Antidiabetic Effects of Red Rice Bran in The Rat Models of Diabetes

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A B S T R A C T

Diabetes mellitus (DM) is a metabolic disease characterized by chronic hyperglycemia than often requires antidiabetic drugs (AD) to control fasting blood glucose (FBG) levels. However, long-term use of AD has side effects. Red rice bran is a natural food containing flavonoids and anthocyanins, rich in antioxidants. However, the antidiabetic effect of rice bran is yet to be understood. This study aimed to determine the effect of ethanol extract of red rice bran (EERRB) on the levels of FBG, insulin, HOMA-IR, HOMA-β, and QUICKI on male rats induced by streptozotocin-nicotinamide (STZ-NA). This study was a pretest-posttest control group design. White male Wistar rats (Rattus norvegicus) (n=35) were divided into five groups randomly i.e P1 negative control (STZ-NA), P2 positive control (STZ-NA and acarbose 1.8 mg/200 g BW), P3, P4, P5 EERRB groups (STZ-NA and EERRB 165, 330, 660 mg/kg BW). The intervention was performed in 21 days. The data were analyzed using one way ANOVA test, paired t-test and Post Hoc test. EERRB effectively reduced FBG and HOMA-IR but increased insulin, HOMA-β and QUICKI in STZ-NA induced diabetic male rats. In conclusion, this shows that EERRB 330 and 660 mg/kgBW/day have the most potent antidiabetic effect.

A B S T R A K

Diabetes melitus (DM) adalah penyakit metabolik yang ditandai dengan hiperglikemia kronis yang seringkali memerlukan obat antidiabetes (OAD) guna membantu mengontrol kadar glukosa darah puasa (GDP). Akan tetapi, penggunaan OAD dalam jangka panjang menimbulkan efek samping. Bekatul beras merah adalah bahan pangan alami dengan kandungan flavonoid dan antosianin yang kaya antioksidan. Namun, belum diketahui secara pasti efek antidiabetes dari bekatul beras merah tersebut. Penelitian ini bertujuan untuk mengetahui efek ekstrak etanol bekatul beras merah (EEBBM) terhadap kadar GDP, insulin, HOMA-IR, HOMA-β, dan QUICKI pada tikus jantan diabetes yang diinduksi Streptopzotocin-Nicotinamide (STZ-NA). Rancangan penelitian berupa pretest-posttest control group design. Tikus putih jantan Wistar (Rattus norvegicus) (n=35) terbagi menjadi lima kelompok secara acak: P1 kelompok kontrol negatif (diinduksi STZ-NA), P2 kelompok kontrol positif (diinduksi STZ-NA dan diberi akarbosa 1,8 mg/200 gBB), P3, P4, dan P5 kelompok EEBBM (diinduksi STZ-NA dan diberi EEBBM 165, 330, dan 660 mg/kgBB). Intervensi dilakukan selama 21 hari. Data dianalisis menggunakan uji one way ANOVA, paired t-test, dan uji Post Hoc. EEBBM efektif menurunkan GDP dan HOMA-IR serta meningkatkan insulin, HOMA-β, dan QUICKI pada tikus jantan model diabetes yang diinduksi STZ-NA. Kesimpulannya menunjukkan bahwa EEBBM330 dan 660 mg/kgBB/hari memiliki efek antidiabetik yang paling kuat.

INTRODUCTION

Diabetes mellitus (DM) is one of the biggest health problems in the world. The prevalence of DM has continually increased from year to year. Globally, there were 537 million people suffered from DM in 2021, increased by 16% compared 74 million in 2019 (International Diabetes Federation, 2021). The prevalence of DM in Indonesia is 10.9% of the entire population (Riskesdas, 2018). There are several types of DM and the most common type of DM (over 90%) in the community is type 2 diabetes mellitus (T2DM).

T2DM is a metabolic disease with a chronic condition characterized by high blood glucose levels (chronic hyperglycemia). The increase in blood glucose occurs due to impaired insulin secretion as a result of oxidative stress and inflammation that affect pancreatic cell dysfunction and lead to insulin resistance (Dietrich et al., 2019; Keane et al., 2015). The deffect in insulin secretion results in disruption of carbohydrate, protein, and fat metabolism (Mahan & Raymond, 2017). Disruption of insulin action indicates a reduced ability of insulin-sensitive tissue to respond to the biological action of insulin on carbohydrate metabolism (the insulin sensitivity is not good) so that glucose homeostasis is not maintained correctly (Dube et al., 2013). Therefore, pharmacological therapy using antidiabetic drugs is needed to control blood glucose for people with diabetes (Bilous & Donelly, 2015). However, long-term use of antidiabetic drugs poses side effects such as indigestion and it is also relatively expensive (Gul et al., 2015; Mahan & Raymond, 2017; Riskesdas, 2018). So, it is necessary to use natural food ingredients that rich in antioxidants as an alternative to antidiabetic drugs, which is safer to consume and affordable for people with DM.

Most of Indonesians consume staple food in the form of milled rice. Milling rice to obtain rice produces a by-product, namely bran (fine powder). Rice bran contributes to the weight of rice milling by around 8-10% or 5 million tons in 2020 (rice production 55.16 million tons) (BPS, 2020; Hartono et al., 2017; Widarta & Arnata, 2014). Rice bran is often wasted due to skin breaking or grinding of rice or used as animal feed. Red rice bran is a type of bran that contains bioactive components such as flavonoids, anthocyanins, vitamin E (tocotrienols and tocopherols), and γ-oryzanol(Esa et al., 2013; Goufo & Trindade, 2014; Tuarita et al., 2017). These bioactive components function as antioxidants that play a role in warding off free radicals and can control blood glucose (Prawitasari, 2019). A previous research revealed that the content of flavonoids and anthocyanins in red rice bran extract, from Magelang, Indonesia was 4.56 mg/g dan 3.40 mg/g, respectively (Nurrohima et al., 2021).

Anthocyanins give red-purple color and become superior compounds in red rice bran, in addition to their benefits for human physiological functions such as antidiabetic(Boue et al., 2016; Wallace & Giusti, 2015). The mechanism of anthocyanins as antidiabetics in controlling blood glucose is through increasing the level of gene expression and glucose-1 transporter (GLUT1) and glucose-4 transporter (GLUT4) mRNA to encourage glucose to enter adipocytes and cells. The increased GLUT1 and GLUT4 showed a positive effect on the regulation of glucose transport (Boue et al., 2016; Sasaki et al., 2007). Ex vivo studies have shown that anthocyanins directly induce insulin secretion in pancreatic cells and in vivo studies showed that they can increase insulin sensitivity in diabetic rats (Apichai et al., 2012). Anthocyanins can inhibit alpha-glucosidase (carbohydrate digesting enzyme) because they are structurally similar to maltose and glucosyl groups. In vitro studies related to red rice bran extract showed that alpha-glucosidase's inhibitory concentration (IC_{50}) was strong(Boue et al., 2016).

To our knowledge, there have been no studies reporting the effect of red rice bran extract from Magelang, Indonesia on the indicators of DM, the pancreatic cells, as well as on insulin resistance and sensitivity. This present study aimed to examine the effect of ethanol extract of red rice bran (EERRB) on the levels of fasting blood glucose (FBG), insulin, HOMA-IR, HOMA-β, and QUICKI.

METHOD

Animals

This study involved 35 male Wistar rats *(Rattus norvegicus)* with bodyweight 150-200 g, eight weeks old, healthy, active and behaved normally, and the levels of FBG after STZ-NA induction were >150 mg/dl (Ghasemi et al., 2014; Husna et al., 2019; Palupi et al., 2019). Adaptation for rats was carried out for seven days. Diabetic models were achieved after five days of induction with a single dose combination of STZ (65 mg/kg BW) and nicotinamide (230 mg/kg BW) intraperitoneally (i.p.). Diabetic rats were divided into five groups (P1-P5) consisting of seven rats per group (based on the Institutional Animal Care and Use Comiittee (IACUC)) (Office of Laboratory Animal Welfare, 2002). P1 was negative control group without intervention; P2 was a positive control group given acarbose; P3, P4, and P5 were given 165, 330, 660 mg/kg BW/day of EERRB, respectively. The rats were reared in a room conditioned by applying a 12/12 light/dark cycle. All groups were fed by using standard Comfeed AD2 and drink *ad libitum*. The intervention was given through a gastric probe and was carried out for 21 days.

Study Setting

The research was conducted in October-November 2021 at the Center for Food and Nutrition Studies (CFNS) Universitas Gadjah Mada (UGM), Yogyakarta, Indonesia. It was a laboratory experiment using a pretest-posttest control group design. Study subject were the Wistar male white rats *(Rattus norvegicus)* as diabetic models.

Preparation of Intervention

Ethanol Extract of Red Rice Bran (EERRB)

The organic and food grade red rice bran used in this study was obtained from PT. Bakti Indonesia, Magelang Regency, Central Java, Indonesia and cultivated in the local area. To make EERRB, rice bran in fine powder was soaked in 96% ethanol in a ratio of 1 gram bran: 6 ml ethanol for seven days and stirred regularly using a shaker (speed 150 rpm) at room temperature. Following this, the ethanol extract was squeezed and filtered using Whatman filter paper no.1 and then it was evaporated using a 30°C rotatory evaporator (Warganegara et al., 2019; Widarta & Arnata, 2014).

EERRB Dosage

The daily anthocyanin requirement of adult humans, is 12.5 mg (Sebastian et al., 2016). After adjusting the anthocyanin content in EERRB (3.40 mg/g) and the conversion factor of experimental animals (0.018), the doses

of EERRB used in this study were 165, 330, and 660 mg/kg BW/day. The middle dose (330 mg/kg BW/day) is a dose in which anthocyanin content is equuivalent to the daily requirement whereas the lower and higher doses were determined by decreasing to a half of middle dose or doubling it.

Acarbose Dosage

The daily dose of acarbose for patients with T2DM is 1x100 mg. The human (70 kg) to rat (200 g) conversion factor was 0.018 that gives the daily dose of acarbose in this study 1.8 mg/200 g BW/day.

Measures and covariates

Evaluation of Fasting Blood Glucose (FBG) Levels

Blood samples were obtained through the retro-orbital sinus. Quantitative measurements were performed by using the Enzymatic Colorimetric Test method Glucose Oxidase Phenol Aminoantipyrina Peroxidase (GOD-PAP) and the unit used was mg/dL.

Evaluation of Insulin Levels

The insulin levels were determined by using the ELISA kit (Zenix-520 Automated Elisa Processor, PT. Sumifin, Indonesia) on blood samples obtained from the retro-orbital sinus. The unit used was pg/ml.

Homeostasis Model Assessment Index and Quantitative Insulin Sensitivity Check Index

The following formulas were used to determine insulin resistance using the Homeostasis Model Assessment Insulin Resistance (HOMA-IR), the level of pancreatic β-cell function using the Homeostasis Model Assessment of Pancreatic Beta Cell Function (HOMA-β), and insulin sensitivity using the Quantitative Insulin Sensitivity Check Index (QUICKI):

$$\text{HOMA-IR (Hirata et al., 2009)} = \frac{insulin\left(\mu\frac{U}{ml}\right) x\ fasting\ blood\ glucose\left(\frac{mg}{dL}\right)}{405} \quad (1)$$

$$\text{HOMA-β (Hirata et al., 2009)} = \frac{\left(360\ x\ insulin\left(\mu\frac{U}{ml}\right)\right)}{fasting\ blood\ glucose\left(\frac{mg}{dL}\right)-63} \quad (2)$$

$$\text{QUICKI (Ma et al., 2014)} = \frac{1}{\left(\log FBG\left(\frac{mg}{dL}\right)+\log\left(insulin\left(\mu\frac{U}{ml}\right)\right)\right)} \quad (3)$$

Data analysis

The results of the normality and homogeneity test showed that the data were normally distributed and the data variation was homogenous (p>0.05), except that the HOMA-β pretest data were nor normally distributed and the HOMA-β and QUICKI posttest data were normally distributed but not homogenous. For data that were normally distributed, One Way ANOVA test was performed to assess the differences between all groups and then followed by the Tukey Post Hoc test (normal and homogenous) or the Games Howell Post Hoc (normal and not homogenous). For data that were not normally distributed, the analysis was performed by using the Kruskal Wallis test followed by the Mann-Whitney test. Paired T-Test (for normally distributed data) or Wilcoxon Signed Rank test (for not normally distributed data) was employed to determine the differences between each group. The results are declared significant or a difference if the p-value <0.05.

RESULTS AND DISCUSSION

Table 1 shows the levels of FBG, insulin, HOMA-IR, HOMA-β, and QUICKI. The highest average of FBG and HOMA-IR was in P1 (271.26±4.07 mg/dL and 8.5±0.18) and the lowest average was in P5 (97.30±3.13 mg/dL and 3.93±0.12). The highest average of insulin levels, HOMA-β, and QUICKI was in P5 (544.70±5.58 pg/ml, 172.74±15.87, and 0.3124±<0.001) and the lowest average was in P1 (422.98±6.93 pg/ml, 21.94±0.56, and 0.2827±<0.001). P5 showed remarkable changes in the decrease of FBG and HOMA-IR and the highest increase in insulin, HOMA-β, and QUICKI.

Effect STZ-NA, Acarbose, and EERRB intervention on FBG Levels

The levels of FBG were not significantly different between the acarbose group (P2) compared to the mid (P4) and high (P5) dose of EERRB intervention groups (Table 2). The levels of FBG after intervention were similar in P2 (102.36 mg/dL) and P4 (103.77 mg/dL), while it was much lower in P5 (97.30 mg/dL). The results showed that antidiabetic effect of EERRB may act through a mechanism similar to acarbose.

Hyperglycemia is a sign of T2DM pathology(DeFronzo et al., 2015). The modeling of rats into T2DM was influenced by STZ-NA induction. Administration of STZ initiated a toxic effect on rat pancreatic β cells so that the rats experienced hyperglycemia, damage or decreased β cells, reduced pancreatic insulin storage, and impaired insulin secretion (Eleazu et al., 2013; Ghasemi et al., 2014). NA given before STZ induction gave a protective effect to anticipate massive damage(Ahangarpour et al., 2018; Husna et al., 2019). In this study, it was proven that the admnistration of STZ-NA in all groups increased FBG levels beyond the normal threshold of 126 mg/dL (Table 1).

The alpha-glucosidase enzyme in the digestive tract can influence the development of T2DM as the enzyme absorbs carbohydrates and breaks them down into glucose (Boue et al., 2016). Antidiabetic drugs, namely acarbose, can carry out alpha-glucosidase's inhibitor (AGIs) (BPOM, 2015; Mahan & Raymond, 2017). Acarbose is often used in the treatment of diabetes. Carbohydrate-digesting enzymes in the small intestine delay carbohydrate absorption and reduce postprandial glucose levels (Mahan & Raymond, 2017). Administration of acarbose in the positive control group (P2) showed decreased FBG levels below the standard limit of 126 mg/dL (Table 1).

EERRB contains flavonoid and anthocyanin compounds as antioxidants. Antioxidants provide an antidiabetic effect by

reducing Reactive Oxygen Species (ROS) production causing oxidative stress so that it can reduce the incidence of inflammation and damage of pancreatic cells (Les et al., 2020). Anthocyanins are a subclass of flavonoids that contribute to the red-purple color of EERRB. Anthocyanins also act synergistically with acarbose, a substance that can inhibit alpha-glucosidase. Previous studies have shown that red bran extract rich in anthocyanins has a more potent inhibitory activity (IC_{50} 8.44 µg/mL) than acarbose (Boue et al., 2016). In addition, there is a relationship between anthocyanins and inhibition of glucose transport from the gastrointestinal tract to blood plasma (Hanamura et al.,

2006). The specific mechanism of anthocyanins in reducing or controlling FBG is by increasing the level of gene expression and GLUT1 and GLUT4 mRNA to push glucose into adipocytes and cells (Boue et al., 2016; Sasaki et al., 2007). This study is in line with previous research showing that foods rich in anthocyanins can reduce the incidence of diabetes, improve blood glucose, and prevent the development of complications related to diabetes(Sun et al., 2012). In another study, giving germinated black rice krisna extract that contain anthocyanin could reduce FBG levels in a group of STZ-NA induced DM rats (Pasaribu et al., 2021).

Table 1
Effect of The Ethanol Extract of Red Rice Bran

Category	Group	Average		Δ Mean ± SD	p
		Pretest (H0) Mean ± SD	Posttest (H22) Mean ± SD		
Fasting Blood Glucose (mg/dL)	P1	269.0±3.96	271.26±4,07	2.26±0.11	0.001[a]*
	P2	269.06±4.12	102.36±3,83	-166.7±0.29	0.001[a]*
	P3	264.77±2.83	130.43±3,13	-134.34±0.3	0.001[a]*
	P4	269.26±4.57	103.77±5,71	-165.49±1.14	0.001[a]*
	P5	270.09±2.60	97.30±3,13	-172.79±0.53	0.001[a]*
	p	0.088[b]	0.001[b]*		
Insulin (pg/ml)	P1	428.43±6.35	422.98±6.93	-5.45±0.58	0.001[a]*
	P2	424.61±9.02	535.80±5.18	111.19±3.84	0.001[a]*
	P3	424.06±7.75	502.91±3.55	78.85±4.2	0.001[a]*
	P4	423.16±6.86	534.16±5.08	111±1.78	0.001[a]*
	P5	418.98±7.25	544.70±5.58	125.72±1.67	0.001[a]*
	p	0.250[b]	0.001[b]*		
HOMA-IR	P1	8.54±0.16	8.5±0.18	-0.04±0.02	0.033[a]*
	P2	8.46±0.11	4.06±0.13	-4.4±0.02	0.001[a]*
	P3	8.32±0.17	4.86±0.09	-3.46±0.08	0.001[a]*
	P4	8.44±0.18	4.11±0.22	-4.33±0.04	0.001[a]*
	P5	8.38±0.18	3.93±0.12	-4.45±0.06	0.001[a]*
	p	0.156[b]	0.001[b]*		
HOMA-β	P1	22.47±0.57	21.94±0.56	-0.53±0.01	0.018[e]*
	P2	22.27±0.87	148.27±15.24	126±14.37	0.001[a]*
	P3	22.70±0.54	80.72±4.15	58.02±3.61	0.001[a]*
	P4	22.17±0.65	144.08±21.49	121.91±20.84	0.001[a]*
	P5	21.85±0.42	172.74±15.87	150.89±15.45	0.001[a]*
	p	0.157[b]	0.001[c]*		
QUICKI	P1	0.2826±<0.001	0.2827±<0.001	0.0001±<0.001	0.047[a]*
	P2	0.2829±<0.001	0.3109±<0.001	0.0280±<0.001	0.001[a]*
	P3	0.2835±<0.001	0.3036±<0.001	0.0201±<0.001	0.001[a]*
	P4	0.2830±<0.001	0.3105±<0.001	0.0275±<0.001	0.001[a]*
	P5	0.2832±<0.001	0.3124±<0.001	0.0292±<0.001	0.001[a]*
	p	0.146[b]	0.001[c]*		

Notes: P1: negative control; P2: positive control; P3: EERRB 165 mg/kgBW; P4: EERRB 330 mg/kg BW; P5: EERRB 660 mg/kg BW; a) paired sample t-test; b) one way anova post hoc tukey; c) one way anovagames howell; d) kruskal wallis; e) wilcoxon signed rank test; *significant (p<0,05)

Effect STZ-NA, Acarbose, and EERRB intervention on Insulin Levels

The intervention for 21 days showed no difference between the positive control group (P2) and P4 (Table 2), while the positive control group compared to P3 and P5 had statistical differences. The group with the intervention of both acarbose and EERRB showed an effect in lowering insulin levels.

The development of diabetes mellitus occurs due to oxidative stress and inflammation in causing hyperglycemia through β cell dysfunction and insulin resistance (Keane et al., 2015). Glucose that exceeds the threshold, which then

accumulates in adipose tissue, muscle, and pancreatic then triggers the excessive production of ROS. An activated stress-sensitive pathway with increased blood gluose leads to insulin resistance and impaired insulin secretion (Banerjee & Vats, 2014).

Using rats as experimental animals, diabetic models were developed by using a chemical substance, namely STZ-NA. STZ is principally cytotoxic to pancreatic β cells. STZ through the glucose-2 transporter (GLUT2) found in the plasma membrane of β cells enters the cell and initiates the visible toxic effect (Husna et al., 2019). The chemical structure of STZ, which has a glucose group, makes it easier to enter the β cell, resulting in damage to the pancreatic β cells. The

damage involves the pathway of free radical formation, nitric oxide production, and DNA methylation (Eleazu et al., 2013). To protect pancreatic cells from these toxic effects, NA is given. As an inhibitor poly ADP ribose polymerase (PARP), NA will inhibit DNA methylation and prevent massive damage to pancreatic cells (Husna et al., 2019). Administration of STZ-NA ultimately causes impaired insulin secretion to insulin resistance(Szkudelski, 2012). This is related to disturbances in cells that reduce insulin synthesis and secretion due to STZ-NA induction in this study was evidenced by the P1 group that was induced by STZ-NA and fed standard feed without any intervention having decreased insulin levels. This statement is in line with previous studies that proved insulin levels in the DM rat group after STZ-NA induced decreased compared to the group without induction (Nurhidajah & Nurrahman, 2017).

In another study, giving germinated black rice to STZ-NA induced rats could increase insulin levels by 16.35% (Nurhidajah & Nurrahman, 2017). In this study, the group of DM rats after the intervention showed an increase in insulin levels compared to the group of DM rats without intervention. The highest increase in insulin levels was 23.08% in the group of rats given EERRB at a dose of 660 mg/kg BW/day. EERRB contains flavonoids and anthocyanins of 4.56 mg/g dan 3.40 mg/g (Nurrohima et al., 2021). Flavonoids and anthocyanins are known to increase insulin secretion, protect cells, and inhibit ROS (Gowd et al., 2017; Mohan & Nandhakumar, 2014). Mechanism of flavonoids increases secretion and protect cells by simulating glucose uptake into peripheral tissues(Brahmachari, 2011). Meanwhile, anthocyanins work by inhibiting dipeptidyl peptidase IV(DPP-IV) activity, which causes an increase in glucose tolerance due to the avoidance of the degradation of incretin glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic (GIP) by increasing the effectiveness of insulin production (Hsieh-Lo et al., 2020).

Tabel 2
Results of Intervention Post Hoc Test Analysis

	P1 (Control +)	P2 (Control -)	P3	P4
Fasting Blood Glucose[a]				
• P2 (Control -)	0.001*		0.001*	0.966
• P3	0.001*	0.001*		0.001*
• P4	0.001*	0.966	0.001*	
• P5	0.001*	0.167	0.001*	0.043*
Insulin[a]				
• P2 (Control -)	0.001*		0.001*	0.979
• P3	0.001*	0.001*		0.001*
• P4	0.001*	0.979	0.001*	
• P5	0.001*	0.032*	0.001*	0.008*
HOMA-IR[a]				
• P2 (Control -)	0.001*		0.001*	0.981
• P3	0.001*	0.001*		0.001*
• P4	0.001*	0.981	0.001*	
• P5	0.001*	0.500	0.001*	0.221
HOMA-β[b]				
• P2 (Control -)	0.001*		0.001*	0.992
• P3	0.001*	0.001*		0.001*
• P4	0.001*	0.992	0.001*	
• P5	0.001*	0.076	0.001*	0.094
QUICKI[b]				
• P2 (Control -)	0.001*		0.001*	0.993
• P3	0.001*	0.001*		0.001*
• P4	0.001*	0.993	0.001*	
• P5	0.001*	0.295	0.001*	0.379

Notes: P3: EERRB 165 mg/kg BW/day; P4: EERRB 330 mg/kgBW/day; P5: EERRB 660 mg/kg BW/day
[a]*one way anova post tukey*
[b]*one way anova games howell*
*) significant ($p<0,05$)

Effect STZ-NA, Acarbose, and EERRB intervention on Insulin Sensitivity Biomarker

The values of HOMA-IR, HOMA-β, and QUICKI are known to have no significant difference between groups P2 compared to P4 and P5 also P4 with P5. Meanwhile, there were significant differences between groups P1 with P2, P3, P4, and P5 (Table 2). These results show that administration of EERRB compared to acarbose control has the same effect in increasing levels of HOMA-β and QUICKI, also lowering HOMA-IR.

HOMA-IR, HOMA-β, and QUICKI levels are indicators to assess insulin resistance, pancreatic β cell function, and insulin sensitivity. Decreased function of pancreatif β cells and insulin resistance is caused by oxidative stress and inflammation, which play a role in developing T2DM. Increased insulin resistance, similar to the statement that HOMA-β and QUICKI decreased.

Based on Table 1, an increase in HOMA-β and QUICKI and a significant decrease in HOMA-IR occured in the group of DM rats with acarbose and EERRB various doses intervention for 21 days. The changes in DM rats without intervention were not significantly different compared to the DM group with intervention. This could be because the administration of cytotoxic STZ causes oxidative stress so that β cells die (necrosis and apoptosis) and decrease insulin synthesis and secretion (Ghasemi et al., 2014). The balance between free radicals and antioxidants at the cellular level is crucial,

otherwise the oxidative stress environment changes sensitivity insulin either by increasing insulin resistance or by impairing glucose tolerance through several cells signaling pathways (Saji et al., 2019).

The administration of EERRB to the DM rat group, which showed an effect on decreasing HOMA-IR and increasing HOMA-β and QUICKI, was thought to be due to the role of bioactive components flavonoids and anthocyanins from EERRB. HOMA-IR with low value and decreased after the intervention showed an association with restoring blood glucose levels and insulin levels. This is in line with research that mulberry fruit extract containing anthocyanins can reduce HOMA-IR and increase insulin sensitivity (Choi et al., 2016). Studies related to several types of extracts with anthocyanin-rich content have shown effective results in improving insulin resistance conditions and increasing insulin sensitivity (Belwal et al., 2017). Anthocyanins are also known to have a protective role against pancreatic β cell dysfunction by modulating antioxidant enzymes (Sancho & Pastore, 2012). Previous studies in vitro and in vivo have shown anthocyanins to protect cells through their antioxidant and modulatory actions against reducing hyperglycemia and stimulating insulin secretion (Gowd et al., 2017; Sancho & Pastore, 2012).

The mechanism of the repair effect was proven in the group of DM rats due to the antioxidant properties of EERRB administration. The antioxidant effect works like the drug acarbose, which inhibits alpha-glucosidase and delays the breakdown of glucose in the digestive tract so that it is not channeled into the blood and prevents increased glucose levels. Anthocyanins against insulin resistance and increase insulin sensitivity by increasing GLUT4 translocation by increasing peroxisome proliferator-activated receptor gamma(PPAR-γ) activity, activating AMP-activated protein kinase (AMPK), and downregulating insulin receptor substrate 1 (IRS-1) phosphorylation (Belwal et al., 2017; Kurimoto et al., 2013).

LIMITATION OF THE STUDY

Increased insulin levels, sensitivity, and improvement of pancreatic β-cell function in T2DM rats with EEBBM intervention for 21 days were proven in this study biochemically and computationally, but the results are not yet known by microscopic histopathology of pancreatic tissue.

CONCLUSIONS AND SUGGESTIONS

Based on dose dependent, EERRB (330 and 660 mg/kg BW/day) exerted the same effect as acarbose (an antidiabetic drug) on decreasing FBG, HOMA-IR, and increasing insulin, HOMA-β, with QUICKI in STZ-NA induced diabetic rats. Meanwhile, EERRB at a dose of 165 mg/kg BW/day showed an effect on DM assessment indicators but not as large or comparable to acarbose. Further research is needed to histopathological examination of pancreas and determine the safety and efficacy of EERRB as an alternative therapy for diabetes in humans.

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ETHICAL CONSIDERATIONS

This research protocol was approved by the Research Ethics Committee, Faculty of Medicine, Universitas Sebelas Maret (No: 58/UN27.06.6.1/KEP/EC/2021).

Conflict of Interest Statement

All authors have no conflict of interest in this article.

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