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Oral Administration of Black Rice (*Oryza Sativa L.*) Ethanol Extract Increased the Number of Pancreas Beta Cells but Not Decreasing the Concentration of Fasting Blood Sugar in Diabetic Male Wistar Rats (*Rattus Norvegicus*)

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ABSTRACT

Black rice ethanol extract contains bioactive compounds (anthocyanins, flavonoids, phenols, tannins and amylose) which have the potential to reduce fasting blood sugar levels and increase the number of pancreatic beta cells in rats with diabetes. This study aims to determine the effect of black rice extract on decreasing fasting blood sugar levels and increasing the number of pancreatic beta cells in alloxan-induced diabetic rats. This was an experimental study with post-test only control group design. The subjects were 36 male rats, all induced with alloxan (25mg/rat). Samples (blood glucose ≥200 mg/dL) were randomly divided into 2 groups, namely the control group, which was administered with a placebo, and the treatment group, which was administered with 80% black rice ethanol extract (*Oryza sativa L. indica*) (16 mg/rat) orally once a day for 14 days. Blood sample was drawn after 26 days to examine the fasting blood glucose and pancreatic histology. The result was analyzed using t-independent test. The number of pancreatic beta cells in the treatment group was found significantly higher compared to the control group (71.8±315.0 versus 37.5±12.2) ($p<0.001$). However, the fasting blood glucose level of the treatment group was not significantly different from the control group (310±113 mg/dL versus 383±146 mg/dL) ($p=0.127$). Based on this study, it can be concluded that black rice (*Oryza sativa L. indica*) ethanol extract increases the number of pancreatic beta cells but does not decrease fasting blood sugar level in male diabetic rats (*Rattus norvegicus*).

Keywords: black rice, pancreatic beta cells, fasting blood glucose, male Wistar rats

INTRODUCTION

Diabetes is often seen as a biological illustration of the premature aging process. The World Health Organization (WHO) predicts an increase in type 2 Diabetes Mellitus (DM) patients in Indonesia from 8.4 million in 2000 to around 21.3 million in 2030 (WHO, 2016). The International Diabetes Federation also shows data that in 2019-2030 there will be an increase in the number of DM patients from 10.7 million to 13.7 million in 2030 (Care, 2022). Diabetes is characterized by hyperglycemia caused by insulin resistance in muscle and liver cells and failure of pancreatic beta cells (Perkeni, 2021). There is a progressive decline in pancreatic beta cell function and chronic insulin resistance, making blood glucose unable to be used effectively resulting in a buildup of glucose in the blood vessels (Baynest, 2015).

To prevent diabetes, non-pharmacological management is needed which includes healthy lifestyle changes such as exercising, knowing diabetes education, healthy eating patterns, such as eating complex carbohydrates, high-fiber foods, limiting saturated fatty acid intake, low-cholesterol foods of less than 200 mg / per day, foods high in protein and most importantly foods high in antioxidants (Decroli, 2020). Antioxidants are substances that can stop free radicals that can damage cells and have a role in reducing oxidative stress. One way to reduce oxidative stress is to consume foods that are high in antioxidants, one of which is black rice extract (Zalukhu, 2016). The black rice extract in this study contains a lot of flavonoids, one of the highest being anthocyanin. In addition to anthocyanins, black rice also contains bioactive compounds, namely phenols, flavonoids, tannins and amylose (Wahyuni, 2015).

The highest anthocyanin levels were found in black rice of the "Cempo Ireng" variety from Sleman-Yogyakarta, Indonesia. From the results of research conducted by the Yogyakarta Agricultural Technology Study Center from January to August 2013 and Ponnappan, the anthocyanin content in Cempo Ireng black rice variety was the highest compared to other rice groups, namely 428.38 mg/100g. (Priska et al., 2018).

Anthocyanins have a role as antioxidants and anti-inflammatories which function to repair pancreatic beta cells in diabetic patients, increase insulin sensitivity and regulate glucose metabolism (Solverson, 2020). Anthocyanins are commonly found in dark colored fruits and vegetables such as whole

grains, nuts, tubers, brown rice, black rice, and black soybeans (Ifadah, 2022). People who eat foods high in anthocyanins have a smaller risk of developing diabetes (Tri Meilawati, 2022). With the high content of anthocyanin class of flavonoids in black rice of the Cempo Ireng variety, this research is expected to provide benefits in preventing diabetes, especially type 2 DM.

METHODS

This research is an experimental study with post-test only control group design (Federer, 2008). The experiment was conducted at the Animal Laboratory Unit of the Pharmacology Department, Faculty of Medicine, Udayana University, Denpasar, Bali for 4 weeks. The sample needed in this experiment was 32 male Wistar rats (n=16), 2.5-3 months old, weighing 200-250 grams. To anticipate drop out, 10% of total sample were added, with the total amount to 36 rats divided into 2 groups: control and treatment group (n=18). The pancreatic beta cells examination was done by histopathological observations using digital analysis methods. Fasting blood glucose was examined using glucometer after the rats were fasted for 8 hours. This research has been approved by the ethics commission of Udayana University, Bali, (B/253/UN14.2.9/PT.01.04/2022).

Statistical analysis was performed with SPSS Version 23.0. Normality test was assessed using Shapiro-Wilk test and homogeneity test was assessed with Levene's test. Comparability test was assessed using t-independent to examine the mean pancreatic beta cell number and fasting blood glucose.

RESULTS

To determine the number of pancreatic beta cells, a histopathological examination of pancreatic tissue was carried out with Gomori Aldehyde Fuschin staining (Figure 1 and 2).

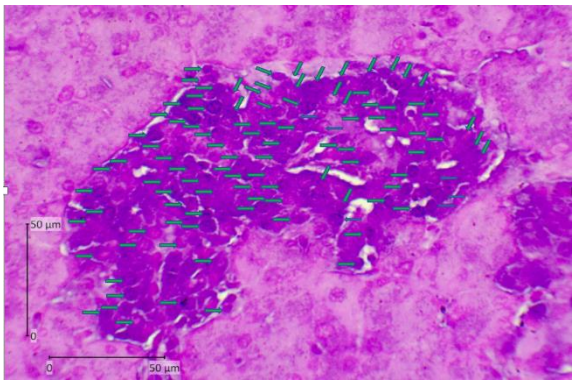

Figure 1. Histopathology of pancreatic beta cell counts in treatment group (400x magnification) were found higher in treatment group than in control group (marked with purple cytoplasmic cells marked with green arrows).

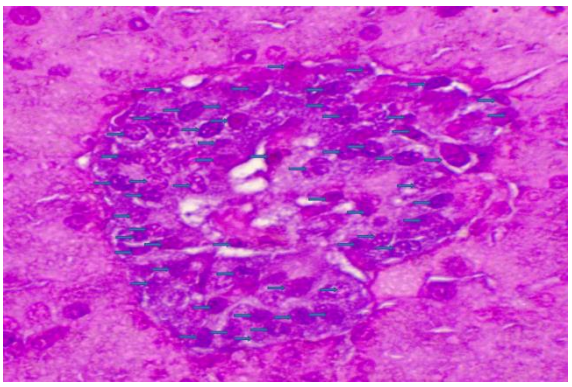

Figure 2. Histopathology of pancreatic beta cells counts in control group (400x magnification) were found less than in treatment group (marked with purple cytoplasmic cells marked with green arrows).

Normality test on pancreatic beta cells number and fasting blood glucose level after 14 days of treatment was done using Shapiro-wilk test. The data were normally distributed.

Based on Levene homogeneity test, it was concluded that data variety of pancreatic beta cells number and fasting blood glucose level was homogeny ($p > 0.05$).

Comparability test on number of pancreatic beta cells and fasting blood glucose level were done using t-independent test. Analysis of comparative test showed significant difference on the mean number of pancreatic beta cells between groups ($p<0.05$) and are presented in Figure 3. Meanwhile, comparative analysis of fasting blood glucose level between groups showed insignificant difference ($p>0.05$) and are presented in Figure 4.

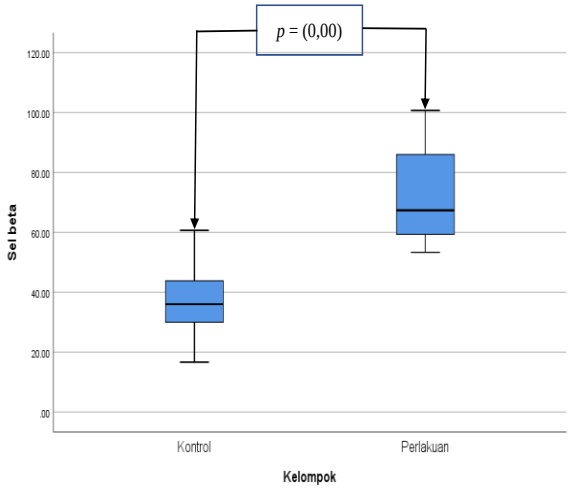

Figure 3. Comparison of Mean Pancreatic Beta Cells (Cells/FOV) between Groups

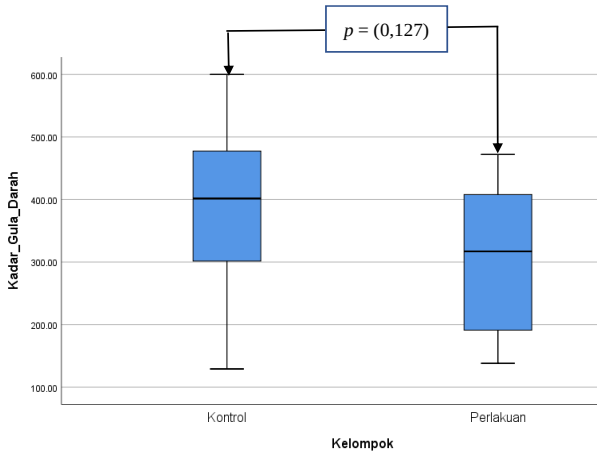

Figure 4. Comparison of Mean Fasting Blood Glucose Level (mg/dL) between Groups

DISCUSSION

The Effect of Administration of Black Rice (*Oryza sativa L. indica*) Ethanol Extract on the Number of Pancreatic Beta Cells

Flavonoids in black rice extract are antioxidants that can release atoms (H) from hydroxyl groups (OH) to form radicals. Flavonoids also act as scavengers for hydroxyl and superhydroxyl radicals that occur in the redox cycle so that they can protect the lipid membrane of pancreatic beta cells and restore insulin receptor sensitivity in cells (Panche *et al*., 2016).

Flavonoids contain genistein which can increase the expression of cyclin D1 which is a protein that can regulate the growth of pancreatic beta cells so that they can improve pancreatic beta cell proliferation (Bahadoran et al., 2013). Black rice extract also has high levels of anthocyanins that can prevent the cutting of supercoiled DNA strands caused by ROS, especially peroxide radicals and hydroxyl radicals in the redox cycle. The hydroxyl group of the main anthocyanin in black rice is Cyanidin-3-glucoside (C3G) which has been shown to reduce adipose inflammation in hyperglycemia in diabetic rats (Wahyuni, 2015). Black rice extract also contains phenol which is a phenolic compound that has one or more hydroxyl groups and functions to increase pancreatic beta cells and maintain insulin levels in cases of diabetes (Diniyah & Lee, 2020).

The Effect of Black Rice (*Oryza sativa L. indica*) Ethanol Extract on Fasting Blood Sugar Levels

In a comparison study conducted by Tantipaiboonwong *et al*. (2017), it was stated that consuming black rice extract at a dose of 50 mg/kg BW can reduce blood sugar levels in diabetic rats after 8 weeks of treatment. The source of black rice was obtained from a local market in Dokkhamtai, Phayao province, Thailand, with a phenolic content of black rice extract of 16,180 mg/100g and a flavonoid content of 9,660 mg/100g (Tantipaiboonwong *et al*., 2017). Whereas in this study the source of black rice was from the Cempo Ireng variety, Sleman, Jogjakarta, with a phenolic content of 2,327.67 mg/100g

and a flavonoid content of 6,687.78 mg/100g. This shows that the place, growing media and farming system of black rice affect the phytochemical content of the extract and the results of the research will also be different.

Diabetes mellitus is very closely related to diet, therefore medical nutrition therapy is the most important thing in the comprehensive management of DM. The concept of managing DM patients is the application of a healthy lifestyle, medical nutrition therapy and physical activity and pharmacological interventions with oral OAD and/or injections. With this concept, it can be concluded that black rice extract in this study is only an additional therapy to control blood sugar levels in DM patients.

CONCLUSION

Administration of 16 mg/g BW of black rice (*Oryza sativa L. indica*) ethanol extract increases the number of pancreatic beta cells but not decreases fasting blood glucose in alloxan-induced diabetic male Wistar rats (*Rattus norvegicus*).

Further research is needed to determine the time and optimal dose of black rice ethanol extract and also optimal level of bioactive compounds in black rice extract administered to male Wistar rats to reduce fasting blood sugar level significantly.

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Conflict Of Interest

All researchers declare that there is no conflict of interest related to this article

Author's Contribution

All authors contribute equally in compiling this research article

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