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The use of Kepok Banana Stem (*Musa paradisiaca*) in diabetes rats does not reduce Malondialdehyde (MDA) levels

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ABSTRACT

Introduction: The prevalence of diabetes mellitus (DM) is still high and is expected to continue to increase. An increase follows this increase in the number of side events, so innovation is needed in DM therapy. DM induces oxidative stress so antioxidants can be an alternative therapy for DM. One source of antioxidants whose use is still limited is the banana plant, especially the banana stem. This study aimed to determine the effectiveness of kepok banana stem extract (*Musa paradisiaca* Var. Balbisiana colla) on MDA levels in diabetic rats.

Methodology/Approach: The study used a post-test-only control group design on 24 male rats (*Rattus novergicus*) Sprague Dawley strain with a bodyweight of 150-250 grams and ± eight weeks old. Rats were divided into four groups, namely the metformin treatment for positive control, distilled water for the negative control, and stem banana extract dose of 200 mg/kg BW and 250 mg/kg BW as treatment group of mice induced diabetes using STZ-NA and given treatment according to the group for 14 days. MDA level measurement using the TBARS method on rat liver. Data were analyzed using the One Way ANOVA comparison test.

Results: MDA levels in the positive control group, negative control, treatment 1 (200 mg), and treatment 2 (250 mg) were 0.9790 + 0.52 mg/dl, 0.7533 + 0.58 mg/dl, 1.2510 + 0.52 mg/dl, 1.5175 + 0.53 mg /dl. There was no statistically significant difference in MDA levels between groups (p=0.182), but there was a decrease in blood glucose levels in the group treatment. The highest decrease in blood glucose levels was in the dose treatment group, 250 mg/kg BW.

Conclusion: The treatment of kepok banana stem extract (Musa paradisiaca Var. Balbisinia colla) for 14 days at 200mg/kg BW and 250mg/kg BW increased malondialdehyde (MDA) levels, although not statistically significant.

Keywords: banana stem, diabetic, malondialdehyde.
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INTRODUCTION

Diabetes mellitus is a condition of metabolic disorder characterized by the emergence of chronic hyperglycemia due to damage to the secretion system and insulin action, so the body has difficulty regulating glucose levels in the blood.[1,2] Chronic hyperglycemia conditions can induce oxidative stress due to the release of free radicals, which then causes an imbalance between pro-oxidants and antioxidants in the body and leads to the development of a disease.[3,4] In addition, free radicals increase the lipid peroxidation process, which produces LOOH as a primary product and malondialdehyde (MDA) as a secondary product. Its levels are a sign of oxidative damage and low antioxidant status in the body.[5-7]

Diabetes mellitus is a disease with a high morbidity rate in the world, with a prevalence of 9.3% of the world's population in 2019 and is expected to continue to increase. The prevalence of diabetes in Indonesia's population aged ≥15 years has increased from 2013 with a prevalence of 1.5% to 2.0% in 2018.[2,8] The increase in prevalence is directly proportional to the increase in the prevalence of the incidence of side effects in anti-diabetic drug therapy, where some drugs cause side effects such as nausea, vomiting, hypoglycemia, dizziness, and tremors, so it is necessary to innovate alternative therapies where the chance of occurrence of side effects is lower. Diabetes mellitus, in the process, induces an imbalance between the production of free radicals and the body's antioxidant mechanism due to hyperglycemia conditions that cause metabolic stress and increase the production of reactive oxygen species (ROS) in the mitochondria.[4,9,10] Oxidative stress can be caused by enzymatic or non-enzymatic, where non-enzymatic contributed by the formation of Advanced Glycation End Products (AGEs) from glucose auto-oxidation, while enzymatic through the Polyol Pathway and Hexosamine Biosynthetic Pathway (HBP) which involves intracellular processes.[11-13]

Oxidative stress in diabetes mellitus can be measured through biomarkers, including Hba1C, fructosamine, malondialdehyde (MDA), levels of important enzymes such as SOD, CAT, GPx, and GSH, as well as an increase in 8-hydroxy-20-deoxyguanosine (8-OHdG).[14-15] MDA is a major biomarker

for lipid damage due to free radicals and oxidative stress. It is formed by destroying polyunsaturated fatty acids (PUFA) in lipid membranes by ROS that appear when the body is under oxidative stress. High levels of MDA in diabetes mellitus indicate the antioxidant status in the body.[16]

Antioxidants are compounds that can inhibit the oxidation process in cells and tissues exposed to free radicals to maintain function, protect pancreatic cells, and reduce the risk of complications in diabetes. Antioxidants are widely found in nature, and one of them is the banana plant, which currently its use is still limited to the fruit and flowers as consumption material. At the same time, the leaves, stems, and roots only become waste. Banana stems have a high glycemic index, are rich in fiber and antioxidants, and have a hypoglycemic effect.[17] This study was conducted to determine the effectiveness of the *Kepok* banana stem (*Musa paradisiaca* Var. Balbisiana colla) against diabetes mellitus therapy by observing malondialdehyde (MDA) levels in diabetes mellitus rats.

METHODS

Study Design

This research was experimental research, with a post-test-only control group design.

Sample selection

The study subjects were 24 white male rats (*Rattus novergicus*) Sprague Dawley strain with a body weight of 150-250 grams and ±8 weeks of age. Rats were divided into four groups: positive control group, negative control group, treatment group dose 1, and treatment group dose 2. The study has been declared ethically feasible by the Research Ethics Commission of the Faculty of Medicine and Health Sciences, University of Muhammadiyah Yogyakarta (FKIK UMY) with letter number 48 /PSK/ Akd.2020.2021/2210201/FKIKUMY.

Assessment

Tools. The tools used in this study include a white rat cage, digital scales Mettler toledo®, sonde, spuit one cc, *Gluco-check* Easytouch®, *gloves*, mask, electric stove Maspion®, Centrifuge EBA 20®, spectrophotometer Jasco V-730®, vortex Thermoscientific®, micropipette Socorex®, tube *Eppendorf*, cuvette, 100 ml tissue pot, measuring flask *pyrex* Iwaki® 100 ml, beaker glass *pyrex* Iwaki®, test tube Iwaki®, minor surgical set, mortar, stamper, spatula, test tube rack and glass tube with lid.

Materials. The materials used in the study included rat feed (A594K pellet), *kapok* banana stem (*Musa paradisiaca* Var. Balbisiana colla), 70% ethanol, streptozotocin (STZ) Bioworld®, nicotinamide (NA) TCI®, metformin, ketamine, distilled water, NaCl 0.9%, phosphate buffer saline (PBS) pH 7.4, citrate buffer 0.1 M, TBA 0.67%, TCA 20%, tetra ethoxy propane (TEP), citrate buffer 0.1 M pH4,5, mouse liver tissue, and fasting blood plasma.

Extract of banana stem. *Kepok* banana stem extract is done by cleaning fresh banana stems, then cut into small pieces and dried in the sun. The dried stems were made into powder with a size of 40 mesh and macerated in 70% ethanol for 24 hours.

Treatment to Rats. Before being given treatment, rats were adapted for seven days, fed A594K pellets, and given water ad libtium twice a day. After the adaptation period, the mice were weighed and divided into four groups by randomization a positive control group, a negative control group, a treatment group at dose 1, and a treatment group at dose 2. The mice were then measured fasting blood glucose (GDP) levels by taking blood samples from a vein. Rats that had been fasted 8-12 hours earlier to determine the initial glucose levels of the rats. Furthermore, rats were induced by nicotinamide (NA) 300 mg/ml NaCl 0.9% intraperitoneally, followed by injection of streptozotocin (STZ) 65 mg/kg BW dissolved in citrate buffer 0.1 pH 4.5 intraperitoneally.[18]

On day 5 post-induction, the rats were checked for fasting blood glucose levels after being fasted for 8-12 hours. The test rats included in the inclusion criteria were rats with diabetes, namely fasting blood glucose levels >135mg/dl. The rats that met the inclusion criteria were then given treatment according to the group, namely group I as a negative control group were fed pellets and aquadest ad libtium, group II as a positive control group, was fed pellets and aquadest ad libtium and metformin 9 mg/100 gr BW/day/rat at 08.00, group III as treatment group 1 was fed pellets and aquadest ad libtium and banana kepok stem extract at a dose of 200 mg/kg BW at 08.00 every day, and finally group IV as treatment group 2 was fed pellets and aquadest ad libtium and kepok banana stem extract at a dose of 250 mg/kgBW at 08.00 every day. The treatment was carried out for 14 days.

Measurement of Malondialdehyde (MDA) Levels in Rat Liver

After the treatment, the rats were rechecked for their blood glucose levels and terminated using ketamine, and their livers were taken. The liver was then cleaned using 0.9% NaCl and stored in tissue pots containing 0.9% NaCl in a freezer at -80°C until testing.[18] Sample preparation was carried out by weighing the liver as much as 400 mg, adding 2 ml of PBS pH 7.4, and ground and homogenizing in an Eppendorf tube. Samples were tested using the TBARS method, where the determination of MDA levels was assessed from the chromatic color that emerged from the TBA-MDA reaction in the sample.[19] In determining the MDA standard, tetra ethoxy propane (TEP) was used, which was diluted 1/80,000 times and poured into test tubes of 30 µL, 50 µL, 70 µL, 90 µL, and 110 µL, then each TEP solution was added until 1 ml using distilled water. Add 0.5 mL of 20% TCA and 1 mL of 0.67% TBA to each tube and shake until homogeneous. Heat the solution in a temperature bath of 95°-100°C for 10 minutes, cool it with running water, and measure its absorbance at 531.5 nm. Make blanks in duplicate.

MDA levels were measured in the sample by taking 1 ml of sample and adding 0.5 ml of 20% TCA, homogenized, and centrifuged at 3000 rpm for 10 minutes. The supernatant sample was taken and added to 1 ml of 0.67% TBA, then heated at a temperature of 95°-100°C for 10 minutes, then cooled with running water. Read the sample absorbance at a wavelength of 531.5 nm and calculate using standard curves.

Statistical Analysis

Data were analyzed using the One Way ANOVA comparison test on differences

in MDA levels of each treatment group to determine the significance and effectiveness of the banana stem extract on MDA levels, followed by a posthoc test and Tuckey test.

RESULTS

Table 1 shows the average blood glucose before and before the treatment. There is a decrease in blood glucose levels. After treatment, the highest reduction was in the treatment group at dose 2 (extract banana stem 250 mg/kg BW), which equals 68.80 ± 135.40 mg/dl. This decrease was greater than the group treatment dose 1 (banana stem extract 200 mg/kg BW).

Table 2 shows the average measurements and calculations of MDA levels in rat livers where the difference in MDA levels between treatment groups was not statistically significant with a p-value = 0.182 (p>0.05).

Table 1. Average GDP Levels and Average Decreased Before and After Treatment.

Groups	Bood glucose (mg/dL) Mean+SD		
	Before treatment	After treatment	Average deceasing
Control (-)	106.17± 11.78	89.17±3.43	17.00
Control (+)	164.75±82.40	123±47.25	41.75
Treatment 200 mg	151.00±97.16	142.17±17.92	8.83
Treatment 250 mg	162.20±38.25	93.4±9.83	68.8

Table 2. MDA levels.

Group	MDA Mean+ SD (mg/dl)	*p*
Positive control	0.9790 ± 0.52	
Negative control	0.7533 ± 0.58	0.182
Treatment 1 (200 mg)	1.2510 ± 0.52	
Treatment 2 (250 mg)	1.5175 ± 0.53	

DISCUSSION

This study showed a statistically significant decrease in blood glucose levels in the treatment group. Several studies have also shown a hypoglycemic effect from administering banana stem extract.[19,20] This study's positive control was metformin, the first-line therapy for type 2 diabetes, administered to patients with normal renal function. Metformin will block cellular respiration, which causes a decrease in ATP and an increase in AMP, resulting in AMP-activated protein kinase (AMPK), then decreases the body's metabolic function so that glucose intake from the blood increases, there is an increase in glycolysis, beta-oxidation of fatty acids, and increases GLUT and glucose utilization blood. This is in accordance with Table 1, wherein in the positive control group, there is a decrease in the average blood glucose levels of rats.

Banana stems have hypoglycemic potential due to a synergistic effect on the compounds contained in banana stems, such as saponins, tannins, flavonoids, and polyphenols, so they can reduce the production of AGEs formed in diabetes mellitus conditions. In addition, antioxidants can improve the proliferation of beta-pancreatic cells, assist insulin secretion, reduce apoptosis, and improve hyperglycemic conditions by regulating glucose metabolism in the liver.[12,19,21,22]

Besides having hypoglycemic potential, the banana stem also contains various antioxidants such as SOD, vitamins, and polyphenolic compounds that can reduce the effects of oxidative stress. In dealing with free radicals, antioxidants are divided into four lines: the first line is enzymatic antioxidants such as SOD, CAT, and GPx, which suppress the production of free radicals. The second line is non-enzymatic antioxidants and comes from nutrients such as vitamin C, tocopherol, and uric acid, which bind radical molecules. Free radicals so that the initiation process is inhibited, the third line is DNA repair enzymes that repair DNA damage caused by free radicals, and the fourth line helps the body's antioxidant adaptation in the face of free radicals.[23-25]

The condition of diabetes mellitus can trigger oxidative stress through glucose auto-oxidation, which produces OH- and the reaction between protein and glucose. This protein and glucose reaction will stimulate the formation of AGEs and free radicals, which then damage the lipid membrane through the mechanism of attracting hydrogen atoms to PUFA bonds in the cell membrane so that the cell becomes damaged (peroxidation) and produces a by-product in the form of malondialdehyde (MDA).[4,5,26]

This study was shown that the MDA levels in rat liver difference in MDA levels between treatment groups were not statistically significant. This is in contrast to previous studies where the administration of banana stem extract reduced MDA levels in diabetic rats. This insignificant difference in MDA levels can be caused by several factors, such as variations in levels and types of antioxidants that affect the binding of ROS or other free radicals or changes in the structure of these antioxidants so that the potential for binding to free radicals is reduced and their ability to reduce MDA is weakened.[27] In addition, the length of treatment given to research subjects can also affect the work results of antioxidant therapy given to the subject. This study showed no significant differences in MDA levels between treatment groups. However, there was a decrease in blood glucose levels in all treatment groups, with the greatest decrease in treatment group 2, which was given 250 mg/kg BW. The insignificant decrease in MDA can be caused by variations in levels and types of antioxidants, especially in their chemical structure, resulting in the binding of free radicals or ROS so that they can reduce MDA levels or changes in antioxidant structure. Thereby reducing the free radical scavenging potential. The decrease in MDA levels was also less significant. This may be due to the effect of treatment duration. A similar study showed that banana stems could reduce MDA levels after 16 weeks of treatment.[28]

CONCLUSION

Based on the results and discussion of the study, it was concluded that the administration of kepok banana stem extract (Musa paradisiaca Var. Balbisinia colla) for 14 days at a dose of 200 mg/kg BW and 250 mg/kg BW did not affect malondialdehyde (MDA) levels, but it could reduce the GDP levels of rats by decreasing the highest dose of 250 mg/kg BW is 68.80 ± 135.40 mg/dl. Further research on the potential of each active compound from banana stems is needed. This is important to help with the treatment and management of diabetes.

CONFLICT OF INTEREST

We declare no conflict of interest.

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ETHICS APPROVAL

The study has been declared ethically feasible by the Research Ethics Commission of the Faculty of Medicine and Health Sciences, University of Muhammadiyah Yogyakarta (FKIK UMY) with letter number 48 /PSK/Akd.2020.2021/2210201/FKIKUMY.

AUTHOR CONTRIBUTION

All authors contributed to the research and writing of this manuscript.

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