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Effect of Germinated Black Rice *Krisna* Extract on Fasting Blood Glucose and Body Weight in Diabetes Mellitus Rats

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ABSTRACT

Background: Diabetes mellitus (DM) is a non-communicable disease that is generally associated with fasting blood glucose (FBG) level of >126 mg/dL and weight loss. Germinated black rice *krisna* (GBRK) extract contains phytochemicals with antioxidant and anti-diabetic properties. The purpose of this study was to assess the impact of GBRK extract on FBG level and body weight (BW) in rat model of DM.

Methods: In an *in vivo* pre- and post-test study 36 male Wistar rats with BW design, of 150-200 g were enrolled and divided into 6 groups, including control (C), negative control (NC), GBRK 535 mg/kg BW (P1), GBRK 1070 mg/kg. BW (P2), and GBRK 2140 mg/kg BW (P3). Diabetes was induced using streptozotocin (STZ) and nicotinamide (NA) and the intervention period was 14 days. Measurement of FBG level was by quantitative enzymatic colorimetric test of Glucose Oxidase Phenol 4-Aminoantipyrine (GOD-PAP) and body weight was determined by a digital scale.

Results: The administration of GBRK extract in P1, P2, and P3 groups could significantly reduce the FBG (p<0.01). GBRK in P1, P2, and P3 groups could also significantly improve the body weight to normal state too (p<0.01).

Conclusion: GBRK extract at doses of 535, 1070, and 2140 mg/kg BW for 14 days was demonstrated to improve metabolic disorders in diabetes by reducing FBG level and improving the BW to the normal state.

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Introduction

In the last few decades, the International Diabetes Federation (IDF) reported in 2019 that cases of diabetes mellitus (DM) have increased in the world, reaching a number of 463 million cases. The growth of the prevalence of DM is believed to increase over the next 11 years to about 578 million cases (1). Based on national statistical reports in Indonesia, the prevalence of DM has increased from 6.9% in 2013 to 8.5% in 2018 (2, 3). DM is a disease of carbohydrate, protein, and fat metabolism resulting in disturbances in the pancreas organ that produces insulin, and is responsible for insulin performance, or both (4). The diagnosis of DM can be established when there is a high blood glucose level (>126 mg/dL) and clinical symptoms such as polyuria,

glucosuria, polydipsia, and polyphagia appear (4-6). DM also has other symptoms such as weight loss that can result in a decline in nutritional status (7).

Basically, weight loss is caused by insulin resistance through cytokines and β-cell dysfunction and with an increased adipocyte inflammation (8). Cytokines produced from adipose tissue will induce inflammation in pancreatic islet cells, thereby affecting weight loss in DM (9). Chronic high glucose levels will lead to an increase in the number of free radicals that can impact on oxidation and disrupt the lipid components of cell membrane and cause lipid peroxidation (10). The increase in free radicals in the body is thought to damage peripheral tissues, thereby damaging insulin receptors or glucose transporters in cell membranes (11). In addition, the free radicals such as hydrogen peroxide, hydroxyl, supra oxide, and nitric oxide can also damage pancreatic cells, resulting in low or no insulin secretion. Theoretically, the free radicals that cause glucose can not enter cells, so they accumulate in the blood and have an impact on increasing blood glucose level (12). Therefore, antioxidants can play an important role to reduce the number of free radicals in DM.

The therapy recommended to treat DM is synthetic drugs, but the use of these drugs still causes side effects that can have interference (4, 11, 12). Currently, alternative treatments using herbal ingredients have been widely developed and used in the treatment of DM by several studies (13-17). Indonesia is an agricultural country and a major producer of various types of rice (18, 19). Black rice is from the species *Oryza sativa L* and the family Poaceae with the genus *Oryza* (20). Germinated black rice variety *krisna* is one of the ingredients that contain phytochemical compounds that are antioxidants. The results of the research by Pasaribu *et al.* revealed that the germinated black rice extract *krisna* (GBRK), from Yogyakarta, Indonesia was shown to have high anthocyanin compounds (48.68 mg/g) and flavonoids (2.08 mg/g) (21). Theoretically, the antidiabetic effect of anthocyanins and flavonoids was demonstrated to be through an antioxidant mechanism, namely reducing the high number of Reactive Oxygen Species (ROS) that can inhibit oxidation in DM (22). The research results of Oyedemi *et al.* proved that flavonoid phytochemical compounds with antioxidants properties can affect body weight and prevent weight loss from DM (23).

The high content of anthocyanin compounds and flavonoids in germinated black rice *krisna* extract can be beneficial in therapy of DM and reduction of blood glucose, improve weight gain, and prevent the weight loss (24, 25). However, research on GBRK against DM is not available in literature. Therefore, this study was conducted to evaluate the effect of GBRKK on fasting blood glucose (FBG) and body weight (BW) in DM rat model.

Materials and Methods

This research has followed the experimental animal research protocol and has been approved by the Research Ethics Committee, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia (Certificate Ethical Clearance no: 18/UN27.06.6.1/KEP/EC/2021, 12 April 2021). This research was conducted at the Central Laboratory of Food and Nutrition Studies, Gadjah Mada University, Indonesia from April to May 2021. This type of research was a laboratory experiment with pre-test and post-test control group design. In an in vivo study, 36 male Wistar rats with BW of 150-200 g were divided into six groups of 6 rats in each group (Table 1). The research animals were male albino Wistar rats aged eight weeks, with body weight of 150-200 g that were provided from the Inter University Center (IUC) field of pre-clinical service and experimental animal development, Central Laboratory of Food and Nutrition Studies, Gadjah Mada University, Indonesia. The rats were kept on light and dark cycle for 12 hours and given standard Comfeed food and drinking *ad libitum*.

The black rice *krisna* was obtained from Sleman Regency, DI Yogyakarta Province, Indonesia. The procedure was sorting the rice, soaking in water in a ratio of 1:1 for water:rice at room temperature for 6

Table 1: The research sample group of germinated black rice *krisna* extract (GBRK).

Group	Days 1-5	Days 6-19
C	NDW	NDW
NC	Induced STZ, NA (ip) & NDW	NDW
Drug	Induced STZ, NA (ip) & NDW	Acarbose (1.8 mg/kgBW) & NDW
P1	Induced STZ, NA (ip) & NDW	GBRK (535 mg/kgBW) & NDW
P2	Induced STZ, NA (ip) & NDW	GBRK (1070 mg/kgBW) & NDW
P3	Induced STZ, NA (ip) & NDW	GBRK (2140 mg/kgBW) & NDW

C (Control), NC (Negative control), Drug (Positive control, acarbose, 1.8 mg/kgBW, P1 (GBRK, 535 mg/kgBW), P2 (GBRK, 1070 mg/kg BW), P3 (GBRK, 2140 mg/kgBW), NDW (Normal Diet and Water), STZ (streptozotocin, 65 mg/kg BW), NA (nicotinamide, 230 mg/kg BW), ip (injeksi intraperitoneal), GBRK (Germinated Black Rice *Krisna* Extract).

hours, placing the rice on a wet cloth, covering with wet cloth in perforated containers, and finally utilizing sprinkle water once every 12 hours for 48 hours until the rice germinates. GBRK was dried in a cabinet dryer at 50°C for 5 hours and for use was ground into flour applying a disk mill and filter 60 mesh (21).

The extraction method was maceration and 80% ethanol solvent was added to GBRK flour in the ratio 1:5. Ethanol was acidifed using 3% citric acid and the ratio of ethanol to citric acid was 85:15. The solution was left for 24 hours and put in an orbital shaker at 150 rpm for 4 hours while stirring. The solution was then centrifuged at 4000 rpm for 30 minutes, filtered with filter paper, while the solution was transferred into a rotary evaporator at 50°C to produce a thick GBRK extract (21).

The research began with adaptation of rats for 7 days. Diabetes was induced in rats using streptozotocin (STZ) at a dose of 65 mg/kg BW and nicotinamide (NA) at a dose of 230 mg/kg BW by intraperitoneal (ip) injection. The rats were acclimatized for 5 days and performed pre-tests, while measuring FBG level and weighing their BW. Rats with FBG level >126 mg/dL were classified as diabetic rats. Rats were given treatment according to their group, namely control (C), negative control (NC), drug acarbose (1.8 mg/kg BW), GBRK extract (535 mg/kg BW, P1), GBRK (1070 mg /kg BW, P2) and GBRK extract (2140 mg/kg BW, P3) for 14 days. Rats that received GBRK extract were given through an oral gavage. All groups were given normal diet and water *ad libitum*. After 14 days of intervention, blood sampling was carried out as post-test to measure the FBG level, the BW. Euthanasia was undertaken using an over-dose of ketamine injection of 120 mg/kg BW.

Measurement of BW was conducted using a fixed digital scale on days 7th and 14th.

The Rats were fasted for 12 hours and still given drinking water *ad libitum* before measuring the FBG level. Blood samples were taken through the retro-orbital. The FBG levels were analyzed utilizing the quantitative enzymatic colorimetric test of Glucose Oxidase Phenol 4-Aminoantipyrine (GOD-PAP) twice as pre-test on day 0 and as post-test on day 14th. Rats on day 14th after measuring BW and FBG were euthanized using ketamine injection of overdoses of 120-150 mg/kg BW ip and cremated. This study was analyzed using SPSS software (version 18, Chicago, IL, USA). All data were tested for normality using the Shapiro-Wilk test to determine the normal distribution. Furthermore, a one-way ANOVA test and Tukey posthoc test were conducted to assess the effect of GBRK on FBG and BW. The results of statistical analysis if the p value was ≤0.05 were considered significant.

Results

FBG was found to be normally distributed (p>0.05) and there was a significant difference between the NC, acarbose, P1, P2, and P3 groups (p<0.05). FBG level in the NC significantly (p<0.01) increased on day 14th (post-test) when compared to the C group. In DM group, when acarbose and GBRK were administered (P1, P2, and P3) for 14 days, a significant reduction in FBG level was noted in comparison to the NC group (p<0.01). Meanwhile, P2 and P3 groups were significantly different (p<0.01; p<0.05) when compared to acarbose group (Figure 1). The doses of GBRK extract that reduced FBG to the most normal state were group P2 at a dose of 1070 mg/kg BW (114 mg/dL) and P3 at a dose of 2140 mg/kg BW (94 mg/dL).

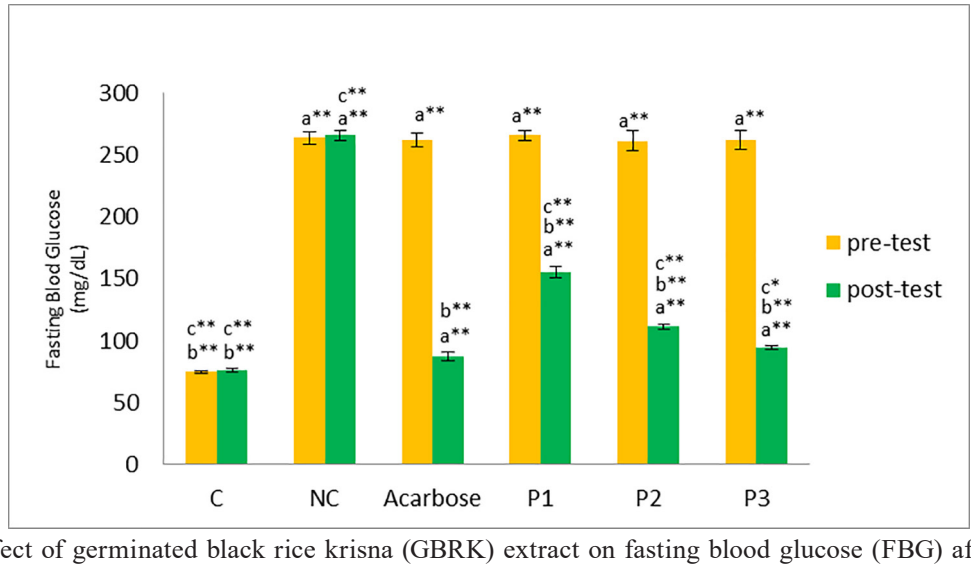

Figure 1: Effect of germinated black rice krisna (GBRK) extract on fasting blood glucose (FBG) after 14 days of intervention. Data were presented as mean±SD (n=6). Different superscripts (*,**) showed significant differences between the groups using ANOVA and Tukey posthoc statistical analysis. a=compared to control group (C), b=compared to NC group (negative control), c=compared to acarbose group, * (p<0.05), **(p<0.01).

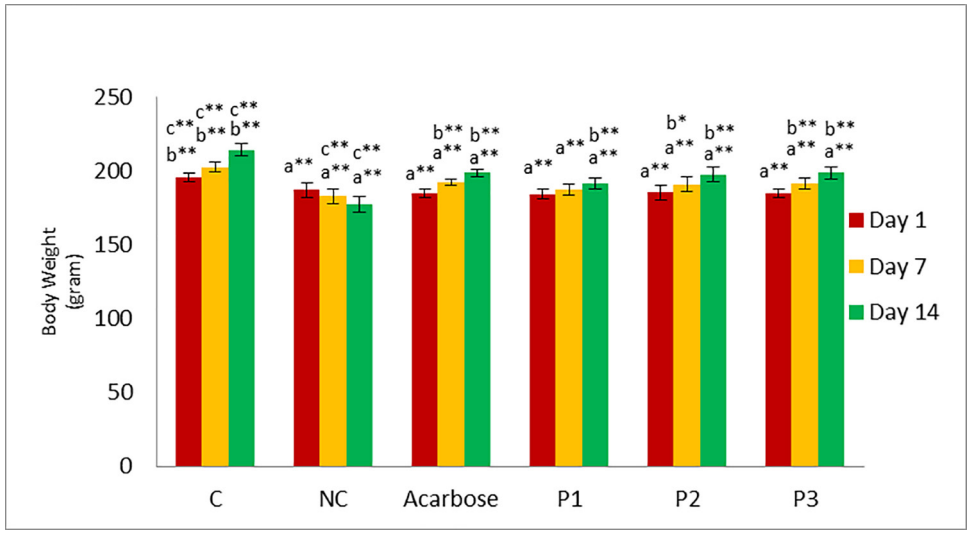

Figure 2: Effect of germinated black rice krisna (GBRK) extract on body weight (BW). Data were presented as mean±SD (n=6). Different superscripts (*,**) showed significant differences between different groups using ANOVA and Tukey post hoc statistical analysis tests. a=compared to control group (C), b=compared to NC group (negative control), c=compared to Acarbose group, Day 1= measurements day 1, Day 7=measurements days 7, Day 14=measurements days 14, *(p<0.05), **(p<0.01).

There was a normal distribution in samplings (p>0.05). There was a significant difference between the NC, acarbose, P1, P2, and P3 groups (p<0.05). The BW in DM rats of NC group significantly decreased on days 7th and 14th (post-test) when compared to the C group (p<0.01). The DM group when received acarbose and GBRK in P3 group for 7 and 14 days, a significant increase was noticed in BW in comparison to the NC group (p<0.01). Furthermore, DM rats receiving GBRK for 14 days in P1 and P2 groups significantly showed an increase in BW when compared to the NC group (p<0.01). The highest dose of GBRK extract to increase BW in diabetic rats was in P3 group at a dose of 2140 mg/kg BW (94 mg/dL). Meanwhile, the increase in BW in diabetic rats in groups receiving GBRK as P1, P2, and P3 groups illustrated the same effect (not different) with the acarbose group (p>0.05) (Figure 2).

Discussion

In our study, STZ was used to induce diabetes in rats using a dose of 65 mg/kg BW and NA at a dose of 230 mg/kg BW that has selective cytotoxicity effects on pancreatic beta cells. It causes DNA methylation, inflammation, pancreatic cell dysfunction and an increase in blood glucose level and increases nitric oxide and free radical (hydrogen peroxide) production (26, 27). Administration of GBRK extract at doses of 1070, and 2140 mg/kg BW continuously for 14 days could significantly reduce FBG levels to normal state below 126 mg/dL, the same as the group given acarbose in STZ and NA-induced DM rats. In contrast, administration of GBRK extract at a dose of 535 mg/kg BW could reduce FBG level, but not to the normal status.

The decreased FBG level in diabetic rats by GBRK extract can be due to bioactive compounds of anthocyanins and flavonoids contents that have anti-diabetic properties (21). It was shown that giving boiled and steamed okra which has flavonoids at a dose of 40 g for 14 days can reduce FBG levels (28). The results of several other studies revealed that anthocyanins and flavonoids in rice which have red, purple, and black pigments and anti-diabetic properties can reduce blood glucose levels towards a normal state (29-30).

The mechanism of these effects shown in diabetic rats to reduce blood glucose levels is the antioxidant properties of anthocyanin and flavonoid compounds in GBRK that decreases the free radicals activities and oxidative damages to pancreatic beta cells (31). These antioxidant effects can repair mitochondria in pancreatic beta cells and decline the blood glucose levels (32). Theoretically, anthocyanins was demonstrated to inhibit the activity of digestive enzymes of α-glucosidase and α–amylase (24, 33), while these enzymes function to absorb polysaccharides in the digestive system. Therefore, if the activity of these two enzymes is inhibited, it would decrease the absorption of polysaccharides and conversion to glucose and would control the FBG level (33, 34).

The decrease in FBG level in our research is in line with a study undertaken by Kang *et al.* reporting that GBRK extract addition to the food at a low dose of 2.5% and a high dose of 5% for 8 weeks in diabetic rats would decrease FBG level (35). According to the research of Chung and Kang,

administration of GBRK extract at a dose of 0.25% (w/w) for 4 weeks can significantly reduce FBG level towards a normal state (36). Our findings showed faster reduction in blood glucose level after 14 days when compared to the study of Chaiyasut *et al.* who found administration of "*Kum Payao*" germinated black rice extract at a dose of 1000 mg/kg BW for 8 weeks could reduce blood glucose level (37). Our study illustrated a faster decrease in FBG level after 2 weeks, because GBRK extract contains anthocyanins and a higher dose was administered.

Our results revealed that on day 0, the BW of diabetic rats was significantly different from the C group, because the BW of normal rats was much higher than other groups (NC, Acarbose, P1, P2, and P3). Theoretically, STZ and NA could induce diabetes in rats and increase the blood glucose level and affect the carbohydrate, protein, and fat metabolism at a molecular level. These metabolic disorders can lead to a weight loss in diabetic rats. The administration of GBRK extract at doses of 535, 1070, 2140 mg/kg BW could significantly increase the BW of diabetic rats on days 7^{th} and 14^{th}, as well as the group received acarbose when compared to NC group. Improved BW in diabetic rats was influenced by GBRK extract and was adjusted to the number of doses given. We are in line with Kazemian *et al.* study reporting that administration of *Capparis spinosa L* extract that contains flavonoid phytochemical compounds can improve weight gain in diabetic rats; while in diabetic rats without any intervention, there was a decrease in BW (38).

The weight gain to normal state can probably be due to the antioxidant content of anthocyanins and flavonoids in GBRK extract. Anthocyanins and flavonoids besides functioning to control blood glucose level can also increase BW and prevent weight loss in diabetes via increasing glycogen synthesis and modulating PI3K/AKT signaling in the liver, muscle, and adipose tissue, as well as a decrease in gluconeogenesis in the liver. Thus, there is no structural protein degradation and muscle wasting to induce weight loss in diabetes (23, 39).

This study did not use the diabetic obese rat model and our findings cannot be utilized in diabetes associated with obesity. However, the research of Noordin *et al.* displayed anthocyanin content in *Hibiscus sabdariffa L.* that could reduce BW in obese rat model (40). Another study showed that administration of herbals containing anthocyanin can reduce BW in obese models induced by high-fat diets through the mechanisms that decrease oxidative stress and inflammation (41, 42). Possibly, GBRK has the same potential in reducing FBG and BW in diabetic rats with obesity, because GBRK has high anthocyanin compounds too (40).

The main strength of this study was use of GBRK as local rice from the province of Yogyakarta, Indonesia, because GBRK is widely produced in Yogyakarta. Meanwhile, the use of rice is still limited and the effect of GBRK on FBG level has not yet been carried out and the results of this study are expected to add new findings to literature to show that local food ingredients such as GBRK has the potential to decrease FBG level in diabetic cases. Although the results of this study showed the positive effect of GBRK on reducing FBG level and improving BW; however, there are still some limitations that are needed to be mentioned. First, this study did not measure total serum antioxidants to evaluate their effect to reduce FBG level based on the antioxidant properties of the bioactive anthocyanins and flavonoids in GBRK. Second, this study did not determine the fat and fat-free masses as the causes of increased BW that are essential to be studied to investigate the effect of GBRK. The increase in BW may be due to changes in fat s and fat-free masses. Therefore, it is necessary to conduct further studies to assess the effect of GBRK on fat mass and total antioxidants in diabetes associated with obesity.

Conclusion

Our findings revealed that administration of GBRK extract at doses of 535, 1070, and 2140 mg/kg BW for 14 days can improve the metabolic disorders in diabetes by reducing the level of FBG and improving BW to a normal state. Further research is needed to exploit the therapeutic effects and the relevant mechanisms of action of GBRK before being used as an antidiabetic drug.

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Conflict of Interest

None declared

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