Banana Peels Extract (Musa Paradisiaca Var Kepok) Decrease MDA in New Zealand White Rabbit with DM Hyperlipidemia

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Banana peels extract (Musa Paradisiaca Var Kepok) Decreased MDA in New Zealand White Rabbit With DM Hyperlipidemia

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Abstract. The incidence of Diabetes Mellitus (DM) is increasing over the year. The drugs of DM cause side effects on an organ and heart. The pathological process of DM hyperlipidemia begins with oxidative stress. It is necessary to think about preventing hyperlipidemia in DM by using herbs as herbal medicines. This study aims to find out the effect of peel extracts of Musa Paradisiaca var. kepok to New Zealand White rabbits with hyperlipidemic in DM. The banana peel extract was obtained from the traditional market in Demak Central Java. Banana peel extraction used maceration with ethanol extract. This study used 27 New Zealand White male rabbits, aged 4 months, with 1.5-2 kg average weights. The sample of the study was divided into three groups. Treatment group 1 (P1), Treatment group 2 (P2) and control group (K). All groups had standard feed and High Fructose Fat Diet (HFFD). Group P1 was given banana peel extract dose of 200 mg/kg body weight/day and group P2was given banana peel extract dose of 400 mg/kg body weight/day and group K was not given banana peel extract. This was an experimental laboratory research with the randomized pretest-posttest control design. The research data were taken three times; pretest, day 45 and day 104. This research was conducted for 104 days at UGM LPPT of unit 4 which has been internationally standardized. MDA levels were measured by the HPLC. The results of the pretest levels of MDA 0, 627, day 45 p= 0.232, day 104 p= 0.028. Ethanol extract of Musa Paradisiaca var. kapok prevents oxidative stress of New Zealand White rabbits with Diabetic Hyperlipidemia.

Keywords: *MDA, oxidative stress, banana peel*

1. Introduction

Diabetes mellitus (DM) is a disease that begins with the process of oxidative stress mechanisms. Oxidative stress is characterized by an imbalance between oxidants and antioxidants in the body. The appearance of oxidative stress in DM occurs through three mechanisms; nonenzymatic glycation in proteins, pathways of sorbitol polyols (aldose reductase), and glucose auto oxidation. The changes in oxidative status are characterized by changes in endogenous antioxidant activity and increased oxidative biomolecular damage.[1, 2] The oxidative stress in DM increases the yield of glycosidation and liposidation in plasma and protein tissue.[3] Diets high in fructose and high in fat (HFFD) can cause oxidative stress in β cells, this also occurs in DM patients.[3-5]

The displacement of the balance of the redox reaction, which is caused by the changes in carbohydrate and lipid metabolism, will increase the formation of ROS from the lipids reaction to lipid oxidation.[4, 6] Therefore, it reduces the antioxidant defense system including GSH. Hyperglycemia will worsen and aggravate the formation of ROS through several mechanisms. ROS will increase the formation of Tumor necrosis factor-α (TNF-α) expression and exacerbate oxidative stress. TNF-α can cause varieties effects such as the insulin resistance by decreasing autophosphorylation (auto-phosphorylation) from insulin receptors, changes in substrate 1 insulin receptors into inhibitors of insulin receptor tyrosine kinase activity, decreased insulin-sensitive glucosetransporters (GLUT-4), increased circulation of fatty acids, altered function β cells, increase triglyceride levels and reduce HDL levels.[7] The results showed that the injection of TNF in healthy test animals would reduce insulin sensitivity caused by hyperglycemia without a decrease in plasma insulin levels. Oxidative stress in people with DM will increase the formation of ROS in the mitochondria which will lead to various complications.[6]

Oxidative stress in DM is characterized by an imbalance between oxidants and antioxidants in the body.[7] Oxidative stress in this research is measured using Malondialdehyde (MDA). Free radicals have a very short half-life that is difficult to measure in a laboratory. Lipid tissue damage due to ROS can be examined using MDA compounds.[7] MDA is a compound produced by lipid peroxidation formed from lipid peroxidation in cell membranes, namely free radical reaction (hydroxy radical) with Poly Unsaturated Fatty Acid (PUFA). MDA elimination from the circulation with the help of aldehyde dehydrogenase and thiokinase enzymes occurs within 2 hours in mice. However, it is 10-30% for semi-permanently attached to the protein and eliminated within 12 hours.[7-9]

Utilization of banana peel which has only been considered as useless waste has shifted the potential to be a highly beneficial herbal medicine extract.[10-13] The aim of this research was to examine the effect of Musa Paradisiaca Var Kepok banana peel extract on oxidative stress of hyperlipidemic DM rabbits. The specific objective of this research is to prove that Musa Paradisiaca Var Kepok banana peel extract could reduce MDA levels in hyperlipidemic DM rabbits. The primacy of this research can improve health status and prevent oxidative stress caused by diabetes hyperlipidemia in order to reduce health costs.

2. Materials and Methods

This research has received a certificate from the animal ethic study of the health maintenance ethics commission at the medical faculty at Diponegoro University/ DR. Kariadi Hospital at Semarang with no 76 / EC / HIFK-RSDK / X / 2017.

2.1. Materials:

2.1.1. Extraction: One of the most popular banana varieties in Indonesia, namely Kepok (Musa paradissiaca var kepok) banana were obtained from the wet markets around Demak central java, Indonesia. All kits of the biological parameters are purchased from Sigma Chemical Company, Egypt.

2.1.2. Rabbit feed: “Champion Rabbit Feed” pellet feed by a composition of 12% moisture content, 18% protein, 5% fat content, 14% crude fiber content, 12% ash content, 0.8-1% calcium and phosphorus 0.6-0, 8%. Feed High Fructose Fat Diet (HFFD) is 150 g per day (75 g each administration) and drink is carried out in ad libitum for all rabbits and given daily during the study.

2.1.3. Preparation of banana peel flour and extraction

Sample collection: The sample used is the Musa Paradisiaca Var Kepok banana peel obtained from a traditional market in Demak district, Central Java, Indonesia.

Preparation of banana peel flour and extraction: One of the most popular banana varieties in Indonesia, namely Kepok (*Musa paradissiaca* var Kepok) banana were obtained from the wet markets around Demak Central Java, Indonesia. Banana peel used is 100 days old with dark green peel characteristics, bright yellow flesh color and removing meat fibers. The extraction process uses the maceration method with ethanol as its solvent. Making banana peel extract, proximate and antioxidant examination were carried out at LPPT unit 1 UGM with international standard ISO 9001-9002

2.1.4. Estimation of moisture, fat, protein, dietary fibre and total ash.

The extract peel sample is analyzed for proximate composition. Percentages of moisture by vacuum oven, ethanol extractives, protein by Kjeldahl nitrogen and ash by direct analysis were determined according to the Association of Official as timed by enzymatic gravimetric method. Total carbohydrates were calculated by difference from the other components from 100.[10, 14, 15]

2.1.5. Preparation of sample extracts in solvents.

For estimated of antioxidant composition, samples is extracted with ethanol. All analysis is carried out in freshly collected extracts peel.

2.1.6. Preparation of experimental animals

Experimental animal care and intervention are carried out in the Experimental Animal Development Unit, Gadjah Mada University, Yogyakarta. The maintenance begins from the period of adaptation, selection, treatment period takes place and completion of the examination of research variables within 3 months.[16] Clinical chemistry examination is carried out at the Integrated Research and Testing Laboratory (LPPT).

2.1.7. Sample

The research sample is a New Zealand White rabbit. Specific populations are male NZW rabbits aged 4, 1.5 to 2.5 kg weight from Tapos Bogor Animal Research Institute. The determination of the number of samples is carried out using Frederer and the laboratory experimental formula of WHO 1993. A total of 27 New Zealand White rabbits are divided into 3 groups, 2 treatment groups and 1 control group so that the minimum number of samples for each group was 9. Thus, the total number of New Zealand White rabbits used for this research is 27 animals.

2.1.8. The Procedure of Research

The control group was given pellet feed and the HFFD diet without being given banana peel extract. The research data was taken during the pre test, day 45 and day 104. MDA examination is carried out by the HPLC method. Data analysis uses Ancova test with control of hyperlipidemia DM markers.

Champion Rabbit Feed pellet uses a composition of 12% moisture content, 18% protein, 5% fat content, 14% crude fiber content, 12% ash content, 0.8-1% calcium and phosphorus 0.6-0.8%, Feeding 150 g of HFFD in a day (75 g each) and drinking is carried out in ad libitum for all rabbits and given daily during the research.[9] The treatment group is given HFFD feed and Musa Pradisiaca Var.kepok peel extract which is diluted using a solution of 0.5% NaCMC Aquabidestilata. The dose of each P1 and P2 is 200 mg/kg and 400 mg/kg for 90 days is given per sonde. The control group is given pellet feed and the HFFD diet without being given banana peel extract. The research data was taken during the pre test, day 45 and day 104. MDA examination is carried out by the HPLC method. Data analysis using ANCOVA test with control of hyperlipidemia DM markers.

3. Result and Discussion

The results of this study indicated that all groups had trends or tendencies to gain weight. The first day of the study, the rabbit's body weight was relatively similar of 1547.80 ±17.35 grams. The average body weight of the 30th day in all groups was relatively the same, amounting to 1793.92±34.47 grams and an increase of 246.11 grams. The average body weight on the 60th day in all groups was relatively the same, which was 2831.77± 31.91 grams with an increase in body weight of 1037.85 grams. The mean body weight on the 90th day in all groups was relatively the same, which was 3207.48 ±45.91 grams with an increase in body weight 375.706 grams. Based on table 1, rabbit body weight increases due to feeding and HFFD.

Table 1 Mean of NZW rabbit's body weight (gram)

no	days	P1	P2	K
1	1	1556,20±20,12	1540,10±18,12	1547,11±13,81
2	30	2707,56±30,13	2571,77±33,81	2458.22±39,46
3	60	2922,22± 39,08	2792,222±23,95	2780,889±32,71
4	90	3289,56±48,96	3207,778±37,98	3125,111±50,80

The results of this research indicate that the pre-test of MDA in P1 is 147.08 U / L for minimum and 313.66 U / L for maximum, while P2 is 60.50 U / L for minimum and 313.09 U / L maximum. The result is gained through the normal data distribution in which p = 0.304. The MDA in 45th day for P1 is 53.30 U / L as minimum and 213.17 U / L as maximum, while P2 is 45.01 U / L for minimum and 162.89 U / L for maximum. The result is gained by the normal data distribution win which p = 0.922. Furthermore, the MDA in 104 days or post-test for P1 is 26.74 U / L as minimum and 156.83 U / L as maximum, while the result of P2 is 49.07 U / L for minimum and 151.51 U / L as the minimum by normal data distribution as p = 0.399.

Based on Table 1, there is increasing value in mean for MDA in all groups on day 45. It also shows that there is a decreasing value on day 104 but the control group (K) is higher than P1 and P2. The results of the ANCOVA test shows day 45 MDA p = 0.232, day 104 p = 0.028. Banana peel extract reduces MDA in P1 and P2 on day 104. The P1 has the lowest MDA mean of the other groups. By dosing 200mg/kg, the extract of banana peel can decrease more MDA than P2 in NZW rabit that suffer hyperlipidemic diabetes.

Table 2. The mean of Malondialdehyde (MDA) (U/L)

	P1	P2	K	*P value*
Pre test	108,9983+_47,54	105,1598+_52,41	66,3949+_23,78	0,627[1]
Day 45	111,8878+_46,90	103,2830+_35,61	76,0317+_33,48	0,232[1]
Day 104 (post tes)	81,5783+_48,08	87,5837+_34,39	101,2879+_35,76	0,028[1]

P[1 uji Ancova

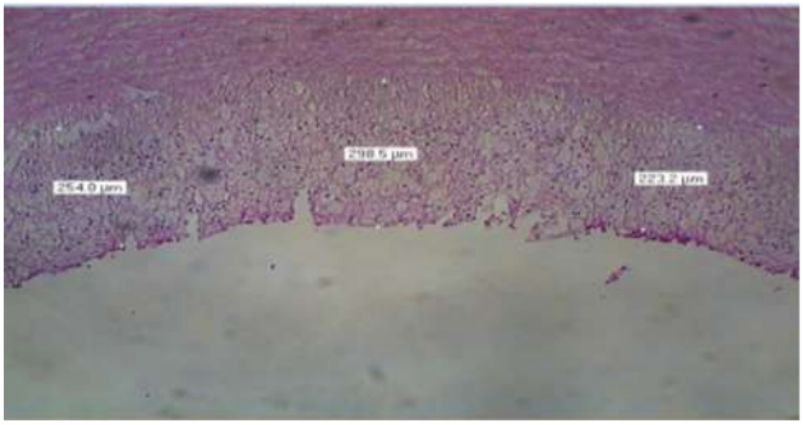
p <0,05 significant

High Fructose Fat Diet (HFFD) can cause oxidative stress in pancreatic β cells. It also happens for those who suffer DM.[1, 9] Oxidative stress causes lipid tissue damage due to ROS. The sign of oxidative stress is measured using MDA markers. Malondialdehyde (MDA) is a compound produced by lipid peroxidation formed from lipid peroxidation in cell membranes, namely free radical (hydroxyl radical) reaction with Poly Unsaturated Fatty Acid (PUFA).[6] This research has shown an increase value in MDA levels for the control group on day 45 and day 104, which proves that HFFD

causes oxidative stress in pancreatic β cells. Increased markers of oxidative stress, namely MDA levels, cause varieties of effects. Those are an increase value in GDP levels, an increase in HomaIR levels, and a decrease value in insulin levels for the control group.

The results of this research prove that the transfer theory of balance redox reactions toward changes in carbohydrate and lipid metabolism cause the increasing of ROS formation from glycation reactions and lipid oxidation.[1] From that result, it reduces the antioxidant defense system including GSH. Hyperglycemia will worsen and aggravate the formation of ROS through several mechanisms. TNF α can cause insulin retention through a decrease in autophosphorylation from insulin receptors. The increasing of TG levels and reduce HDL levels.[2] This research proves that the HFFD diet as a source of oxidative stress can increase MDA levels.[6, 9] This process will be accompanied by an increase in hyperlipidemic DM markers in the control group. However, P1 and P2 can decrease MDA and hyperlipidemia DM markers. That process happens because P1 and P2 get the intake of peel extraction of Musa Paradisiaca Var. Kepok as an antioxidant

Increasing MDA levels as a marker of oxidative stress has proven a lipid tissue damage, which is caused by ROS, can be examined using MDA compounds which are the result of lipid peroxidation. Malondialdehyde is the most common among reactive *aldehyde* derived from lipid peroxidation. It is significantly increasing in the blood and peripheral mononuclear cells. Aldehyde is the cause of the cytotoxic release process, then it interacts and induces oxidative stress in cells and LDL molecules. Moreover, it increases the risk of cardiovascular damage.[2, 6] The HFFD diet induces oxidative stress, activates SOD and tissue in the liver and decreases CAT activity and GSH in the serum. The lipid peroxidation produces MDA. The mean MDA in the control group is seen to be higher compared to the treatment groups. The provision of banana peel extract reduces MDA levels. The results of this research are in accordance with similar studies, namely the research of Ambon banana peel extract and kepok banana peel having an influence on the total cholesterol level of male white rats, Sprague Dawley strain. Giving kepok bananas for 21 days at a dose of 9 g / 200 mg/day can make the MDA levels of the metabolic pre-syndrome lower. According to the increase of SOD in plasma, it also decreases free radicals including MDA levels. Polyphenols reduce the absorption of MDA in atherosclerosis. Because absorption is incomplete, flavonoids work in the large intestine through metabolism and release it with feces.[12, 17, 18]



Picture 1 Aorta histology of control group

The control group's histology showed a thickening of the aortic intima tunica caused by oxidative stress. Oxidative stress occurs due to the provision of diet High Fructose Fat Diet (HFFD).

4. Conclusion

The peel extract of Musa paradisiaca var. kepok works to reduce MDA for hyperlipidemia New Zealand White Diabetes rabbit.

5. Acknowledgment

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