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Effects of mixture powder of black rice (Oryza sativa L indica), red beans (Phaseolus vulgaris L), and moringa leaves (Moringa oleifera L) on blood glucose concentration in hyperglycemic Rats

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ABSTRACT

Background: Diabetes mellitus, increased blood glucose or hyperglycemia, is associated with increased oxidative stress and cardiovascular diseases. This condition will further cause carbohydrate and fat metabolism change, resulting in the decreased antioxidant defense system. Black rice, red beans, and moringa leaves contain oleic acid, butyric amino acid, antioxidants, phytic acid, and arginine, which can improve insulin sensitivity, and blood glucose homeostasis.
Objective: This study aimed to analyze the effect of betamelor (black rice, red beans, and moringa leaves) on blood glucose in rats
Materials and Methods: The design of this research was experimental research with pre-and post-control group design. A total of 20 Sprague Dawley female rats were divided into four groups, namely standard feed (PS), 80% feed of betamelor (PB8), 50% feed of betamelor (PB5), and 20% feed of betamelor (PB2). Betamelor intervention was given as much as 5% of weight for 28 days. Fasting Blood Glucose (FBG) levels were measured using the GOD-PAP method. Blood glucose data were analyzed by Analysis of Variance (ANOVA) at a 95% confidence level and using Duncan's test.
Results: There were differences in FBG between groups after the intervention of betamelor. The results showed that after 28 days of intervention, betamelor decreased the serum glucose concentration from 122.69 mg / dL to 97.70 mg / dL (20.37%) in the PB8 group and from 123.91 mg / dL to 113.28 mg / dL (8.58%) in the PB5 group, but the standard diet (PS) increased by 5.73%. This result can be applied to reduce blood glucose levels in obese and patients with metabolic syndrome.
Conclusions: There was a significant effect of giving a mixture of black rice, red beans, and Moringa leaves on fasting blood sugar in rats.

Keywords: Black rice; Red beans; Moringa leaves; Fasting blood glucose

BACKGROUND

The changes in lifestyle and diet of carbohydrate-based foods into high-fat content foods leads to the rise of degenerative diseases such as coronary heart disease, hypertension, and diabetes mellitus. An increase in blood glucose or hyperglycemia is a sign of a metabolic disorder known as diabetes mellitus and is associated with the rise of oxidative stress and complications in the vascular system. It will further cause the alteration in carbohydrate and fat metabolism resulting in an impairment of the oxidation defense system. In which this condition stimulates the increased formation of Reactive Oxygen Species (ROS) induce β-pancreatic cell dysfunction.1

One of the antioxidant defense systems is Superoxide Dismutase (SOD), the enzyme involved in the earliest and most potent detoxification in cells.2 The antioxidant enzyme itself is an essential enzyme which is able to eliminate radicals, then may protect cells against toxic of aerobic metabolism byproducts.3 The use of oral medicine of diabetes mellitus accompanied by the use of natural ingredients already become common practice yet, and around 1050 anti-Diabetes Mellitus plants have been studied.4 Some plants commonly used as a source of functional foods and already developed to help in controlling blood sugar are black rice, red beans, and moringa leaves. Ingredients contain oleic acid, amino butyric acid, antioxidants, pitic acid and arginine which are proven to reduce the rate of oxidative stress, thereby increasing antioxidants in the body.

Following Walter and Marchesan (2011), phenolic compounds are concentrated higher in the black rice pericarp.5 Bioactive compounds work as antioxidants in rice, including phenolic, flavonoid,

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anthocyanin, proanthocyanidin, tocopherol, tocotrienol, γ- oryzanol and phytate.6 Aleuron and endospermic in black rice could produce the high-intensity anthocyanin result a deep purple to dark color.7 However, black rice contains less protein and fiber, and this lack can be corrected by substitution of red beans and Moringa leaves. Kidney beans contain fat (15.80%), dietary fiber (3.60%), and carbohydrates (49.00%).8 The soluble fiber in red beans may reduce blood glucose.9 These beans also possess a high antioxidant capacity. The antioxidant capacity of EC50 red bean skin extract reaches 294.78 mg/ml.10 Al-Malki et al. (2015) found that antioxidant and antidiabetic activity in Moringa leaf extract showed potential as an antidiabetic agent in streptozotocin induced mice.17 Tantipaiboonwong et al. (2017), who have reported the antihyperglycemic and anti-hyperlipidemic effects of red rice extract and black rice extract to streptozotocin induced mice. Kidney beans contain arginine about 600 mg/100g.25 Arginine acts as an antidiabetic by regenerating pancreatic β cells to improve the stimulation of insulin secretion.26 Moringa leaves contain 6.7% protein, 1.7% fat and 14.3% carbohydrates.

Rat is one of rodents often used as models in studying blood sugar levels. This is because rats have a significant similarity in hematology and genome with humans.11 Studies related to black rice, red beans and Moringa leaves, which associated with the potential and influence on health, are still rarely performed. It is critical to assess whether there is an impact of giving black rice, red beans, and moringa leaves on blood sugar levels in rats.

This study aims to determine the effect of feeding developed from black rice flour, red beans, and Moringa leaves on rat blood glucose. Precisely, the objectives of this study, i.e., 1) determine differences in body weight 2) determine the average consumption of rat feeding during the intervention period; 3) analyze the effect of treatment on reducing feed consumption; 4) investigate the impact of treatment on rat blood glucose.

MATERIALS AND METHODS

This was an experimental study using a complete random design (CRD) with a pre-post control group. This study was conducted in March-May 2020, involving several laboratories. Making the black rice, red beans, and moringa leaves flours were carried out in the Food Processing Laboratory of Kupang's Health Polytechnic of Health Ministry's. The feed production was carried out at the Animal Feed Industry Laboratory, Kupang Agricultural Polytechnic. The intervention towards the experimental animal and blood collection was executed at the Biosciences Laboratory of the University of Nusa Cendana Kupang, while blood analysis conducted at the Health Laboratory of East Nusa Tenggara. The proximate analysis was carried out at the Saraswanti Indo Genetech Laboratory in Bogor, West Java.

The number of experimental animals used in this study was 20 rats, which were calculated according to the Federer formula (1997): (n-1) (t-1) ≥15 (n is the number of samples needed and t is the number of treatments). All those 20 rodents were divided into four groups, namely five rats of the standart feed (PS), five rats intervened by 80% mixture powder (PB8), five rats intervened by 50% mixture powder (PB5), and five rats intervened by 20% mixture powder (PB2). The animals used were Sprague Dawley female rats met the inclusion criteria, such as normal and healthy, aged 5-6 months, the weight of 150-200 g, open eyes, reddish-white skin, agile, and have soft, clean, dense, smooth, not fall, and shiny-haired. The exclusion criteria were that the rat died during the intervention, behavior changes (did not want to eat, drink and limp), weight loss of > 5%. Equipment used for rat care consists of a cage filled with husks equipped with food containers and drinking bottles, iron cage enclosures, digital scales for weighing rats and leftovers, and a set of cage cleaning tools. This study had received ethical approval from the Animal Ethics Commission, Faculty of Veterinary Medicine, University of Nusa Cendana No.168 / UN15.8.1 / PP / 2020.

The first stage of the research was the feed formulations from black rice, red beans, and moringa leaves. The composition of ingredients was following the diet of people with diabetes mellitus. Producing mixture powder began with making black rice flour, red beans flour, and moringa leaves powder, then sieved with 80 mesh size. Feed material in the form of bravo 512 standard solid feed was mixed until homogeneous. After that, a little water added until the feed mixture was half wet, then formed to obtain pellet feed. The wet pellet then dried in an oven (T 40ºC), then put into a plastic bag and kept in a refrigerator until the intervention time to experimental rats.

During the adaptation period, intended to ensure the experimental animals were in a healthy condition before the intervention, which was carried out in individual cages, given standard feed, and ad libitum drinking water for about ten days. Bravo 512 standard feed contains 12% water, 19.5-21.5% crude protein, min 5% crude fat, max 5% crude fiber, max 7% ash, 0.9 - 1.1% calcium, 0.6 – 0.9% phosphorus, and energy

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about 3125kcal/Kg. Intake and residual feed were weighed and recorded every day, and body weighing was done once a week. A high glucose feed of 2cc/200g of body weight/day was given through sonde for two weeks to create hyperglycemic rats. The treatment was performed for 28 days, i.e., subjected a standard feed, 80% mixture powder, 50% mixture powder, and 20% mixture powder. Furthermore, those rodents fasted for 10 - 12 hours, then two cc of blood samples per each were taken through the eye veins (ocular arteries) using a microhematocrit capillary tubes after anesthetized with ketamine 10 mg/kg rat. Blood serum glucose analysis was executed according to the GOD-PAP method.

The data of blood glucose were analyzed by Analysis of Variance (ANOVA) at a 95% confidence level. If it shows the significance response, then Duncan's Multiple Range Test was applied. Data processing and analysis were performed using Microsoft Excel and SPSS for Windows version 22.0.

RESULTS AND DISCUSSION

Proximate Composition

The results of the proximate analysis of black rice, red beans, and moringa leaves can be seen in Table 1. It shows that the ingredients used complement nutritional value each other mainly on the parameters of carbohydrate, protein, fat and dietary fibre.

Table 1. Proximate Composition of Mixture Powder

Parameters	PB Mixture 80%	PB Mixture 50%	PB Mixture 20%
Protein (g)	6.3 ± 0.91	10.1 ± 1.26	13.87 ± 2.03
Total fats (g)	0.8 ± 0.17	1.54 ± 0.49	2.28 ± 0.94
Carbohydrate (g)	25.3 ± 3.21	36.5 ± 2.64	47.9 ± 5.16
Fibre (g)	18.6 ± 2.33	13.8 ± 1.75	9.1 ± 1.02
Energy (kcal)	133.5 ± 18.01	200.2 ± 20.01	267.6 ± 37.22

Body Weight

Body weighing was done weekly to determine the amount of standard feed, high glucose feed (weeks 1 and 2), and mixture powder feed (weeks 3 to 6). The effect of feeding treatment on the body weight of rats can be seen in table 2. At the end of acclimatization, the average body weight of the rats did not have a significant difference between treatment groups. During the high-fructose diet for 7 days, the body weight of the rats increased significantly compared to the acclimatization period, but not significantly between treatments. During the mixture powder intervention period for 21 days, the body weight of the rats experienced a significant increase compared to the high fructose diet and there were also significant differences between the treatment groups. It was probably caused by the influence of blood drawn through the eye veins (ocular arteries), which results in pain and low appetite, recognized from the decrease in feed intake. In the following days, there was a slow improvement in body weight in line with an increase in feed intake.

Table 2. Body weight of rats fed a high-fat diet and treated with mixture powder for 10 weeks

Group	Acclimatization mean ± SD (g)	HGD mean ± SD (g)	Intervention of mixture powder mean ± SD (g)	P^2 (Acclimatization)	P^1 HGD1-Intervention
PS	190.82 ± 8.81	202.33 ± 5.79	227.62 ± 4.95	0.048	0.000
PB8	192.27 ± 6.47	201.48 ± 7.40	210.13 ± 3.19		
PB5	190.38 ± 6.72	202.18 ± 4.51	219.68 ± 3.57		
PB2	189.91 ± 7.63	203.76 ± 6.24	224.27 ± 5.51		
P^1	0.821	0.294	0.008		

HGD : High Glucose Diet, [1]One way Anova test, [2] Paired t-test

Table 3. Concentration (mg/dL) of fasting blood glucose of female Sprague dawley rats that were administered graded doses of mixture powder

Group	Acclimatization mean ± SD (mg/dl)	HGD mean ± SD (mg/dl)	P[2]	△Increase (mg/dl) (%)	P[1]
PS	78.41 ± 2.60	124.10 ± 3.71	0.000	45.69 ± 1.51 (58.27%)	0.071
PB8	80.12 ± 1.53	122.69 ± 2.06	0.000	42.57 ± 2.07 (53.13%)	
PB5	79.64 ± 1.29	123.91 ± 3.49	0.000	44.27 ± 1.92 (55.58%)	
PB2	78.29 ± 2.31	124.53 ± 3.84	0.000	46.24 ± 1.89 (59.06%)	
P[1]	0.369	0.493			

HGD : High Glucose Diet, [1] One way Anova test, [2] Paired t-test

Table 4. Concentration (mg/dL) of fasting blood glucose of female Sprague dawley rats that were administered graded doses of betamelor.

Group	Acclimatization mean ± SD (mg/dl)	HGD mean ± SD (mg/dl)	P[2]	△Increase (mg/dl) (%)	P[1]
PS	124,10 ± 3,71	131,21 ± 2,46	0,004	7,11 ± 2,18 (5,73%)	0,007
PB8	122,69 ± 2,06	97,70 ± 1,92	0,000	-24,99 ± 1,84 (-20,37%)	
PB5	123,91 ± 3,49	113,28 ± 1,17	0,000	-10,63 ± 2,35 (-8,58%)	
PB2	124,53 ± 3,84	126,96 ± 2,38	0,042	2,43 ± 1,72 (1,95%)	
P[1]	0,493	0,493			

HGD: High Glucose Diet, [1] One-way Anova test, [2] Paired t-test

Fasting Blood Glucose Level Before and After Observation

Blood glucose levels of the rats at the end of the aclimatization stage and after HGD feeding for each group can be seen in Table 3. Significantly increased blood sugar levels occurred in all groups after intervened by HGD with a significant value ($p > 0.05$) based on the analysis of one-way test Anova. The average value of fasting blood glucose levels in the intervention of mixture powder 80% (PB8) and mixture powder 50% (PB5) experienced a significant decrease ($p < 0.05$). While the response by standard feed as control and mixture powder 20% (PB2) experienced an increase in blood glucose levels. There were differences in rat blood glucose levels between groups after the administration of mixture powder based on the *Kruskal Wallis test*. The PB8 treatment group experienced the most substantial decline in blood glucose levels which was -24.99 ± 1.84 (-20.37%) mg/dl (Table 4). Concentration (mg/dL) of fasting blood glucose of female Sprague dawley rats that were pre and post administered graded doses of *mixture powder*.

Body weight in mice increased significantly for all groups from the acclimatization period to the high fructose diet phase and the high fructose diet phase to the betamelor intervention phase.Weight loss in the experimental animal was significantly increased for all groups on the acclimatization, high glucose diet, and mixture powder intervention. There was no significant difference in body weight among the groups in either phase of acclimatization or a diet high in glucose, but there were substantial differences in the intervention period. The bodyweight improvement was due to the consumption of standard and high glucose feed before the intervention. The differences in the different treatments during the intervention cause the alterations that have a significant effect on the amount of energy intake so that excess consumption will be stored as fat reserves and increase bodyweight. In the acclimatization phase, the standard feed intake of each group was not significantly different. In the phase of the provision of a high glucose diet, there were significant differences between the treatment groups and the standard feed, the feeding intake of the control group had the highest compared to other groups.

Significant differences in feed intake occurred between groups. The control group had a higher feed intake than the treatment group. The consumption of the control group tended to remain since acclimatization. The treatment group of 50% and 20% mixture powder increased but not significant, and the 80% mixture powder group experienced a decrease in feed intake. It was due to the administration of mixture powder in the treatment groups that contains a high fibre composition, lead to full quickly compared to the standard feed group. Reducing intake in the intervention phase compared to the conditioning phase of treatment groups was due to the high fibre effect of diet caused by the rise of the hormone leptin, so the appetite decreased in the intervention phase.12 In the treatment groups, the standard feed intake was lower than in the conditioning period because it was in the adaptation stage. During the intervention phase, the usual feed intake of treatment groups was lower than the other groups because the stomach was quickly filled due to high fibre feeding.

Blood glucose levels were elevated in the test animals as the result of conditioning the provision of a high glucose diet after a period of adaptation completed with values > 120 mg/dl. It can be interpreted as metabolic syndrome, according to Crescenzo (2014) in Italia, the metabolic syndrome can be realized by high glucose feeding to increase blood glucose levels.13 Excess fructose intake in the liver will be metabolized into fat. Fructose may lead to a failure of insulin signal to reduce the synthesis of glycogen and increase gluconeogenesis, resulting in blood glucose elevation.13

Furthermore, this study revealed that the average fasting blood glucose values in the PB8 and PB5 groups experienced a significant slope after mixture powder intervention ($p < 0.05$). The results of this study were in accordance with Mbikay's literature review study (2012), the administration of moringa leaves for diabetes mellitus causes a potentially reducing blood sugar levels.14 Compounds that was an essential key allegedly in the antihyperglycemic activity in extracts of moringa (M. oleifera) is oleic acid. Oleic acid, or commonly called omega-9, is one of the best types of fat. Oleic acid also is known as one of monounsaturated oil (MUFA) because the body can synthesize from the nutritional compounds consumed, so it also including in non-essential fatty acids.14

In the research conducted by Aly et al. (2016) using GC-MS analysis, identified eight fatty acids contained in Moringa seed oil including palmitic acid, stearic acid, arachidonic acid, behenic acid, palmitoleic acid, linoleic acid, linolenic acid, and oleic acid.15 Besides, there were more than 50 phytochemical contents possessed in Moringa seed oil. According to Busari et al. (2015), the hypoglycemic activity of Moringa seed oil is caused by the presence of fatty acids in the oil.16 Monounsaturated fatty acids have a tendency to improve the function of beta-cell secretors, reduce the disruption of beta-cell activity and proliferation. Al-Malki et al. (2015) found that antioxidant and antidiabetic activity in Moringa leaf extract showed potential as an antidiabetic agent in streptozotocin-induced mice.17

Blood glucose levels in this study also found a decrease might be caused by the presence of black rice extract in the mixture flour. It is in line with the study by Tantipaiboonwong et al. (2017), who have reported the antihyperglycemic and anti-hyperlipidemic effects of red rice extract and black rice extract to streptozotocin-induced mice. The results of the study revealed that consumption of black rice extracts of 50 mg/kg body weight or red rice extract of 100 mg/kg body weight significantly reduced blood sugar levels after eight weeks.18 Observation by Chaiyasut et al. (2017) showed that germinated black rice extract could increase the aminobutyric acid content, total antioxidant capacity, and levels of antioxidant enzymes in diabetic rats and showed antidiabetic activity both before and after administration of streptozotocin in rats as animal models.19

A study conducted by Chung, et al. (2016) also found that pigmented rice has inhibitory enzymes responsible for diabetes. The results showed that those pigments contained phenol and had a significant enzyme inhibitory activity. Bioactive compounds that cause pigmentation in rice are anthocyanin and proanthocyanidin.20 Kasim et al. (2005) revealed that black rice has the highest antioxidant activity compared to the red and white rice. Naturally, black rice contains pigments with high antioxidant activity (anthocyanin).21 Hosoda, et al (2018) reported that black rice and brown rice contain anthocyanin and proanthocyanidin, which are potentially used as antioxidants sources other than as a starch source in ruminants.22 Bioactive compounds in pigmented rice may reduce the oxidative stress, prevent cancer, cardiovascular, complications and potential diabetes, and others.5,22

In this study, mixture powder as a mixture of flours in an intervention feed in which one constituent is red beans, so the slope of fasting blood sugar values in an animal model may be related to the red beans. Kidney beans (*Vigna angularis*) are good sources of fiber, every 100 grams of dried red beans provides about 4 grams of fiber consisting of both soluble and insoluble fiber.23 Soluble fiber significantly lowering blood sugar, due to the soluble fiber is able to substantially reduce the glycemic response 23,24. This beans like other legumes, contain several components of inhibitor substances such as phytic acid, tannin, trypsin inhibitors, and oligosaccharides. The diet treatment using this legume may reduce glucose absorption by 48.43%. As for the soy diet, it can reduce absorption by 45.84% .25 Water-soluble fiber can form a viscous solution; the higher viscosity in the intestine leads the slower absorption of glucose by the small intestine. The viscous soluble fiber can reduce postprandial blood sugar and insulin levels. Based on the results of several studies, three different doses of red beans might lower blood glucose levels in male Wistar rats that given glucose load.24

Kidney beans contain arginine about 600 mg/100g.25 Arginine acts as an antidiabetic by regenerating pancreatic β cells to improve the stimulation of insulin secretion.26 Arginine as an amino acid of hormonal terminal GLP-1 (Glucagon-Like-Peptide-1) served as proinsulin gene expression and insulin synthesis stimulator. After insulin secretion occurs, glucose levels in the circulation immediately decrease; thus, the effect of GLP-1 will disappear by itself.27 Kidney beans; a staple food recommended for diabetic patients contains high protein and fiber, and low carbohydrates. Starch granules from these legumes have a unique structure and hydrolysis of starch in the small intestine relatively slow, thus postprandial glycemic response will be delayed.27 Yao's (2014) finding that the mechanism of red beans in hyperglycemia related to the effect of protein and phenolic compounds in it. Besides, this experiment also proved that extruded red bean protein and polysaccharides play a role in α-glucosidase inhibition.28 The subjects used were mice with *Sparague Dawley* strains, could not study the mechanism of diabetes mellitus. in further research, humans can use as samples. Further research is necessary to test the complete phytochemical contents of mixture powder of black rice, red beans, moringa leaves and to analyze its effect on other anti-inflammatory and antioxidant parameters in diabetes mellitus to deepen our understanding on the role and mechanisms of mixture powder of black rice, red beans, moringa leaves in diabetes mellitus

CONCLUSIONS

According to this study, the mixture of black rice, red beans, and moringa leaves as mixture flour reduce the fasting blood glucose reduction in rats significantly. Further research is needed to examine the complete phytochemical content of mixed black rice, kidney bean, moringa leaf powder and analyze their effect on anti-inflammatory and other antioxidant parameters in diabetes mellitus to deepen understanding of the role and mechanism of mixed powder black rice, kidney beans, moringa leaves in diabetes mellitus.

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