Control Of Lipid Profile On Diabetes Mellitus Animal Models With Watercress And Black Rice Bran

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Abstract. Fluctuations of blood glucose levels in people with diabetes is a major cause of developing complications from hyperglycemia ability to form the free radicals. Hyperglycemia condition that causes glucose autooxidation, protein glycation, and polyol pathway activation so as to accelerate the formation of reactive oxygen compounds, further increasing the modification of proteins, lipids, and DNA in various tissues. Various studies have been done, but they rarely take advantage of local foodstuffs as a functional food therapy. Raw foods contain a powerful antioxidant that is a combination of watercress and black rice bran. This study aimed to analyze the effect of functional food supplements to changes in the lipid profile of experimental animals . The results showed that levels of total cholesterol and triglyceride levels decreased significantly after being given the supplement. There is a significant increase in HDL levels in experimental animals after supplementation for 4 weeks, however, supplementation is not significant effect on LDL levels decrease.

INTRODUCTION

The costs of health services, including those for medicating degenerative diseases, are very expensive, so people tend to prevent or cure diseases by minimizing the consumption of medicine. This situation happens because the price of medicine is unreachable to buy for many people. And, it gets worse due to the improving knowledge on bad effects of consuming medicine in long periods. Therefore, people mostly consume natural medicine, whether in forms of food, drink, *jamu* or herbal drink, herbal medicine or food supplement to cure the diseases.

Food or drink consumed for the components are useful for health, but not categorized as either medicine or *jamu*, is called functional food or drink. The ingredients of functional food are mostly from vegetable materials, usually used for they contain active substances that can resist the progressivity of a disease. Nowadays, one of diseases with increasing numbers of patients and that is able to cause complication if suffered in long period is Diabetes Mellitus (DM).

Diabetes Mellitus (DM) is a metabolic disorder with high prevalence or mortality. IDF (International Diabetes Foundation) data shows that in 2003, the number of patients of DM type 1 and 2 was 194 million people worldwide and will increase up to 334 million people by 2025 (Wild et al, 2004). The Glucose level in the blood of DM a patient that keeps higher than the normal limit will cause hyperglycemia. This condition can create glucose autoxidation, protein glycation, and also polyol pathway activation, which then can accelerate the formation of reactive oxygen compounds that increase the modifications of protein, lipid and DNA in various body tissues. Consequently, there is an imbalance between the protective antioxidant and free radical production, which finally results in the oxidative stress. The antioxidant imbalance can be decreased by consuming food that is source of antioxidant.

Natural antioxidants can be obtained from vegetable foodstuffs, e.g. watercress and the residue of washing rice called black rice bran. Both are rich of vitamins, minerals and bioactive substances. Thus, they have strong antioxidant effects. One of antioxidant types related to DM is *anthocyanin*. Anthocyanin is a substance with high antioxidant activities that functions as the predator for free radicals and has potency to destroy metals. Another function of anthocyanin is that it is thought to be able to decrease oxidative stress that can destruct tissues, so it can keep the function of β cells and protect the progression of insulin resistance in the body of a DM patient.

Watercress contains vitamin A, vitamin B, vitamin C, vitamin E, vitamin K, folic acid, calcium, magnesium, phosphor, potassium, sodium, and also some bioactive substances such as beta carotene, lutein, and *zeaxanthine* (Hollman PC, Katan MB, 1999 : Hertog MG, Kromhout D, Aravanis C, et al., 1995.; Song, Yiqing. Manson, Joann E. Buring, et al., 2005.; Anjaneyulu, M., Chopra, K., Kaur, Indupal I., 2003. ; Kwon O, Eck P, et al., 2007). The black rice bran contains various nutrients and bioactive substances, such as vitamin B, vitamin E, folic acid, zinc, iron, metal, copper, selenium, manganese, *polyphenol* and *anthocyanin*.

Natural antioxidants can be obtained from vegetable foodstuffs, e.g. watercress and the residue of washing rice called black rice bran. Both are rich of vitamins, minerals and bioactive substances. Thus, they have strong antioxidant effects. The result of a research done by Syamsianah and Anggraini (2014) shows that watercress contains C vitamin (29.17 mg/100g), magnesium (2706,22 mg/kg), iron substance (127, 35 mg/kg), zinc (73,33 mg/kg), and some bioactive substances such as carotene (470,34 mg/g) and quercetin (107,11 mg/kg). The research on a diabetic mouse inducted streptozotozin concludes that quercetin is potential to be used as antidepressant for DM patients. Besides, it can reduce glucose absorption in the small intestine (Anjaneyulu, et al., 2003.; Kwon, et al., 2007).

Another kind of antioxidant substances related to the DM disease is anthocyanin, which is thought to be able to reduce oxidative stress that can destruct cells so that it can protect function of β cells and protects the progression of insulin resistance in the body of a DM patient. Besides containing anthocyanin (630,38 mg/100g), black rice bran also contains various kinds of nutrients, such as vitamin C (41,29 mg/100g), zinc (77,34 mg/kg), and iron as much as 102,04 mg/kg (Syamsianah and Anggraini, 2014). Setiawan and Suhartono (2005) states that vitamin C can play a role as the inhibitor of aldose reductase enzyme in the mechanism of DM.

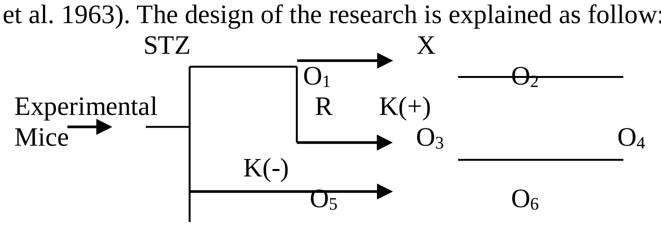
The composition of C vitamin, zinc, Magnesium and quercetin active substance get the highest increase when the two kinds of food, i.e. watercress and black rice bran, are mixed in vitaral in accordance with the same ratio. This is proven in the research conducted by Syamsiana and Anggraini (2014) that the mixture of 1 portion of watercress and 1 portion of black rice bran part (1 : 1) contains vitamin C (83,17mg/100g), zinc (99,43 mg/kg), magnesium (3767,10 mg/kg) and quercetin 9217,85 mg/kg) higher than the mixture of both with the ratio of either 1 : 2 or 2 : 1.

Referring to the research, some research on animals needs to be conducted in order to examine the effectiveness of anti-hyperglycemia functional food supplement as a preventive effort toward the increase of oxidative stress caused by the fluctuation of blood glucose level in a DM patient.

RESEARCH METHOD

1. Research Type and Design

The type of the research is experimental research with Randomized Pretest- Posttest Control Group Design (Campbell, et al. 1963). The design of the research is explained as follow:



Picture 2. Research Design

Explanation:

$O_{1,3,5}$ = Lipid Profile before Treatment

$O_{2,4,6}$ = Lipid Profile after treatment

X = The functional food giving

K(+) = Positive control, inducted by STZ (65mg/kg BB) + Nicotinamide (230mg/kg BB) and given by standard food.

K(-) = Negative control, mice given standard food.

a. Populations and Experimental Animal Samples

The population of the research is Male Wistar mice, 3-4 months, with weight 200-300 grams. The samples of the research are divided into 3 groups and the first group is given a treatment, the second group is determined as the positive control group, and the last group is the negative control group.

The determination of the research samples refer to the WHO standard (1993), which says that research samples for animals need minimally 5 animals. Therefore, this research uses 7 mice for each group as the samples, either for the treatment group or control group. The technique of sample taking uses random sampling technique.

The criterion of sampling inclusion is determined by considering some aspects, such as: (1). Weight; the weights of the mice are between 200-300 grams and the weights must be still around 200-300 grams before getting treatment or not having weight loss. (2). Body condition; the samples must be in healthy conditions and they must not show abnormal anatomies, (3). Blood glucose level; blood glucose level in normal condition is around 80 – 120 mg/dl, blood glucose level on fasting time <110mg/dl, blood glucose level on fasting time after induction STZ ≥ 200mg/dl. The Sampling exclusion criteria are: (1). The mice get sick during the research, (2). The mice die when the research is ongoing.

b. Data Analysis

Data analysis in this research is done univariately and bivariately. Univariate analysis is used to describe mean, median and standard deviation score from the investigated variable which is lipid profile data including total cholesterol level, triglyceride, HDL, and LDL.

Bivariate analysis is used to examine the influence of the functional food feeding toward lipid profile used the *T-test* statistic. The lipid profile difference for each treatment is analyzed through an *Independent Sample-Test*. A *Paired-T test* is conducted to analyze the difference of lipid profile before and after giving functional food to each treatment group in order to test the effect of functional food to the lipid profile of the experimental animals.

THE RESULT AND DISCUSSION OF RESEARCH

The Lipid Profile of the Experimental Animals

The analysis of lipid profile is conducted at the beginning and the end of the treatment, including the total cholesterol level, triglyceride level, HDL level and LDL level. The result of the lipid profile analysis is shown in Tables 2, 3, 4, and 5.

The Total Cholesterol Level

The cholesterol levels of the experimental animals increase, whereas the cholesterol levels of the DM experimental animals given functional food decrease down to 111 mg/dl. The result of the statistical test using Paired T-test shows that there is a significant difference between the total cholesterol level before treatment and that after treatment of the DM experimental animals given functional food during 4 weeks (p=0,008). The statistical test of the *Independent T-test* shows the difference of total cholesterol level between the DM experimental animals DM given standard food and the DM experimental animals given functional food (p=0,000). The mean score of the total cholesterol level is shown in Table 2.

Table 1. The mean of the Total Cholesterol Level Before and After Treatment

Treatment Group	Pre-Treatment Cholesterol (mg/dl)	Post-treatment Cholesterol (mg/dl)	Cholesterol Difference Total (mg/dl)
K-1 (normal, healthy)	106,6±2,7	104,8±2,0	-1,8±4,0
K-2 (DM type2)	233,0±5,2	239,9±10,6	6,9±14,7
P-3 (functional food)	242,8±8,6	131,8±3,2	-111,0±7,3

The Triglyceride Level

The Triglyceride levels of the experimental animals DM given standard food increase during the research, whereas the triglyceride levels of the experimental animals given functional food during 4 weeks reduce down to

56.3 mg/dl. The explanation of the triglyceride levels of the experimental animals is shown in Table 3. The statistical analysis shows that there is a significant difference in triglyceride level after getting treatment (p=0,012) between the DM experimental animals given standard food and the DM experimental animals given functional food.

Table 2. The mean of the Triglyceride Level Before and After Treatment

Treatment Group	Pre-Treatment TG (mg/dl)	Post-Treatment TG (mg/dl)	TG Difference (mg/dl)
K-1 (normal, healthy)	71,0±2,3	67,6±4,2	-3,3±4,9
K-2 (DM type 2)	133,3±3,9	138,1±8,0	4,7±9,6
P-3 (functional food)	136,7±10,0	80,4±3,7	-56,3±8,6

The HDL Level

The HDL levels of the DM experimental animals given standard food increase a little bit during the research, whereas the experimental animals given functional food during 4 weeks increase up to 53, 9 mg/dl. The explanation of the triglyceride levels of the experimental animals is shown in Table 4.

Table 3. The Mean of HDL Level Before and After Treatment

Treatment Group	Pre-Treatment HDL (mg/dl)	Post-Treatment HDL (mg/dl)	HDL Difference (mg/dl)
K-1 (normal, healthy)	44,7±2,2	41,9±1,0	-2,8±2,9
K-2 (DM type 2)	17,5±1,9	20,9±1,9	3,4±3,0
P-3 (Functional Food)	17,5±1,8	71,4±3,5	53,9±3,6

The result of the statistical test using Paired T-test shows that there is a significant difference of HDL levels before and after treatment for the DM experimental animals given functional food during 4 weeks (p=0,001). *T-test Independent Sample Statistical test* shows the difference of HDL levels after treatment between the DM experimental animals only given standard food and the DM experimental animals given functional food (p=0,000).

The LDL Level

The LDL levels of the DM experimental animals given standard food increase during the research, whereas the animals given functional food during 4 weeks reduce 9,9 mg/dl. The description of the triglyceride levels of the experimental animals is shown in Table 5.

Table 4. The Mean of the LDL Level Before and After Treatment

Treatment Group	Pre-Treatment LDL (mg/dl)	Post-Treatment LDL (mg/dl)	LDL Difference (mg/dl)
K-1 (Normal, Healthy)	59,6±2,9	59,4±5,7	-2,0±7,3

K-2 (DM type 2)	89,0±2,5	108,6±9,4	19,6±11,3
P-3 (Functional Food)	88,5±6,2	78,6±4,7	-9,9±5,7

The statistical analysis shows that a significant difference occurs in LDL level after getting treatment (p=0,000) between the DM experimental animals given standard food and the DM experimental animals given functional food. However, the difference of the LDL levels before and after getting treatment in the DM experimental animals given functional food is not significant (p=0,319).

CONCLUSION

The formula of functional food made of watercress and black rice bran in accordance with the same composition ratio between both materials is able to reduce the blood glucose level, total cholesterol level, triglyceride level and LDL level of the DM experimental animals. The real effect is also shown by the increase of the HDL levels of the DM experimental animals after getting functional food.

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