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The Beneficial Effect of Watercress (*Nasturtium officinale*) and Black Rice (*Oryza sativa L.*) Bran Supplementation in Glycemic Improvement

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Abstract: ***This study was to analyze the effect of watercress (WC) and black rice bran (BRB) in glycemic improvement of rat induced by Streptozotocin-nicotinamide (STZ-Na). Thirty-five male Wistar rat were randomly divided into 5 groups; healthy rat (K-), group induced by STZ-Na (K+), group given WC (P1), group given BRB (P2), group given WC + BRB (P3) for 4 weeks. Fasting blood glucose was measured by GOD-PAP method. Either WC or BRB can improve glycemic status of P1, P2, P3. Combination of WC and BRB shows synergistic effect and is more effective in improving glycemic status than WC or BRB alone.***

Keywords: watercress, black rice bran, glycaemia status

1. Introduction

Hyperglycemia is a condition of blood glucose exceeding the normal limit characterized by fasting blood sugar ≥ 126 mg / dl (≥ 7.0 mmol / L) or 2 hours ≥ 200 mg / dl (≥ 1.1 mmol / L) glucose[1], [2]. High levels of blood glucose in a long period can increase the ability of hyperglycemia in oxidative modification of various substrates and consequences in formation of free radicals. The condition of hyperglycemia leads to ROS overproduction in endothelial cells (mitochondria) and biochemical reactions that produce free radical products [3].

Watercress is a shrub, rich in nutrients, tastes good, and is found in various countries, but it also contains bioactive substances. Vitamins contained in watercress are vitamins A, B1, B2, C, E, K, beta carotene and folic acid. Mineral contents in watercress are calcium, sulfur, magnesium, phosphorus, potassium, sodium, iodine, zinc, selenium, iron, and beta carotene. Watercress is one of vegetables that contains high flavonoid that are quercetin and kaempferol. Watercress contains the highest levels of vitamin C, flavonoids, and variability in composition and antioxidant species comapred to vegetable cabbage groups[4].

Black rice bran, the result of milling of the outer skin portion of black rice, also contains phytochemical components. The previous study showed that black rice bran extract had high phenolic content and it had a potency as a free radical predator [5]–[7]. A study conducted to identify components found in black rice bran found that there was anthocyanin, a flavonoid component of water-soluble pigment, which is beneficial for health because it has a high antioxidant capacity. The types of anthocyanin found in black rice are cyanidin and peonidin [8], [9]. The blackish purple color of black rice arises from the presence of pigment, known as anthocyanin. Bran is part of the process of rice milling process in particular the second process of milling, while nutrients and bioactive substances concentrated in the insides of the bran have the highest nutrient and bioactive content compared to other rice[6], [10]. The role of phytochemical substances contained in bran (including black rice bran) acts as an anti-inflammatory, anti-bacterial, and as an antioxidant that prevent various diseases, such as coronary heart disease, cancer, and diabetes, and hyperglycemia [11]. Rice containing pigments or colored rice including black rice is the best source of antioxidants with the strongest antioxidant activity [12]. Other results suggested that in the bran there is also bioactive substance γ-oryzanol, and routine treatment of oryzanol may cause protective effects against hyperglycemia, diabetes with complications of nerve damage [13]. This study aimed to analyzed the beneficial effect of WC, BRB, and combination both of them towards glycemic status.

2. Research Method

2.1. Materials

This study used watercress (WC) and black rice brand (BRB) powder. WC was obtained from watercress farmers in Candi village, Bandungan district, Semarang, Central Java. BRB is derived from organic black rice harvesting in Ngampel Village, Gentungan, Mojogedang District, Karanganyar.

2.2. Methods

Male Wistar rats were divided into 5 groups, negative control (K-): the healthy rats, positive control (K+): hyperglycemia rats caused by Streptozotocin-Nicotinamide (STZ-Na induction), P1 was hyperglicemia rats by given WC intake, P2 was hyperglicemia rats by given BRB intake, and P3 was hyperglicemia rats by given combination of WC and BRB. WC, BRB, and combination of WC and BRB were administered (five days after STZ-Na induction) by oral gavage WC as much as (g/kg/body weight) 9.5; 9.37; and 4.75 of WC and 4.69 of BRB, respectiely [14], [15]

Fasting blood glucose (FBG) levels before and after treatment was assessed by GOD-PAP method. The experiment was conducted for 28 days at Animal Laboratory of UGM Center for Food and Nutrition Studies. Ethical Clearence research was obtained from Ethical Clearance Commission for Preclinical Research of UGM Integrated Research and Testing Laboratory.

2.3. Statistical Analysis

All values are expressed as mean ± SD. Kruskal-Wallis followed by Mann-Whitney was used to calculate statistical significance. SPSS 21 software was used to analyse all the statistical analysis. The data differences were considered significant at $P<0.05$ and confidence interval 95%.

3. Result and Discussion

All rats were still alive during the study. In the end of acclimatization, FBG level of groups in average was 75.54 mg/dl. Table 1 shows that FBG levels in K(-) group tended to be normal (<110 mg / dl) during the study, whereas the fasting blood glucose levels of K(+) remained high (>200 mg/dl). FBG level of the treatment group decreased after the experimental hyperglycemia was given WC + BRB intake for 4 weeks. The mean of fasting blood glucose level of P1, P2, P3 groups decreased from 238.10 ± 6.01 mg /dl to 112.59 ± 8.20 mg /dl. The results of statistical analysis showed that the administration of WC and BRB had a very significant effect ($P<0.001$) in the status of glycaemia. The mean of fasting blood glucose level of P1, P2, P3 were lower compared to K(+).

Table 1: The effect of WC, BRB, and the combination of WC and BRB towards FBG of rat

Group	*Initial*	*Final*	*P*
P1	236,3±6,9	108,1±4,1	0.000*
P2	240,4±6,2	122,1±5,8	0.000*
P3	237,6±5,0	107,6±4,0	0.000*
K(+)	235,8±6,6	235,9±6,5	0.853
K(-)	75,8±3,2	77,7±1,7	0.014*

**p<0.05 = significant level before and after treatment*

The status of hyperglycemia improved in treatment groups which was characterized by decreased blood glucose level. The combination of WC and BRB in STZ-Na-induced diabetic rat reduced FBG effectively. Furthermore, it was marked by an almost equal value for the difference in average decrease in fasting blood glucose levels in P1 and P3 groups, with statistical test results showing no significant differences as shown in Fig 1.

The condition of hyperglycemia is characterized by elevated fasting blood glucose levels beyond the normal threshold. Fluctuations in FBG indicate uncontrolled blood glucose levels that, if persistent, lead to both micro and macro vascular complications.

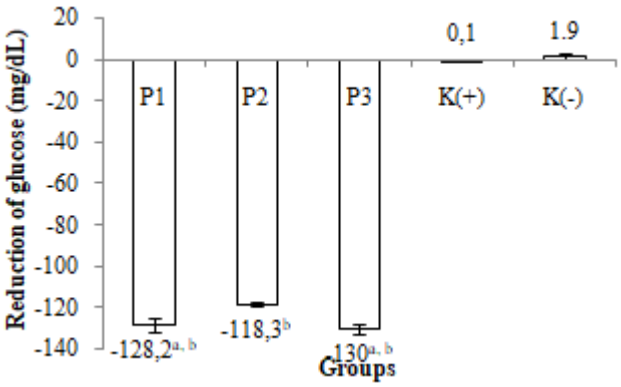

Figure 1: Reduction of FBG (mg/dL). Data are represented as mean ± SD for 5 groups. [a]- Significant compared with P2; [b]- significant compared with K(+) P<0.05 determined by Mann-Whitney test.

Controlling diet is the best way to maintain blood glucose levels. Hyperglycemia-induced ROS production and its complication like diabetic retinopathy, neuropathy, and cardiopathy can be inhibited by antioxidant compounds through trapping single electron to free radicals or through reducing ROS enzymatically[16]. High vitamins, minerals, and antioxidants consumption from WC and BRB powders is capable to reduce FBG of rat. This study elucidated that WC treatment showed almost equal decreasing of FBG compared to WC+BRB treatment, but the highest decrease still occurred in WC + BRB group. The combination of WC and BRB has potential to have association of nutrients and antioxidants from WC and BRB, therefore the ability to inhibit the oxidative damage caused by free radicals becomes stronger. According to Sharma et al (2011), one of the factors related to the pathogenesis of hyperglycemia is increased production of ROS by oxidative stress which further affects antioxidant oxidation ability and influence oxygen consumption. These conditions also results in uncontrolled hyperglycemia due to disruption of carbohydrate, fat, and protein metabolism[17].

Watercress contains quercetin, an active substance in the form of bioflavonoids that have the potential as a therapeutic substancerelated as a free radical predator, inhibitor of oxidation of xanthine and lipid peroxidation in vitro. Both alone and together quercetin ascorbic acid can reduce oxidative damage. Research on diabetic animals found that quercetin with its antioxidant action can regenerate pancreatic cell damage and increase insulin sensitivity so that it is useful as an anti-diabetic[18].

Anthocyanin, also found in black rice bran, are the pigments found in foodstuffs and it causes blue, red and purple on fruits, vegetables, or flowers. Furthermore, anthocyanin is an active substance which also acts as an antioxidant. Some researchers say that anthocyanin has anti-inflammatory, anti-carcinogenic, anti-obesity, vasoprotective, and anti-diabetic effects. Another in vivo study found that anthocyanin increased plasma antioxidant capacity, improved postprandial serum antioxidant status, and repaired DNA linked to axidative damage in healthy men after consuming high-fat foods[19].

4. Conclusion

Provision of WC and BRB in hyperglycemia Wistar rats has beneficial effect in FBG improvement. Combination of WC and BRB shows the most effective treatment towards FBG reduction. The further study is needed to analyzed the combination of WC and BRB effect towards cellular biomarkers to investigate the possibility mechanisms of the combination WC and BRB in FBG reduction.

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