	29.37 ± 1.82
100	41.90 ± 1.64

EEBRB, ethanol extract of black rice bran.

The results showed five series of EEBRB concentrations (6.125, 12.5, 25, 50, and 100 mg%) able to decrease glucose levels as a result of the metabolism of sucrose, it demonstrates the ability of EEBRB in inhibiting the enzyme (Table 1).

Effect of BRB to the Decrease in the Blood Sugar of White Rats

Rats induced by alloxan at dose 150 mg/kg BW, could increase blood sugar levels to an average of 404.6 ± 166.3 mg/dL. Diabetic rats with blood glucose levels > 200 mg/dL were resulted on days 2-4 after alloxan induced. A dose of 100 mg/kg BW of EEBRB was able to decrease blood sugar started from day 4 and effectively on day 10 ($p < 0.05$). The treatment dose of 200 mg/kg BW decresed blood glucoe to 131.33 mg/dL at day 10 (Table 2).

Insulin Level of EEBRB-Treated Rats

Treatment of diabetic rats on day 10 indicated that EEBRB could increase the level of insulin significantly. A dose of 200 mg/kg BW of EEBRB could increase insulin level significantly compared to negative control and normal control to 15.2 ± 9.5 ng/dL [Table 3]. On the basis of baseline data, rats treated with EEBRB dose 200 mg/kg BW consistently indicated the increase of insulin level.

Histopathology of Pancreas in Treated Rats

This experiment was performed to show regeneration or repair of pancreas cell in treated rats for 10 days. The results showed alloxan treatment of 150 mg/kg BW (negative control) indicated inflammation of the pancreatic beta cells. The groups administered EEBRB dose of 50, 100, and 200 mg/kg showed regenerative cell. Pancreatic beta cells showed repairing back to normal on treatment of dose of 100 mg/kg BW (Figure 1).

Discussion

The inhibitory activity of the enzyme α-glucosidase (Table 1), with IC_{50} values was 121 mg% leads to antidiabetic effect of EEBRB due to inhibition of the breakdown of carbohydrates into monosaccharides, this leads to a decrease in blood glucose

Table 2: Average blood glucose level in treated rats

Groups	glucose levels (mg/dL) before and after treatment				
	Early Levels	day 0	day 4	day 7	day 10
	83	83	90	161	126
Normal control (I)	92	116	112	122	149
	155	152	88	136	152
X ± SD	110 ± 39.23	117 ± 34.51	96.67 ± 13.31	139.67 ± 19.75	142.3 ± 14.22
II	190	230	545	469	447
Negative control (II)	120	206	137	147	133
	132	519	457	418	306
X ± SD	147.33 ± 37.43	318.33 ± 174.19	397.67 ± 214.71	344.67 ± 173.07	295.33 ± 157.27
III	134	414	475	193	345
EEBRB 50 mg/kg BW (III)	100	669	637	547	583
	116	243	195	184	169
X ± SD	116.67 ± 17.00	442 ± 214.37	451.67 ± 198.03	308 ± 207.02	365.67 ± 20..77
IV	138	240	214	141	124
EEBRB 100 mg/kg BW (IV)	181	474	154	218	152
	149	486	430	184	141
X ± SD	156 ± 22.33	400 ± 138.69	266 ± 145.61	151 ± 38.58	139 ± 14.10
V	118	347	213	108	130
EEBRB 200 mg/kg BW (V)	136	341	167	122	124
	204	686	431	174	140
X ± SD	152.67 ± 45.35	458 ± 197.47	270.33 ± 141.02	134.67 ± 34.77	131.33 ± 8.08

EEBRB, ethanol extract of black rice bran; SD, standard deviation.

Table 3: The average of insulin level in treated rats

Groups	Average of insulin level (ng/mL), X ± SD		
	Baseline	Alloxan induced	After 10-day treatment
Normal control (I)	6.5 ± 2.9	6.5 ± 2.9	6.3 ± 4.5
Negative control (II)	12.9 ± 9.9	1.4 ± 2.3	4.5 ± 2.3
EEBRB 50 mg/kg BW (III)	23.4 ± 22.2	15.1 ± 8.2	6.5 ± 5.9
EEBRB 100 mg/kg BW (IV)	16.8 ± 5.5	1.8 ± 2.4	11.5 ± 3.5
EEBRB 200 mg/kg BW (V)	13.7 ± 9.2	13.5 ± 11.7	15.2 ± 9.5

levels. This effect probably was contributed by the content of C3G, which is a specific inhibitor of the enzyme glucosidase in the intestine. The position of the cyaniding-3-O is an important factor for modulating inhibition of glucosidase and α-amylase.[12]

Other studies have shown that flavonoids anthocyanins in black rice may lower blood glucose levels.[5] This experiment showed EEBRB dose 200 mg/kg BW could decrease glucose level to 131.33 ± 8.08 mg/dL compare to its glucose level on day 0 (458 ± 197.47 mg/dL). These results were confirmed by the observation that insulin levels showed a significant improvement in the treatment group EEBRB 200mg/kg BW (Table 3). Consistently, EEBRB demonstrated the ability to induce the repair or regeneration of pancreatic beta cells (Figure 1).

Some studies support the potential EEBRB as an antidiabetic. One of the compounds contained in EEBRB is C3G. This compound is a derivative of anthocyanins were able to reduce the concentration of blood glucose and improve insulin sensitivity in type 2 diabetic mice through a mechanism upregulating glucose transporter 4[5] and downregulating retinol-binding protein 4 in adipose tissue.[13] C3G also demonstrated the prevention of insulin resistance in adipocytes via signal transduction inhibition of c-Jun NH2-terminal kinase.[14]. Recent studies have shown that C3G and cyanidin-3-galactoside can directly stimulate the secretion of insulin from the pancreas. Flavonoid compounds work by improving pancreatic beta cells.[8]. In addition, the mechanism of flavonoids as antidiabetic through stimulation of glucose uptake into peripheral tissues.[15]

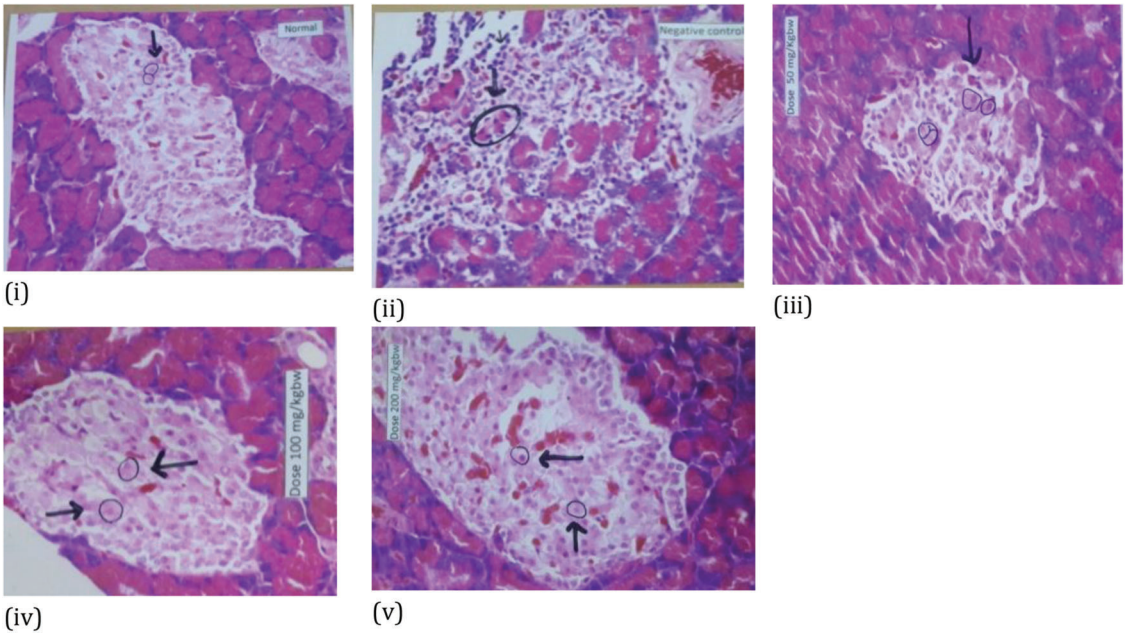

Figure 1: Stained histopathology of pancreas observed under microscope 40 ×. (i) Normal control, (ii) negative control, indicated inflammation and degeneration of beta-cells-treated rats with ethanol extract of black rice bran (EEBRB) 50 mg/kg BW, (iii) EEBRB 100 mg/kg BW, and (iv) EEBRB 200 mg/kg BW indicated cells back to normal.

Conclusion

This experiment concludes that EEBRB can decrease blood glucose level on alloxan-induced diabetic. The mechanism of antidiabetic effect of EEBRB through (a) α-glucosidase inhibition activity with IC_{50} is 121 mg%, (b) EEBRB 200 mg/kg BW stimulates insulin level to 15.2 ± 9.5 ng/mL, and (c) EEBRB doses 50, 100, and 200 mg/kg BW stimulate the regeneration of pancreatic beta cells.

Acknowledgments

This experiment was funded by Ministry of Research Technology and Higher Education through "Hibah Bersaing" scheme and was supported by Universitas Muhammadiyah Surakarta.

References

1. Zhang M, Zhang RF, Zhang FX, Liu RH. Phenolic profiles and antioxidant activity of black rice bran of different commercially available varieties. J Agric Food Chem. 2010;58(13):7580–7.
2. Ichikawa H, Ichiyanagi T, Xu B, Yoshii Y, Nakajima M, Konishi T. Antioxidant activity of anthocyanin extract from purple black rice. J Med Food. 2001;4(4):211–8.
3. Raghvendra V, Shakya A, Hedaytullah M, Arya GS, Mishra A, et al. Chemical and potential aspects of anthocyanins-a water-soluble vacuolar flavonoid pigments: a review. IJPSRR. 2011;6(1):28–33.
4. Tsuda TK, Shiga K, Ohsima S, Kawakishi T. Inhibition of lipid peroxidation and the active oxygen radical scavenging effect of anthocyanin pigments isolated from Phaseolus vulgaris L Biochem Pharmacol. 1996;52(7):1033–10.
5. Inaguma T, Han J, Isoda H Improvement of insulin resistance by cyanidin-3-glucoside, anthocyanin from black beans through the up-regulation of GLUT4 gene expression BMC Proc. 2011;5(Suppl 8): 21 Available athttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3284935&tool=pmcentrez&rendertype=abstract. Last accessed on October 26, 2015.
6. Jang H-H, Park M-Y, Kim H-W, Lee Y-M, Hwang K-A, Park J-H, et al. Black rice (*Oryza sativa L.*) extract attenuates hepatic steatosis in C57BL/6 J mice fed a high-fat diet via fatty acid oxidation Nutr Metab (Lond). 2012;9(1):27 Available athttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3366884&tool=pmcentrez&rendertype=abstract.
7. Wahyuni AS, Munawwaroh R. Economic value added of black rice bran as antidiabetic:Pharmacology and Phytochemistry Studies, 2014.
8. Tapas AR, Sakarkar D, Kakde RB. Flavonoids as nutraceuticals: a review. Trop J Pharm Res. 2008;7(3):1089–99.
9. Mataputun PS, Rorong JA, Pontoh J. Alpha Glucosidase inhibitory activity of extract of bark of Matoa (Pometia Spp) as antihyperglicemic agent. J MIPA USRAT. 2013;2(2):119–23.
10. Moradi-Afrapoli F, Asghari B, Saeidnia S, Ajani Y, Mirjani M, Malmir M, et al. In vitro alpha-glucosidase inhibitory activity of phenolic constituents from aerial parts of Polygonum hyrcanicum. DARU J Pharm Sci. 2012;20(1):37.
11. Borges de Melo E, da Silveira Gomes A, Carvalho I. α- and β-Glucosidase inhibitors: chemical structure and biological activity. Tetrahedron. 2006;62(44):10277–302.
12. Akkarachiyasit S, Charoenlertkul P, Yibchok-Anun S, Adisakwattana S. Inhibitory activities of cyanidin and its glycosides and synergistic effect with acarbose against intestinal α-glucosidase and pancreatic α-amylase Int J Mol Sci. 2010;11(9):3387–96. Available at http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2956102&tool=pmcentrez&rendertype=abstract.
13. Sasaki R, Nishimura N, Hoshino H, Isa Y, Kadowaki M, Ichi T, et al. Cyanidin 3-glucoside ameliorates hyperglycemia and insulin sensitivity due to downregulation of retinol binding protein 4 expression in diabetic mice Biochem Pharmacol. 2007;74(11):1619–27. Available at http://www.sciencedirect.com/science/article/pii/S000629520700545X.
14. Guo H, Ling W, Wang Q, Liu C, Hu Y, Xia M, et al. Effect of anthocyanin-rich extract from black rice (Oryza sativa L. indica) on hyperlipidemia and insulin resistance in fructose-fed rats. Plant Foods Hum Nutr. 2007;62(1):1–6.
15. Brahmachari G. Six bio-flavonoids with promising antidiabetic potentials: a critical survey. Res Signpost Chall Scope Nat Prod Med Chem. 2011;37661(2):187–212.

How to cite this article: Wahyuni AS, Munawaroh R, Da'i M. Antidiabetic mechanism of ethanol extract of black rice bran on diabetic rats. Natl J Physiol Pharm Pharmacol 2016;6:106-110

Source of Support: Nil, **Conflict of Interest:** None declared.