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The Effect of Banana Pell (*Musa paradisiaca* L) Ethanol Extract on The Blood Glucose Level of Diabetic Mice with Alloxan Induction

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ABSTRACT

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia due to abnormalities in insulin secretion, insufficient insulin hormones, or the inability to use insulin effectively. One of the traditional medicine use with DM is to control their blood glucose levels using herbal plants, namely the banana peel plant (Musa paradisiaca L.) which is rich in flavonoids, alkaloids, and tannins. This study aims to determine the effect of ethanol extract banana peel EEBP on glucose levels in mice with alloxan induction. The design of this study was a laboratory experiment, using male white mice (Mus musculus L) Balb/c strain, which were induced with alloxan at a dose of 200 mg/kgBW. The mice were divided into six groups, namely normal, negative (CMC Na 0.5%), positive (glibenclamide 5 mg/kgBW), and three groups of EEBP at a dose of 100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW. Blood glucose levels were evaluated on day 3 after induction, as well as day 7 and 14 after treatment. Blood glucose level data were analyzed using one-way ANOVA, followed by LSD post hoc test with 95% confidence level. The 400 mg/kgBW dose of banana peel ethanol extract showed the highest percentage reduction in blood glucose levels, namely 65.83% ± 0.800, compared to other doses. Based on LSD statistical test, doses of 200 mg/kgBW and 400 mg/kgBW of EEBP showed no significant difference compared to the positive control (p>0.05). The optimal dose for reducing blood glucose levels in DM mice induced by alloxan is the treatment of EEBP in doses of 200 mg/kgBW and 400 mg/kgBW.

INTRODUCTION

The number of people with diabetes mellitus in 2019 worldwide reached 463 million (93.3%), according to a report from the International Diabetes Federation (IDF). This number is expected to increase by 51%, to 700 million people (10.9%) by 2045 (IDF, 2019). Indonesia is among the top ten countries in the world with a prevalence of type 2 DM cases at 8.6% of the population (Safitri *et al.*, 2022). In 2021, the East Java Provincial Health Office reported 929,535 diabetes mellitus cases. Of these, 867,257 sufferers, or 93 percent of the

total, received medical treatment (Sutomo *et al.*, 2023).

Hyperglycemia is a symptom of the metabolic disorder known as diabetes mellitus. Causes of hyperglycemia may include abnormal insulin secretion, insufficient insulin hormone, or the inability to use insulin effectively (Idf, 2021). Diabetes mellitus can affect almost all systems of the human body, from the skin to the heart, which can lead to various complications. due to its often unnoticed effects, the disease is often referred to as the "Silent Killer" (Hestiana *Et Al.*, 2017).

Hyperglycemia can trigger glucose autooxidation, protein glycation, and polyol metabolic pathway activity, all of which can accelerate the formation of reactive oxygen species (ROS). These ROS can also damage cells in a chemical process known as lipid peroxidation (Nurmalasari, et al., 2021). Insulin is produced by pancreatic beta cells and functions to lower glucose levels. When blood glucose levels rise too high, insulin will store glucose in the liver (Khatimah et al., 2022).

The use of herbal plants can now be an alternative to control blood glucose levels, as they are considered safer, more affordable, and have lower side effects than synthetic drugs (Herman *et al.*, 2019). Based on observations in humans, herbal plants have been identified and known to contain compounds that can help prevent disease, cure, and perform certain biological functions (Sinambela *et al.*, 2022). One of the plants used is banana (*Musa paradisiaca* L.).

All parts of the banana plant can be utilized from the fruit, leaves to the peel. The banana plant is a versatile plant, which is often consumed as a source of nutrition. One part of the banana that often becomes waste and is not utilized is the peel. Waste banana peel showed antioxidant activity of 95.14% (Panjaitan *et al.*, 2017).

Banana antioxidants are generally higher in ripe banana peel fruits than when unripe (Mentari et al., 2019). Banana peel content functions as a free radical scavenger, namely flavonoids and phenols that can reduce glucose levels (Hasma et al., 2019). Flavonoids reduce oxidative stress and reactive oxygen species (ROS), protect pancreatic beta cells, and increase insulin receptor sensitivity (Panjaitan et al., 2017). In previous studies, research has been conducted with the title of the effect of banana stem extract on blood glucose levels, the similarity of phytochemical content in one plant allows the fruit peel to have similar activities (Wenas et al., 2019). This study aims to determine the effect of ethanol extract banana peel EEBP on glucose levels in mice with alloxan induction.

METHODS

This research utilizes a laboratory experimental study. Tools used included ultrasonic, glucometer (Easy Touch GCU®), glucose strips (Easy Touch GCU®), syringe, sonde needle (Onemed®), injection syringe (Onemed®), rotary evaporator (Intra®), blender (Philips®). analytical (Ohaus®), filter paper, porcelain waterbath, and various other glassware. Materials used included 70% ethanol, alloxan monohydrate (Sigma Aldrich®), CMC Na 0.5% (Merck®), glibenclamide (Renabetic®), and banana peel (Musa paradisiaca L.) simplisia from Gumuk Mas, Jember Region.

Determination of Banana Peel (*Musa paradisiaca* L.) plants was carried out at the Ahmad Dahlan University Biology Laboratory with the number 464/Lab.Bio/B/XII/2023.

Simplisia Preparation and Extraction

Banana peels were cleaned, cut into small pieces (0.5 cm), dried for 48 hours, then dried again in an oven at 50°C until the weight stabilized. The next stage is dry sorting, and smoothing of simplisia, where with the smaller size of simplisia contact and diffusion into particles becomes easier and absorption is optimal (Anggitasari *et al.*, 2023).

Banana peel simplisia powder of 300 g was extracted by the Ultrasonic Assisted Extraction (UAE) method using 3 liters of 70% ethanol solvent, in a ratio of (1:10). The sample was sonicated for 30 minutes at 30°C, the sample was filtered using filter paper. After that, the liquid extract was solidified using a rotational evaporator at 40°C, then evaporated on a waterbath, at 50°C until thick (Widyastutik *et al.*, 2022). The following formula can be used to calculate the percentage yield (Senduk *et al.*, 2020).

$$\%$$
Yield = $\frac{\text{weight of extract}}{\text{weight of raw materials}} \times 100\%$

Phytochemical Screening

The tube method or color test is used to conduct qualitative research on the phytochemistry of EEBP. The aim is to identify the content of secondary metabolite compounds in banana peel, such as alkaloids, flavonoids, saponins, and tannins, by observing the color changes that occur (Nababan *et al.*, 2022).

Animal Preparation

This study received ethical approval from the Universitas dr. Soebandi Jember with number: 116/KEPK/UDS/II/2024. This study involved thirty male mice aged two to three months. For 7 days, the mice were placed in cages at 25°C to adapt.

Antidiabetic Test

A total of 30 mice were kept hydrated after fasting for 16 hours. To ensure their normal condition, glucose levels were measured as To values. Except for the control group induced with normal saline, groups of mice induced all were intraperitoneally with alloxan at a dose of 200 mg/kgBW (Widyasti et al., 2019). After testing glucose levels on the third day, mice were considered diabetic if their glucose levels exceeded 200 mg/dL (Suri et al., 2022). The EEBP was given to mice at a dose of 100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW in the treatment, negative control, and positive control groups. Treatment was given once a day for fourteen days (Table 1). On the seventh and fourteenth days after treatment, glucose levels were measured. This formula was used to calculate the percentage reduction in glucose levels (Amir et al., 2019).

% Decrease =
$$\frac{T1-Tt}{T1} \times 100\%$$

Description:

 T_1 =Glucose level measurement pretreatment T_t =Glucose level measurement past treatment

Table 1. Treatment group of test animals		
Group	Treatment	
Normal	Normal saline dose 0.9 %	
Negative Control	CMC Na dose 0.5%	
Positive Control	Glibenclamide dose 0.65 mg/kgBW	
Group I	EEBP at a dose of 100 mg/kgBW	
Group II	EEBP at a dose of 200 mg/kgBW	
Group III	EEBP at a dose of 400 mg/kgBW	

Data Analysis

This study analyzed data using IBM SPSS 22 statistical software. Glucose levels were measured on the first and third days using paired T-test. To measure the percentage reduction in blood glucose levels between groups, one-way ANOVA test was used. Before conducting the LSD test, normality and homogeneity tests were conducted to determine the differences between each group. If the p value was <0.05, the groups were considered significantly different.

RESULT AND DISCUSSION

Extraction of Banana Peel

Based on table 2, the viscous extract from EEBP weighs 76.71 grams, resulting in a total yield of 25.57%. Thus, the yield of EEBP is considered good because the value exceeds 10% (Walid *et al.*, 2023).

Table 2. Results of banana peel extraction preparation

Simplisia powder (g)	Extract (g)	Yield (%)
300	76.71	25.57

Phytochemical Screening Result

Secondary metabolites in eebp were identified through phytochemical screening. the results of the screening are presented in Table 3.

Table 3. Phytochemical screening results of EEBP

Identification	Reagent	Result	Interpret ation
Alkaloids	Dragend rof	Orange brown precipi tate	+
Flavonoids	Magnesi um +HCl concentr ated	Yellow	+
Tannins	FeCl ₃ 10%	Blackis h green	+
Saponins	Hot water + 2N HCI	There is 1-10 cm of froth	-

Description:

- +: Compound present
- -: No. compound

Phytochemical screening showed that EEBP contains alkaloids, flavonoids, and tannins as secondary metabolites. The presence of these secondary metabolites was detected through changes in the color of the extract solution after adding the appropriate reagents. The alkaloid test is characterized by the formation of an orange brown precipitate, the flavonoid test is characterized by the formation of a yellow color, while the tannin test is characterized by the formation of a blackish green color (Nababan et al., 2022). The results of phytochemical screening are almost similar to the findings of research conducted by (Nababan et al., 2022), except that the screening results that have been carried out have differences in saponin compounds that have negative results. This is due to differences in extraction methods, types of solvents, and sampling sites. In the previous study, the extraction was carried out by maceration method using 96% ethanol solvent. According to other research, the sonication process transfers energy to the solvent, causing agitation and friction in the liquid which generates heat. Too long extraction duration can damage the extract content. As a result, the saponin yield decreased after 30 minutes using the UAE method (Adiwibowo et al., 2020).

Effect of Alloxan Induction on Blood Glucose Levels of Mice

Except for the normal group, all groups of mice were induced with alloxan. The normal group was only induced with saline solution. Before intraperitoneal (i.p) induction, alloxan was dissolved quickly in normal saline solution at a dose of 200 mg/kgBW (Widyasti *et al.,* 2019). This step was taken to ensure that blood conditions remained normal on the first day. The alloxan solution was prepared in cold saline solution (Hasim *et al.,* 2020). On the third day, glucose was measured to ensure that the mice had developed diabetes. The results of alloxan induction are presented in Table 4.

Table 4. Mean blood glucose pre and post alloxan induction

	Mean blood glucose level			
Group	$(mg/dL) \pm SE$			
	Pre-induction	Post- induction		
Normal	91 ± 3.10	116.75 ± 6,27		
Diabetes	104.35 ± 2.85	384.15±13.46*		

*significantly different from pre-induction, with Paired t-test analysis, p<0.05

All mice that have undergone DM are treated orally according to the group. After alloxan induction, blood glucose levels increased to 384.15 ± 13.46 mg/dL. Alloxan can damage pancreatic endocrine cells, especially beta cells, resulting in increased blood glucose levels. As a result, insulin secretion into the blood decreases. Alloxan compounds cause necrosis and degeneration in about 40% to 50% of beta cells (Dachi *et al.*, 2022). Mice that do not receive treatment and are only fed and drink water show normal glucose levels (Fitrya *et al.*, 2022).

Effect of Treatment of EEBP on Blood Glucose Levels of DM Mice

Glucose levels were measured on the third day after induction, as well as the seventh and fourteenth days after treatment. The results showed that oral administration of a suspension of EEBP in various doses succeeded in reducing glucose levels after treatment.

Table 5. Mean blood glucose levels of all groups after treatment

Treatment	Mean blood glucose level (mg/dL) ± SE		
Groups	Day 0	Day 7	Day 14
Normal	116.75 ±	124.00±	123.25 ±
control	6.28	4.69	2.14
Negative	355.75 ±	442.75 ±	535.75 ±
control	41.00	24.30	17.99
Positive	424.75 ±	227.25 ±	124.5 ±
control	36.25	8.75	2.217
EEBP 100	401.25 ±	254.25 ±	242.75 ±
mg/kgBW	9.213	27.57	13.21
EEBP 200	392.75 ±	285.75 ±	159.25 ±
mg/kgBW	27.15	19.92	10.61
EEBP 400	346.25 ±	247.00 ±	118.00±
mg/kgBW	22.48	19.39	6.57

This finding indicates that the ethanol extract of banana peel has antidiabetic properties that are effective in reducing glucose levels. On the fourteenth day after treatment, the positive control group showed a decrease in average glucose levels of 124.5 ± 2.217 mg/dL, treatment 1 showed 242.75 ± 13.21 mg/dL, treatment 2 showed 159.25 ± 10.61 mg/dL, and treatment 3 showed 118.00 ± 6.57 mg/dL, all of which showed a decrease in glucose levels.

Table 6. Percentage reduction in blood glucose levels of all groups on post treatment

Mean percentage reduction in blood glucose level (%) ±SE	
-6.34 ± 5.01a	
-54.79 ± 12.77 ^b	
70.08 ± 2.36°	
39.49 ± 3.06^{d}	
59.42 ± 0.13°	
65.83± 0.800°	

Notes: Normal control different with positive control

Negative control different with positive control

EEBP 100 mg/kgBW different with positive control

Positive control not different with EEBP doses of 200 and 400 mg/kgbw

Glucose levels did not decrease in the normal and the negative control group that was only given CMC-Na, as shown in Table 6. The normal and negative controlgroup did not show an antihyperglycemic effect, so it did not succeed in reducing glucose levels (Akuba *et al.*, 2022).

The treatment of 100 mg/kgBW of banana peel ethanol extract showed the lowest percentage reduction in glucose levels compared to the doses of 200 mg/kgBW, and 400 mg/kgBW. The higher the dose of EEBP given, shows a more significant decrease in glucose levels, because higher doses produce a more significant decrease in glucose levels (Febriani et al., 2024). Doses of EEBP of 200 mg/kgBW and 400 mg/kgBW each showed effectiveness in reducing blood glucose levels of mice, equivalent to the positive control glibenclamide. However, the results of this study showed that a dose of 200 mg/kgBW proved effective in reducing glucose levels in mice.

Glibenclamide is a second-generation oral diabetes drug utilized for the treatment of type 2 DM that belongs to the sulfonylurea class (Kulsum *et al.*, 2022). The mechanism of action of glibenclamide is to promote the release of the hormone insulin from beta cell granules in the pancreatic islets of Langerhans. This causes depolarization of the beta cell membrane, resulting in the opening of calcium channels. Calcium ions then enter the beta cells, stimulating the insulin-containing granules, causing insulin release (Brata *et al.*, 2022).

EEBP which contains antidiabetic compounds such as alkaloids, flavonoids, and tannins, can help mice reduce glucose levels by repairing damaged pancreatic beta cells and stimulating sympathetic nerves to increase insulin secretion (Risqia et al., 2023). Flavonoids can reduce glucose levels because

absorbed glycoside compounds increase the solubility of glucose, so that glucose is more easily secreted through urine, and circulatory glucose levels decrease (Wenas *et al.*, 2019). Tannins also stimulate antioxidant enzymes that support pancreatic beta cell regeneration and act as free radical scavenging agents. It helps lower glucose levels by preventing the colon from absorbing glucose and stopping adipogenesis (Munawwaroh *et al.*, 2022). The more compounds present in banana peel, the better the activity, so the dose of ethanol extract of banana peel shows a stronger antidiabetic effect.

CONCLUSIONS

The study showed that the dose of EEBP of 200 mg/kgBW is the optimal dose to reduce glucose levels in mice suffering from diabetes mellitus and induced with alloxan with blood glucose level reduce 59.42 ± 0.13 %. The percent blood glucose level significant difference with negative control and not significant difference with positive control are glibenclamide.

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AUTHORS' CONTRIBUTIONS

All authors was responsible for research design, data collection, and data analysis.

All authors contributes to the initial manuscriptwriting, editing, and revision ofe the article.

All authors provided supervision, validation of the research results, and final approval of the manuscript for publication.

All authors have read and approved the final version of the manuscript.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest that could influence the results or interpretation of this study.

ATHICAL CONSIDERATION

Plagiarism:

No part of this study has been copied without a acknowledgement of the source. All references and citations have been provided in accordance with academic rules.

Data Fabrication:

The data presented in this study was obtained directly from the results of the research conducted, without any alteration, fabrication, or manipulation.

Multiple publications:

This research is an original work that has not been previously published in other journals or is in the process of consideration in other journals simultaneously.

Ethics Committee Approval:

This study received ethical approval from the Universitas dr. Soebandi Jember with number: 116/KEPK/UDS/II/2024.

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