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The Effect of Hydroethanol Extract of Ambon Banana peel (*Musa paradisiaca* L.) on reduction blood glucose levels in type 2 Diabetes Mellitus rats

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ABSTRACT

Background: Diabetes mellitus is a metabolic disorder caused by the pancreas not being able to produce enough of the hormone insulin, insulin action or both. The current treatment of diabetes mellitus with oral anti-hyperglycemic drugs and long-term insulin is feared to worsen the patient's condition so that alternative treatments using herbal ingredients such as Ambon banana peel are needed.

Aim: To determine the effect of hydroethanol extract from Ambon banana peel (*Musa paradisiaca* L.) on reduction of blood glucose levels in streptozotocin-induced type 2 diabetes mellitus rats.

Methods: An experimental research pre-test and post-test control group design using 24 male Wistar rats, which were divided randomly into 4 groups. Negative control, positive control, hydroethanol extract of Ambon banana peel dose 400 mg / KgBB, hydroethanol extract of Ambon banana peel dose 800 mg / KgBB treatment for 21 days (po). On the 36th day, blood was drawn through the orbital plexus to test blood glucose levels.

Results: It was showed that the administration of hydroethanol extract of Ambon banana peel (*Musa paradisiaca* L.) at a dose of 400 mg / kgBB was the optimal dose to reduce blood glucose levels in rats with type 2 diabetes mellitus ($p = 0.032$).

Conclusion: Giving hydroethanol extract of Ambon banana peel at a dose of 400 mg / KgBW in rats induced by streptozotocin can significantly reduce blood glucose levels.

Keywords: Ambon banana peel, blood glucose, hydroethanol extract, *Musa paradisiaca* L.

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder caused by the pancreas not being able to produce enough of the hormone insulin (insulin secretion disorder), insulin action or both^{1,2}. The characteristics of DM are an increase in blood glucose levels (hyperglycemia). There are 2 types of diabetes mellitus, namely diabetes mellitus type 1 and 2. Diabetes mellitus type 1 is diabetes characterized by a lack of insulin production. Diabetes mellitus type 2 is a type of diabetes caused by insulin resistance accompanied by insulin deficiency¹.

Diabetes mellitus has become a serious threat to global health, 2016 WHO data shows 70% of the world's total deaths and more than half of the disease burden are caused by DM. Indonesia is the 6th country in the world after China, India, the United States, Brazil and Mexico with around 10.3 million people with diabetes aged 20-79 years. The International Diabetes Federation (IDF) Atlas 2017 reports an increase in the epidemiology of DM in Indonesia, around 95% of the number of cases are type 2 diabetes. Riskesdas data shows a significant increase in prevalence, from 6.9% in 2013 to 8.5% in year 2018³.

The management of DM begins with implementing a healthy lifestyle such as a dietary diet and physical activity as well as pharmacological therapy in the form of oral antidiabetic drugs and / or injections. The workings of oral anti-hyperglycemic drugs used today in the form of hyper insulin secretion, improve insulin sensitivity, inhibiting the absorption of glucose in the digestive tract, an inhibitor of

dipeptidyl peptidase IV inhibitor of sodium glucose cotransporter^{2,4}.

Regardless from pharmacological therapy options currently available, many herbal medicine have been recommended for the treatment of diabetes. Herbal medicines are widely prescribed because of their effectiveness, fewer side effects and relatively low cost.⁵ Some traditional herbal medicines that have been researched and used for the treatment of diabetes include Brotowali (*Tinospora crispa*) with the results of *Borapetoside A* in Brotowali which have a hypoglycemic effect, which can increase glycogen content and reduce plasma glucose levels⁶. *Cinnamaldehyde* in cinnamon (*Cinnamomum burmannii*) shows a gluco lipid-lowering effect in diabetic animals by increasing glucose absorption and increasing insulin sensitivity⁷. Bay leaf extract (*Syzygium polyanthum*) was able to reduce fasting blood glucose and postprandial glucose levels⁸.

Research on herbs that are useful in reducing blood glucose levels is growing, one of which is the use of bananas as an alternative to traditional medicine in reducing blood glucose levels. In Ariani & Linawati's research (2017) states that giving Ambon banana fruit juice can reduce blood glucose levels of male Wistar rats which in Ambon banana (*Musa paradisiaca* L.) contains flavonoids and tannins which are hypoglycemic by increasing glycogenesis⁹. Ambon banana peel (*Musa paradisiaca* L.) can be used to lower blood glucose levels. Imam *et al.* (2011) reported that Ambon banana peel has a hypoglycemic effect because it stimulates insulin and

glucose utilization¹⁰. Banana peels contain higher antioxidant activity than the fruit. Fitrianiingsih *et al.* (2012) reported the types of antioxidant compounds that can be isolated from banana skin, namely flavonoids¹¹.

In the current extract preparation, water is used as the main solvent. Apart from water, alcohol (ethanol) is a solvent that is often used in most extracts. Alcohol, together with water, forms a mixture of hydroalcohol (water and ethanol) which dissolves alcohol-soluble and water-soluble substances, this is a very useful property in the extraction of active ingredients from crude pharmaceuticals. Hydroalcohol mixtures are perhaps the most flexible of the solvents. In a mixture of these two agents, the active elements can be selectively dissolved and extracted or left to remain, according to their solubility characteristics. The flexible combination of these two agents forms a solvent mixture that is most suitable for the extraction of the active elements from a particular substance¹².

From the background presented, research on Ambon banana peel extract has been carried out, but the extract used is in the form of water extract from Ambon banana peel. Research on banana peel extract with hydroethanol solvent has never been carried out to the best of the researcher's knowledge, therefore researchers are interested in conducting research on the effect of hydroethanol extract from Ambon banana peel on reducing blood glucose levels in type 2 diabetes mellitus rats.

MATERIALS AND METHODS

Experimental research was conducted in Pharmacy Laboratory Wahid Hasyim University for the manufacture of extracts and Integrated Research Laboratory Unit IV Gadjah Mada University from adaptation of experimental animals to examination of blood samples. The study was conducted from July 2019 - February 2020. The study used a sample of 24 male Wistar rats with a body weight of 180-200 grams which were divided into 4 groups, each consisting of 6 rats. All experimental animals after an adaptation period of 7 days were given streptozotocin

(STZ) at a dose of 40mg / Kg, then the negative control group (K1) received standard feed and drink in the form of BR-II, the positive control group (K2) received metformin 500mg / KgBW, treatment group 1 (P1) obtained 400mg / KgBB of Ambon banana peel extract and treatment group 2 (P2) obtained 800mg/ KgBB of Ambon banana peel extract. All experimental animals were given standard BR-II feed *ad libitum* and given Ambon banana peel extract 3 times a day. Laboratory examinations were carried out on the 14th day after adaptation and induction of STZ, all groups of rats were blood drawn on the retroorbital plexus to check their blood glucose levels before treatment (*pre-test*). On day 35, all groups of rats were blood drawn on the retroorbital plexus to see blood glucose levels in rats that were not given Ambon banana peel extract and those given Ambon banana peel extract (*post-test*). Measurement of blood glucose levels was carried out using the method *Glucose Oxidase – Aminoantipyrine Peroxidase* (GOD-PAP) using spectrophotometry which was read at a wavelength of 546 nm. The research has been approved by the Medical and Health Research Ethics Commission (KEPK) of the Faculty of Medicine, Diponegoro University.

RESULT

The results of the examination of blood glucose levels of rats before and after giving Ambon banana peel extract could be shown in Table 1 and Figure 1.

The results of One Way Anovabetween blood glucose level *pretest*, *post test* and differences in blood glucose level showed that there were significant differences as shown in Table 2.

Post-Hoc test showed significant differences between research groups could be seen in Table 3 and Table 4. The difference between the positive control group and the Ambon banana peel extract at a dose 400 mg / kgBW and Ambon banana peel extract at a dose of 800 mg / kgBW did not show a significant difference (Table 5.), it meant that the Ambon banana peel extract at a dose of 400 mg / kg and 800 mg / kgBB had the same effect. Table 6 showed that Ambon banana peel extract at a dose of 400 mg / kgBW could reduce blood glucose level optimally.

Table 1. The mean of blood glucose levels pre-test and post-test

Variable	Group			
	Negative Control Mean±SD	Positive Control Mean±SD	Ambon Banana Peel Extract Dose 400 mg / kgBW Mean±SD	Ambon Banana Peel Extract Dose 800 mg / kgBW Mean±SD
Blood glucose level (pre-test)	278.26±51.96	391.25±74.36	407.93±41.53	426.35±56.42
Levels of blood glucose (post-test)	343.83±50.02	282.78±47.78	276.95±43.32	296.76±25.42
Δblood glucose level	+65.56	-109.13	-130.98	-129.58

Table 2. One Way Anova research group

Variable	Group				Sig.
	Negative Control Mean±SD	Positive Control Mean±SD	Ambon Banana Peel Extract Dose 400 mg / kgBW Mean±SD	Ambon Banana Peel Extract Dose 800 mg / kgBW Mean±SD	
Blood glucose level (pre-test)	27.26±51.96	391.25±74.36	407.93±41.53	426.35±56.42	0.001*
Blood glucose levels (post-test)	343.83±50.02	282.78±47.78	276.95±43.32	296.76±25.42	0.040*
Δblood glucose levels	+65.56	-109.13	-130.98	-129.58	0.003*

Note: * Significant (p <0.05)

Table 3. The result of Post-Hoc test blood glucose levels pre-test

	Negative Control	Positive Control	Ambon Banana Peel Extract Dose 400 mg / kgBW	Ambon Banana Peel Extract Dose 800 mg / kgBW
Negative Control	-	0.003*	0.001*	0.000*
Positive Control	0.003	-	0.634	0.310
Ambon Banana Peel Extract Dose 400mg/kgBW	*0.001*	0.634	-	0.584
Ambon Banana Peel Extract Dose 800mg/kgBW	0.000*	0.310	0.584	-

Note: * Significant (p <0.05)

Table 4: The result of Post-Hoc test blood glucose levels post-test

	Negative Control	Positive Control	Ambon Banana Peel Extract Dose 400 mg / kgBW	Ambon Banana Peel Extract Dose 800 mg / kgBW
Negative Control	-	0.017*	0.010*	0.059
Positive Control	0.017*	-	0.806	0.558
Ambon Banana Peel Extract Dose 400mg/kgBW	0.010*	0.806	-	0.409
Ambon Banana Peel Extract Dose 800mg/kgBW	0.059	0.558	0.409	-

Note: * Significant (p <0.05)

Table 5: The results of the Post-Hoc test on the difference between the pre-test and post-test blood glucose levels

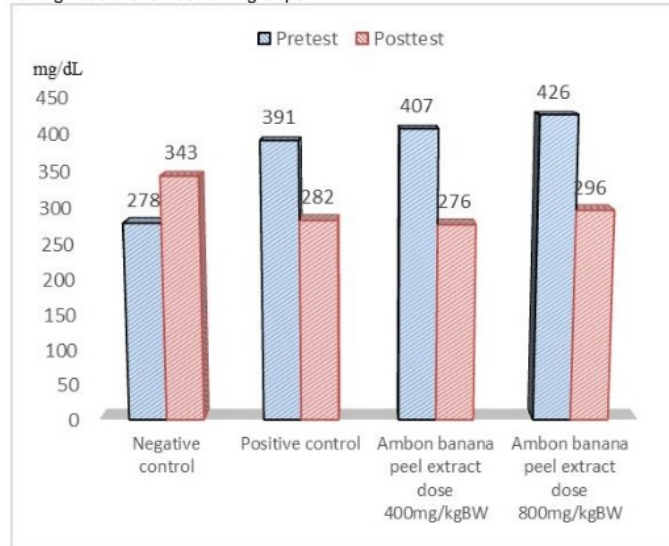
	Negative Control	Positive Control	Ambon Banana Peel Extract Dose 400 mg / kgBW	Ambon Banana Peel Extract Dose 800 mg / kgBW
Negative Control	-	0.019*	0.001*	0.001*
Positive Control	0.019*	-	0.215	0.244
Ambon Banana Peel Extract Dose 400mg/kgBW	0.001*	0.215	-	0.935
Ambon Banana Peel Extract Dose 800mg/kgBW	0.001	0.244	0.935	-

Note: * Significant (p <0.05)

Table 6: Paired t-test results of pre-test and post-test blood glucose levels

Group	Pre-test Post-test	p	Significance of
Negative Control		0.000	Significant
Positive Control		0.002	Significant
Ambon Banana Peel Extract Dose 400 mg / kgBW		0.032	Significant
Ambon Banana Peel Extract Dose 800 mg / kgBW		0.191	Not Significant

Figure 1. Graph the average blood glucose level research groups



DISCUSSION

Diabetes mellitus is a serious chronic disease occurs when the pancreas does not produce enough insulin or when the body cannot use insulin effectively. Diabetes mellitus is a condition where the concentration of glucose in the blood is above normal or is known as hyperglycemia.¹³ This study tested the activity of the hydroethanol extract of Ambon banana peel (*Musa paradisiaca L.*) on reducing blood glucose levels in type 2 diabetes mellitus rats. The hydroethanol extract of Ambon banana peel was obtained bypercolation extraction process using 70% ethanol solvent. The choice of 70% ethanol as a solvent is because based on 70% ethanol it can attract both polar and non-polar compounds such as alkaloids, flavonoids, saponins, tannins and steroids.¹⁴ Percolation is considered as an extraction method in this study because the advantage of this method is that the process of taking nutritious substances in simplicia is more perfect¹⁵.

This study used 24 male rats with an average weight of 182 grams which were divided into 4 groups, that were negative control, positive control, treatment group 1 and treatment group 2. Before being given STZ induction, the initial blood glucose levels were measured in each group with using a glucometer brand *Autocheck* and the rats were fasted for 12 hours to keep blood glucose levels stable due to the influence of food intake¹⁶.

Measurement of initial blood glucose levels was carried out before giving the treatment as a value of initial blood glucose levels and the measurement results showed that the average blood glucose levels of the rats were 43 ± 6.51 mg / dl, so it was certain that the rats were in normal condition, then given STZ induction. Hyperglycemia induction in rats using STZ at the cellular level can cause failure of glucose oxidation and interfere with insulin biosynthesis and synthesis. The result of STZ induction can cause beta cells to lose response to glucose.¹⁷ Measurement of blood glucose levels was carried out 7 days after STZ induction (*pre-test*) using GOD-PAP spectrophotometry. The measurement results show that giving STZ can increase blood glucose levels (*p-value* = 0.001). A significant increase in blood glucose levels in rats indicates a hyperglycemia state in accordance with Kenneth's 2008 study that rats experienced hyperglycemia when their blood glucose levels were > 200 mg / dl. These results are also in accordance with the research conducted by Eleazu, Kate Chinedum showing that STZ treatment in rats with blood glucose levels > 200 mg / dl after STZ induction characterized by glycosuria is considered a diabetic condition¹⁸.

Hyperglycemic rats were then given treatment according to the group for 21 days. In this study, 4 treatment groups were given, namely positive control, negative control and treatment (extract according to dose). The positive control in this study was metformin, this is necessary to see the effect of oral antidiabetic drugs that have proven their efficacy to reduce blood glucose levels.¹ The test material used in this study was the hydroethanol extract of Ambon banana peel with a dose of 400 mg / kg and 800 mg / kg, giving the test material three times a day orally using a gastric swab for 21 consecutive days and measuring blood glucose levels on day 36th (*post-test*).

The results of the mean blood glucose levels can be seen in Table 1. The measurement data showed a decrease in blood glucose levels in each group. The profile of the mean blood glucose levels of rats in Figure 1 shows that the positive control and treatment showed a decrease in blood glucose levels, while the negative control had an increase so that it can be seen that after treatment, all groups experienced a decrease in blood glucose levels except the negative control group. Based on Table 2 shows varying results because the body resistance of each test animal is different against STZ which causes varied hyperglycemia conditions. Decreased blood glucose levels in the positive control group, namely metformin, which is the first-line therapy in type 2 diabetes mellitus, the main way it works is to reduce hyperglycemia, increase glucose absorption and insulin sensitivity by inhibiting gluconeogenesis¹⁹.

The treatment group with the hydroethanol extract of Ambon banana peel (*Musa paradisiaca L.*) at a dose of 400 mg / kgBW showed a significant decrease in blood glucose levels before and after treatment, but in the treatment group with hydroethanol extract a dose of 800 mg / kgBW showed a decrease insignificant (Table 6). After the paired *t-test* was confirmed that the Ambon banana peel extract at a dose of 800 mg / kgBW between the *pre-test* and *post-test* showed insignificant differences. This means that between treatment and no treatment at the dose of 800 mg / kgBB of ambon banana peel extract does not have an effect in reducing blood glucose levels so that the 400 mg / kgBB dose of Ambon banana peel extract becomes the optimum dose in reducing blood glucose levels which is equivalent in function to positive control (metformin).

The decrease in blood glucose levels is due to the synergistic effect of the bioactive compounds contained, including flavonoids, saponins and tannins. Ambon banana skin has antioxidant activity. Based on the research of Someya (2002) in Peni Sri (2012), the types of antioxidants that can be isolated from banana skin are flavonoids. Flavonoids are phenolic compounds that are owned by many plants. Flavonoids as potential antihyperglycemic agents because flavonoids function as glucosidase inhibitors.²⁰ In addition, flavonoids have a mechanism in the inhibition of phosphodiesterase so that cAMP (*cyclic Adenosine Monophosphate*) in pancreatic beta cells increase.²¹ This will stimulate the release of PKA (Protein Kinase A). Increased levels of cAMP (*cyclic Adenosine Monophosphate*) will cause closure of the K⁺ channel in the plasma membrane of beta cells. This situation will lead to membrane depolarization and the opening of Ca channels thereby accelerating the entry of Ca ions into the cell. The increase in Ca ions in the cytoplasm of beta cells will cause an increase in insulin secretion by pancreatic beta cells^{22,1}.

The hydroethanol extract of Ambon banana peel had a significant effect on reducing blood glucose levels. In this study, it showed that the most effective concentration of hydroethanol extract from Ambon banana peel was not the highest dose, but the dose of 400 mg / kgBW was able to reduce blood glucose levels. Increasing the dose of the drug should increase the response in proportion to the increased dose. However, with increasing doses, the increase in response will eventually decrease because the optimum dose has been reached. This often happens because the components of compounds in natural

medicinal substances are not single but consist of various bioactive compounds that work synergistically to cause effects¹¹.

CONCLUSION

The hidroetanol extract of Ambon banana peel (*Musa paradisiaca L.*) can reduce blood glucose levels in type 2 diabetes mellitus rats induced by streptozotocin. Hydroethanol extract of Ambon banana peel (*Musa paradisiaca L.*) at a dose of 400 mg / kgBW is the optimal dose to reduce blood glucose levels in hyperglycemic rats.

Conflict of interest: No conflict interests

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