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HYPOGLYCEMIA POTENTIAL OF ETHANOL EXTRACT OF MELINJO LEAVES (*Gnetum gnemon* L.) ON WISTAR MALE WHITE RATS

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ABSTRACT

Male rats were used in the study to evaluate the ethanol extract of melinjo leaf (Gnetum gnemon L.) for hypoglycemia after being given alloxan and having their pancreatic histology observed. Secondary metabolites with antidiabetic properties found in melinjo leaf include flavonoids, tannins, saponins, and phenolics. This study sought to ascertain the ethanol extract of melinjo leaves' ED50 as well as its impact on lowering blood glucose levels and monitoring pancreatic histology. The doses of melinjo leaf ethanol extract were varied to 125, 250, and 500 mg/kgBW. An insulin dosage of 1 IU/kgBW served as the positive control, a 0.5% Na CMC suspension served as the negative control, and a normal control group did not receive any therapy. Rats were tested by utilizing a DTN-410-K photometer to measure their fasting blood glucose levels on days 0, 10, 15, and 20 using the GOD-PAP enzymatic method. Hematoxylin-eosin staining was used in histopathology preparations, which were prepared in accordance with established protocols. Melinjo leaf ethanol extract dosages of 125, 250, and 500 mg/kgBW resulted in three treatment groups with corresponding the percentage of blood glucose reduction (%BGR) of 31.48, 34.39, and 42.90%, whereas the positive control had an average BGR of 40.68%. Melinjo leaf ethanol extract has an ED50 of 720.86 mg/kgBW. According to the histological image, the positive control group and the three treatment groups showed improvement, whereas the negative group's Langerhans islet endocrine cells showed necrosis. The 500 mg/kg BW dosage group shows the greatest improvement.

Keywords: alloxan, GOD-PAP, hypoglycemia, melinjo leaves, pancreas

INTRODUCTION

The chronic metabolic disease known as diabetes mellitus (DM) is brought on by either insufficient insulin production by the pancreas or ineffective insulin utilization by the body, which raises blood glucose levels (hyperglycemia). One hormone that controls blood sugar balance is insulin.

Diabetes management aims to maintain blood glucose levels within the normal range and can be done non-pharmacologically and pharmacologically. Diet, physical training, and weight control are examples of non-pharmacological management. Giving insulin and/or oral hyperglycemic medications are examples of

pharmacological therapy (Mutu and Yuda, 2019).

With minimal side effects, the Indonesian government is currently encouraging the use of traditional medicine (Leonita and Muliani, 2015).. The widespread use of traditional medicines by the community is because they are natural, easy to obtain, and inexpensive, the use of traditional medicines also does not produce side effects as often occurs in chemical treatments, in addition, many people still think that the use of traditional medicines is safer than the use of synthetic drugs (Handayani and Natasia, 2018). Numerous plants are frequently employed as traditional remedies. The melinjo plant, which has numerous health benefits, is one of them.

Melinjo (Gnetum gnemon L.) is a member of the Gnetaceae family originating from Southeast Asia, especially Indonesia. The chemical content of melinjo, especially in the seeds and leaves, includes saponins, flavonoids and tannins (Tanamal et al., 2017). Melinjo has many health benefits, such as lowering blood sugar, preventing cancer, being an antioxidant, highly nutritious, and inhibiting the aging process (Ira and Cikra, 2015). According to Parhusip (2011), flavonoids and stillbenoids from melinjo (Gnetum gnemon L.) are active components that can function as antioxidants.

According to Ulfa et al. (2018), giving melinjo seed ethanol extract at a dosage of 250 mg/kgBW demonstrated a decrease in triglyceride levels by 17.86% on the 15th day in mice given standard feed and 2 mL/day of cow brain. According to Ira and Cikra (2015), 50% infusion of melinjo seeds (Gnetum gnemon L.) was administered, and the results a decrease in glucose levels of 33.41%±28.84% in type 2 diabetes mellitus mice induced by 40% dextrose monohydrate.

Flavonoids are substances found in melinjo leaves that can help lower blood sugar levels. Flavonoids have antidiabetic properties because they are antioxidants that can bind free radicals, reducing oxidative stress, lowering insulin retention, and preventing damage and malfunction to pancreatic β cells (Gajalakshmi et al., 2018).

The way flavonoids work to reduce blood sugar levels is to reduce glucose absorption by inhibiting GLUT 2 intestinal mucosa and increasing glucose utilization in peripheral tissues and skeletal muscles by suppressing the glycogenolysis and gluconeogenesis pathways (Edi, 2020). Based on the description above, a study was performed on the reduction of blood glucose levels in alloxan-induced mice in vivo. Blood sugar levels were measured using the spectrophotometric method with the Glucose Oxidase Phenol 4-Aminophenazone (GOD-

PAP) reagent kit. This study was conducted not only to observe the decrease in blood sugar levels from the ethanol extract of melinjo leaves (Gnetum gnemon L.), but also to determine the effective dose (ED50), Area Under Curve (AUC), and to characterize the ethanol extract of melinjo leaves and see the histopathology of the pancreas in alloxan-induced mice.

METHODS

Materials

The tools used in this study were maceration equipment such as maceration containers, rotary evaporators (IKA®), centrifugators with a speed of 2500 rpm (IEC®), analytical scales with an accuracy of up to 4 digits behind the decimal point (Ohaus®), DNT-410-K photometer (Dialab®), animal test maintenance equipment set, rat surgical equipment (Olympus®), per-oral syringes or sondes (Ministry of Health of the Republic of Indonesia), data analysis software (SPSS®), 10 µL, 100 µL, and 1000 µL micropipettes (Eppendorf®, Accumax® and Labopette®), hematocrit pipettes (Nesco®), glass funnels (Pyrex®), measuring flasks (Pyrex®), and other laboratory glassware (Pyrex® and Iwaki®). In this investigation, the plants utilized were the leaves of the melinjo plant

(Gnetum gnemon L.) collected from the Inderalaya region, South Sumatra and determined at the Laboratory of the Plant Conservation Center, Purwodadi Botanical Gardens (LIPI), Purwodadi, Pasuruan, East Java. The test animals used in this study were male white rats of the Wistar strain weighing between 130-200 g and aged 2-3 months. The test rats used were healthy, not disabled and behaved normally, obtained from the research rat breeder Abdul Tikus Palembang in Palembang.

The materials used include 96% ethanol (PT. Dira Sonita Palembang), GF254 silica plate (Merck & Co.), injection syringe (Onemed®), Whatman paper No. 45 porosity 0.45 µm, non-EDTA (Ethylene Diamine Tetra Acetate) vacutainer tube (Vaculab®), Mayer, Wagner, Liebermann-Bourchard and Dragendorf reagents (PT. Bratachem Palembang), Magnesium metal (PT. Bratachem Palembang), Aluminum chloride (Merck & Co.), Iron (III) chloride (PT. Bratachem Palembang), glucose oxidase phenol-aminophenazone (GOD-PAP) kit (Diasys®), alloxan (Sigma-Aldrich®), Insulin (Solostar®), Sodium Carboxyl Methyl Cellulose (Na CMC) 0.5%, alcohol, paraffin, xylol (PT. Bratachem Palembang), hematoxylin-eosin (HE) (Merck & Co.), standard rat food, and distilled water (PT. Bratachem Palembang).

Research Path

1. Producing Melinjo Leaf Ethanol Extract

Preparation begins with processing 4 kg of fresh melinjo leaves into simplicia powder through procedure that includes drying, chopping, washing, wet sorting, and dry sorting. The maceration method, which uses a 96% ethanol solvent for three days, protected from light while frequently stirring, sprinkling, and squeezing. For two days, the residue is re-macerated with 96% ethanol.

Maceration result obtained is then filtered with Whatman paper and concentrated at 500^{o}Celsius in a rotating evaporator. The yield value of the ethanol extract of melinjo leaves is then determined after the leaves are evaporated in an oven set to 400C until a thick extract is produced.

2. Phytochemical Screening of Extracts

2.1 Flavonoids

1 g of melinjo leaf extract was added with five minutes of heating and five mL of ethanol. After filtering the extraction findings, ten drops of concentrated HCl were applied to the filtrate. Up to 0.1 g of magnesium powder was added. Flavonoids are indicated by a reddish-orange to reddish-purple hue, whereas chalcones, flavones, and aurones are indicated by a yellow-orange hue (Depkes RI, 1995).

2.2 Alkaloids

1 g of melinjo leaf extract was added with 9 mL of water and 1 mL of 2 N hydrochloric acid, heated for 2 minutes, cooled, and filtered. The results of the filtrate were identified. For the initial identification, two drops of Wagner reagent were applied to a test tube containing one milliliter of filtrate. Brown sediment was found in the positive results. For the second identification, two drops of Mayer reagent were applied to a test tube containing one milliliter of filtrate. White sediment was found in the positive results. For the third identification, two drops of Dragendorff reagent were applied to a test tube containing one milliliter of filtrate. Orange sediment is present when the results are positive (Depkes RI, 1995).).

2.3 Saponin

Ten mL of boiling water were added to a test tube containing one gram of melinjo leaf extract, allowed to cool, and then violently shaken for ten seconds. If foam forms up to 1–10 cm and does not go away when 1 drop of 2 N hydrochloric acid is applied, the results are positive and include saponin. (Depkes RI, 1995).

2.4 Phenolic and Tannin

1 gram of melinjo leaf extract was diluted in ten milliliters of distilled water, boiled for five minutes, and then filtered in order to perform phenolic and tannin tests. Four to five drops of $FeCl_3$ were added to

the filtrate. The presence of dark blue or blackish green indicates positive results for phenol chemicals (Harborne, 1987). Positive tannin findings are indicated by the presence of green violet. Another method is to add 5 mL of filtrate to 1% gelatin and 1 mL of 10% NaCl solution to form a white precipitate indicating positive results for tannins (Iriany et al., 2021).

2.5 Terpenoids and Steroids

1 g of melinjo leaf extract was added with 5 mL of ether solution on a porcelain plate. The residue was added with Lieberman-Burchard reagent containing anhydrous acetic acid and concentrated sulfuric acid (2:1). Positive results for terpenoids show a brown or blue violet color. Positive steroid results show green or blue color (Harborne, 1987).

3. Flavonoid Identification using TLC

Flavonoid identification is carried out with a 96% ethanol extract solution of melinjo leaves which is spotted on a TLC plate made of silica gel measuring 1 x 5 cm with an upper and lower limit of 0.5 cm which has been made using a pencil. The TLC plate is then eluted using eluent-n-hexane:ethyl acetate (3:2) until a clear spot is obtained. The eluted TLC plate is observed for spots under UV light 254 and 366 nm after being sprayed with aluminum chloride. Flavonoids contained in the identified extract will fluoresce yellow, green or blue at UV 366 nm (Bladt, 2009).

4. Extract Characterization

4.1 Organoleptic

Organoleptic determination was carried out by observing the shape, color, odor and taste of the melinjo leaf extract used (Depkes RI, 2000).

4.2 Water Content

A sample of 1 gram was put into a porcelain cup, then closed. Then it was dried using an oven at a temperature of 1050C for 3 hours, then cooled in a desiccator and then weighed. The drying process was repeated with the oven for 30 minutes until a constant weight was obtained. The water content was calculated as a percentage of the initial sample (Depkes RI, 2000).

4.3 Total Ash Content

A sample of 1 gram was weighed carefully into a porcelain crucible that had been incandescent and the weight of the empty crucible was weighed. The sample was incandescent using a furnace slowly with the temperature gradually increased to 6000C ± 250C until the charcoal ran out. Then cooled in a desiccator and weighed until the weight remains constant. The total ash content is calculated as a percentage of the initial sample weight (Depkes RI, 2000).

4.4 Water-Soluble Compound Content

For 24 hours, one gram of the sample was macerated with twenty milliliters of

water-chloroform LP solvent (1:1), then filtered. The filtrate was taken and then evaporated in a tared cup by leaving it until the solvent evaporated and the residue remained. Furthermore, the residue was heated at a temperature of 1050C until the weight remained constant. The content of compounds dissolved in water was expressed as a proportion of the original sample weight (Depkes RI, 2000).

4.5 Ethanol-Soluble Compound Levels

Twenty milliliters of 96% ethanol solvent were used to macerate a one-gram sample for twenty-four hours, then filtered. The filtrate was taken and then evaporated in a tared cup by letting it stand until the solvent evaporated and the residue remained. Furthermore, the residue was heated at a temperature of 1050C until the weight remained constant. The levels of compounds dissolved in ethanol were calculated as a percentage of the initial extract weight (Depkes RI, 2000).

5. Preparation of Test Animals

The test animals to be used in this study were 4 rats in each group. As many as 2 rats were added in each group to prevent sample shortages if the test animals died during the study.

Table 1. Test groups for treatment

Group	Treatment
Normal	Not given treatment
Negative control	Alloxan 130 mg/kgBW + suspension Na CMC 0,5 %
Positive control	Alloxan 130 mg/kgBW + insulin dose 1 IU/kgBW
Group 1	Alloxan 130 mg/kgBW + suspension EEML 125 mg/kgBW
Group 2	Alloxan 130 mg/kgBW + suspension EEML 250 mg/kgBW
Group 3	Alloxan 130 mg/kgBW + suspension EEML 500 mg/kgBW

5.1 Alloxan Induction

The test animals were fasted for 8-12 hours while still being given water, then the rat were induced with alloxan 130 mg/kgBW intraperitoneally. The rat's body weight and blood sugar levels were measured first before induction to determine the initial body weight and initial glucose levels, the blood glucose levels of the rat were checked on the third day after alloxan induction to determine which test animals had experienced diabetes mellitus. If the rat's blood glucose levels had not reached 200 mg/dL, they were re-induced with alloxan until the rat's blood glucose levels had constant ≥ 200 mg/dL.

5.2 Antidiabetic Activity Test

The rat that had been declared to have DM (levels of blood glucose ≥ 200 mg/dL) were split up into six groups, each group was given different treatments. Normal control (not treated), positive control given insulin 1 IU/kgBW, negative control given Na CMC suspension 0.5%, and treatment

group with 3 different doses, namely 125, 250 and 500 mg/kgBW Fasting blood glucose of mice (pre prandial) was measured on days 0, 5, 10, 15, and 20.

5.3 Blood Glucose Level Measurement

Rat normal blood glucose levels were measured by taking blood from rat that had been fasted for ± 18 hours (drinking was still given). Blood was taken via the retroorbital plexus from the eye vein as much as 0.5-1 mL using a hematocrit pipette. Furthermore, the blood was collected in a non-EDTA vacutainer tube and centrifuged at a speed of 2500 rpm for 10 minutes to separate the serum from the blood. Serum was taken as much as 10 µL with a micro pipette. Serum was added with 1 mL of GOD-PAP reagent. Then incubate for 10 minutes at a temperature of 25-30°C. Furthermore, measurements were made using a DIALAB DTN-410-K photometer at a maximum λ of 500nm. After all data was obtained, the AUC and ED50 values were calculated. One mouse from each group was dissected to take its pancreas for histopathological observation.

Data Analysis

Statistical testing was carried out quantitatively by comparing data on the decrease blood glucose levels in the group receiving therapy with the positive control group. Initial analysis of data on the decrease in blood glucose levels with a descriptive normality test (Shapiro-Wilk) to ascertain if the information was distributed normally. One-way ANOVA with a 95% confidence level was used for parametric statistical analysis to determine whether the data was regularly distributed. The results of the analysis obtained showed significant differences followed by the LSD post hoc test. If the data on the decrease in blood glucose levels were not normally distributed (in the Shapiro-Wilk test), a transformation was carried out on the data. The data transformation was then tested for normality again with Shapiro-Wilk. Analysis of normally distributed data then used one-way ANOVA. The program used for data processing is SPSS® software.

RESULTS AND DISCUSSION

The raw materials obtained before the processing of the simplicia were determined first in order to ensure the truth of the identity of the plant. Different raw materials can affect the pharmacological effects of plants so that determination is needed to avoid errors in collecting materials. The Purwodadi Botanical Gardens' (LIPI), Purwodadi, Pasuruan, East Java, Plant Conservation Center Laboratory served as the site for the determination procedure. The findings of the determination indicated that the sample was melinjo or which has the Latin name *Gnetum gnemon* L. which is included in the Gnetaceae family.

1. Extract Preparation

Extraction of melinjo leaves was use the maceration process, because it is a cold extraction technique, making it suitable for materials that are not heat resistant such as flavonoids and the tools are simple. The solvent that was utilized in this study was ethanol solvent which is a universal solvent because it can extract polar and non-polar components (Harborne, 1987). The appropriate ratio of water to ethanol will facilitate the diffusion of components out of the cell so that 96% ethanol is chosen because the greater the amount of ethanol, the more efficient it is in degrading non-polar cell walls so that more polyphenols will be extracted (Puluh et al., 2019).

The amount of thick extract of melinjo leaves was obtained as much as 86.59 g with a yield of 8.659%. The percentage yield is carried out to predict the weight of the simplex for making a certain amount of extract, in addition to showing the possible amount of the extract's chemical constituents and how well the solvent and extraction procedure work to extract the simplex's constituents (Wahyuni, 2021).

2. Phytochemical Test

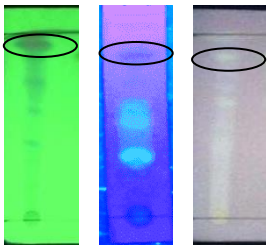
The results of the phytochemical test in Table 2. show a positive reaction in the flavonoid, phenolic, tannin, and saponin tests. However, it showed a negative reaction in the terpenoid, steroid, and alkaloid tests.

Table 2. Phytochemical screening results

Secondary Metabolites	Extracts
Flavonoid	+
Alkaloid	
- Wagner	+
- Mayer	-
- Dragendorff	-
Saponin	+
Phenolik	+
Tannin	+
Terpenoid	-
Steroid	-

Note: (+) positive result, (-) negative result

2.1 Identification of Flavonoids using TLC



Note:
stationary phase = silica gel GF_{254}
mobile phase = *n*-heksana:etil acetate (3:2)

Figure 1. TLC results of flavonoid ethanol extract of melinjo leaves (a) UV 254 nm (b) UV 366 nm (c) After spraying AlCl3

Identification of flavonoids by TLC using n-hexane:ethyl acetate with a ratio (3:2) based on the polarity level of this solvent compound as a mobile phase that will be used to see the presence of flavonoid compounds that are polar to semi-polar. The test results on the TLC plate that has been eluted with n-hexane and ethyl acetate eluents (3:2) sprayed with AlCl3 spot detectors produce yellow spots. Positive results contain flavonoids if the stain is yellow (Markham, 1988). Spraying has the principle of the presence of AlCl3 spot

detectors because it binds to aluminum to form a stable yellow complex.

3. Phytochemical Characterization

Table 3 indicates that the ethanol extract of melinjo leaves is a thick, dark green liquid with a characteristic bitter flavor and leaf odor. The leaves' chlorophyll content gives them their blackish green hue. The ethanol extract of melinjo leaves is what gives the extract its unique smell and bitter flavor which contain several secondary metabolites. Based on Table 4. it is known that the water content has met the requirements, namely <10%. The same thing also happens to the levels of water-soluble and ethanol-soluble extracts, where the value of the water-soluble extract content is smaller than the ethanol-soluble water content. This shows that the number of polar compounds (water-soluble) such as saponins in the extract is less than the compounds that are ethanol-soluble and semi-polar such as alkaloids, flavonoids to non-polar such as steroids and triterpenoids.

Table 3. Organoleptic results

Parameters	Result
Extract identity:	
-- Extract name	Ethanol extract of melinjo leaves
-- Latin name	Gnetum gnemon L.
-- Plant part	Leaves
Extract organoleptic:	
- Shape	Thick extract
- Color	Blackish green
- Smell	Typical
- Taste	Bitter

Table 4. Extract characterization results

Test Parameters	Test Result (%) ± SD	Requirements of the Indonesian Ministry of Health (2000)
Water content	6,071 ± 0,1420	<10 %
Water soluble content	41,3±0,0102	>18,2%
Ethanol soluble content	81,7±0,0035	>15%
Ash content	2,34 ± 0,8504	-

4. Alloxan Induction

Initial glucose levels were measured first before alloxan induction. This aims to ensure that the test animal subjects used are healthy and do not suffer from diabetes mellitus and as comparative data for blood glucose levels after alloxan induction and after treatment. The first range of blood glucose levels was 90–120 mg/dL.

The levels of blood glucose were checked after 3 days of alloxan induction.

The study employed rat blood glucose levels greater than 200 mg/dL.

If it has not reached 200 mg/dL, it is re-induced with alloxan until the levels of blood glucose are stable above 200 mg/dL. The normal group was not induced with alloxan like The treatment group and the control group, they were only given standard drinks and food that did not affect the rat's elevated blood glucose levels, causing the blood glucose levels to the rat remained normal. Figure 2 provide information on the rise in blood glucose.

It is evident from the comparison of blood glucose levels that alloxan induction causes blood glucose levels to rise. This happens because alloxan compounds have specific cytotoxic properties on β Langerhans cells and alloxan also generates radical groups that damage β Langerhans cells due to increased free radicals in the body. Damage to β Langerhans cells will be followed by a decrease in insulin hormone secretion which can cause glycogenesis reactions and glucose transport in cells to decrease. Conversely, glycogenolysis becomes uncontrolled, resulting in increased blood glucose levels.

Because alloxan compounds increase the permeability of pancreatic β cell membranes, they can potentially harm them. Insulin levels can drop as a result of damage to the pancreatic β cells caused by membrane damage. By increasing its permeability, alloxan's mode of action in vitro demonstrates that it will cause the release of calcium ions from mitochondrial organelles, so interfering with the oxidation process of cells, tissues, and organs. Cell death starts when calcium ions leave the mitochondria and disturb equilibrium (Pertiwi et al., 2021).

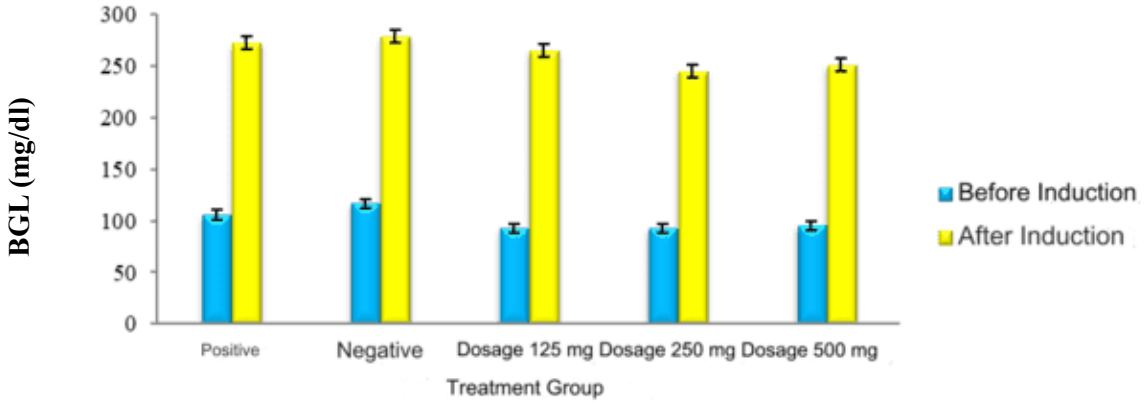


Figure 2. Blood glucose level comparison before and after alloxan induction

5. Antidiabetic Activity Test

After the mice were declared to have diabetes, the next step was testing on 6 test groups, namely normal control, positive control given insulin 1 IU/kgBW, negative control given Na CMC 0.5%, treatment

groups with doses of 125, 250 and 500 mg/kgBW. The testing was carried out for 20 days with a sampling interval of 5 days, specifically days 0, 5, 10, 15, and 20. Blood glucose levels were measured using a DIALAB DTN-410-K photometer at a wavelength of 500 nm. Data on the results of blood glucose measurements can be seen in Table 6.

Table 6. Results of average blood glucose levels

Groups	Average Blood Glucose Levels (BGL)				
	BGL after induction (day 0)	BGL treatment			
		Day-5	Day-10	Day-15	Day-20
Normal	109,045±5,07	108,911 ±7,82	105,927 ±7,99	105,007 ±7,27	102,07 ±6,23
Positive	272,233±21,31	204,468 ±11,99	159,79 ±9,64	111,014 ±15,68	90,1135 ±9,61
Negative	278,405±21,26	277,89 ±21,22	276,528 ±21,50	275,65 ±22,69	274,658 ±19,72
Dose 125 mg/kgBW	264,578±20,50	227,23 ±26,64	194,743 ±26,17	144,635 ±9,99	118,49 ± 9,20
Dose 250 mg/kgBW	244,56±29,02	211,29 ±29,68	189,993 ±19,29	146,623 ±11,69	111,498 ±10,01
Dose 500 mg/kgBW	251,003±24,75	191,11 ±17,04	156,493 ±17,45	113,623 ±16,22	90,177 ±6,51

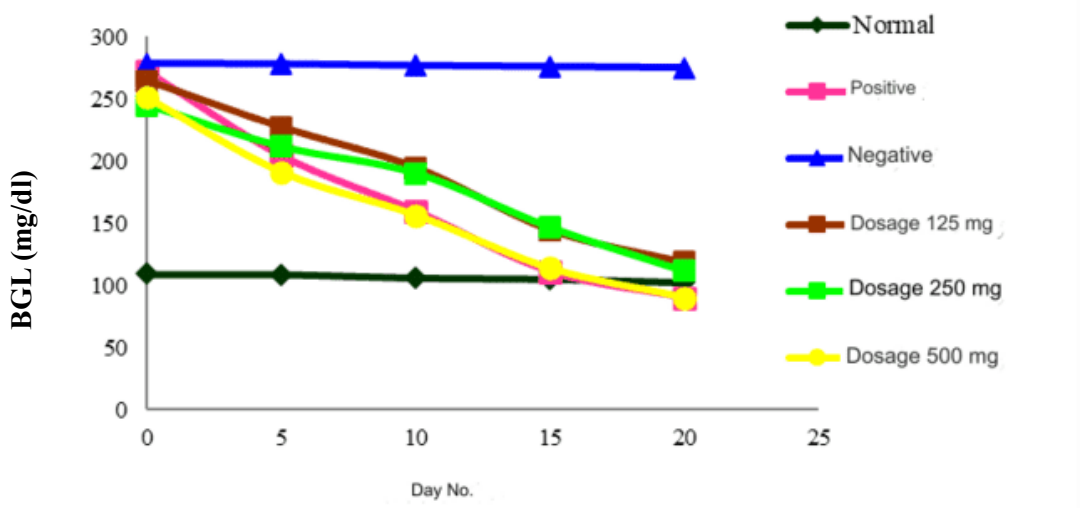


Figure 3. Graph of the correlation between each group's average blood glucose levels following alloxan induction

It is evident from the preceding graph that the normal group's blood glucose levels are comparatively constant at less than 126 mg/dL. This is because alloxan did not induce the normal group and was only given standard food and drink in order to maintain normal fasting blood glucose levels. Charisma (2017) states that normal fasting blood glucose levels are less than 126 mg/dL.

The levels of blood glucose of rat in the negative group were stable in DM conditions (≥200 mg/dL). This is because alloxan was used to produce the negative control group which was then given a 0.5% Na CMC suspension which is inert and has no efficacious effects including antidiabetic effects so as not to interfere with the mice's declining blood glucose levels. Following alloxan induction, blood glucose levels responded in a fluctuating manner before stabilizing into a diabetic condition.

Immediately following alloxan exposure, there is a brief (1-2 minute) increase of insulin production, which precedes the insulin secretion inhibition phase. the time when blood glucose levels drop as a result of short-term insulin secretion stimulation. A brief rise in ATP in beta cells and a brief release of insulin are caused by the initial decrease in ATP consumption brought on by glucokinase's inhibition of glucose phosphorylation. However, the phase of rising blood glucose levels is caused by alloxan's selective suppression of insulin release by the beta cell glucose sensor glucokinase (Floris et al., 2024).

Blood glucose levels dropped in the positive control group. This is because after being induced by alloxan, an insulin analog injection was given at a dose of 1 IU/kgBW in order to lower blood sugar levels (Piero et al., 2015). Insulin's actual mode of action involves transferring blood glucose into cells so that it can be converted into energy. The insulin analog used is Lantus® Solostar® (insulin glargine) which is a long-acting insulin with a duration of 24 hours. The reason for using insulin glargine is because its duration of action is longer and its use is once a day which is adjusted to the administration of extracts to the treatment group. The treatment groups' blood glucose levels with doses of 125, 250, and 500 mg/kgBW decreased. Because the melinjo leaf extract contained flavonoids, saponins, and tannins, among other secondary metabolites, the blood glucose levels in the treatment group dropped. Having antihyperglycemic properties, flavonoids was potential antidiabetic agents with flavonoid antioxidant mechanisms that help prevent damage and repair pancreatic β cells, slow down cell necrosis to reduce lipid peroxidation, and improve vascularization to promote β cell regeneration (Beroual et al., 2017).

Flavonoids as antidiabetics show a working mechanism by increasing insulin sensitivity with their ability to inhibit damage to pancreatic β cells from chain peroxidation reactions caused by Reactive Oxygen Species. Additionally, flavonoids can help boost insulin secretion and rejuvenate pancreatic β cells (Kinanti et al., 2023). Investigations by Sang et al. (2023) shows that tannins work to lower blood

glucose levels by triggering the insulin-mediated signaling system, glucose transport is increased. The way saponins function is by regenerating the pancreas, which increases the amount of islets of Langerhans and pancreatic β cells, which in turn enhances insulin output. Lower blood glucose levels will be aided by increased insulin secretion (Hernawati et al., 2019).

6. *Area Under Curve* (AUC) value

Table 7. Average data AUC_{0-20} and %BGR

Kelompok	AUC_{0-20}	%BGR
Normal	2127,01	-
Positive	3282,22	40,6791
Negative	5532,99	0
Dose 125 mg/kgBW	3790,71	31,4891
Dose 250 mg/kgBW	3629,67	34,3996
Dose 500 mg/kgBW	3159,07	43,9048

Calculating the Area Under Curve (AUC) value determines changes changes in blood sugar levels between days 0 and 20. Each treatment group's variations in blood glucose levels are determined by computing the area under the curve from day 0 to day 20 (AUC0-20). The average data for AUC0-20 and %BGR can be seen in Table 7. The AUC0-20 value has an inverse relationship with the percentage of blood glucose reduction (%BGR), where the greater the %BGR, the better the antidiabetic activity and vice versa. The test group's ability to reduce blood glucose levels is better if its AUC value is smaller. Based on this statement, the group with the smallest AUC value and the largest %PKGD is the 500 mg/kg BW dose group.

7. Effective Dose (ED_{50})

Finding the dose at which 50% of test mice's blood glucose levels can drop is the goal of determining the ethanol extract of melinjo leaves' ED50 value. The linear regression between the therapeutic dose and the percentage drop in blood glucose levels (%BGR) is used to determine the ED50 value (Figure4).

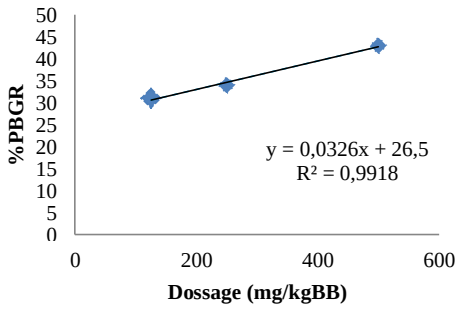


Figure 4. Linear regression graph between dose and %BGR

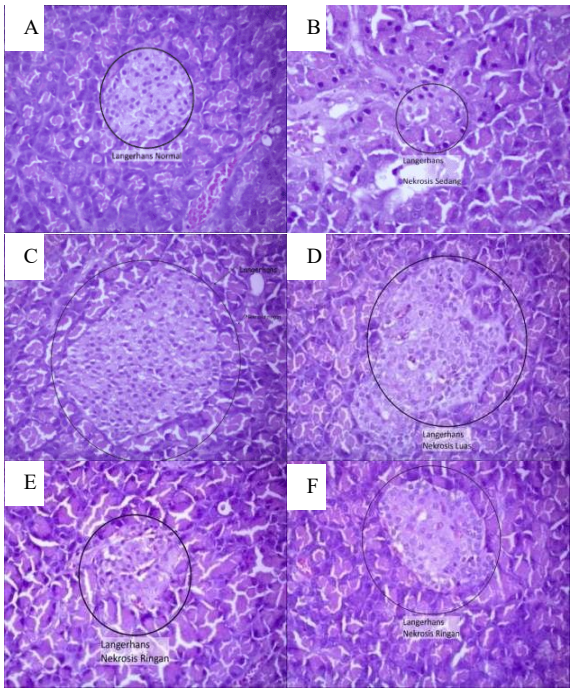
The linear equation obtained is y = 0.0326 x + 26.5 with an R2 value of 0.9918. Based on the calculation results, the ethanol extract of melinjo leaves has an ED50 of 720.86 mg/kgBW. This is because this study used a crude extract, but the use of drugs from natural ingredients in the form of this extract is safer than the use of synthetic drugs and easier in the application of the administration route than the use of insulin. Therefore, this ethanol extract of melinjo leaves is expected to be an alternative in

supporting the safe and comfortable treatment of type 1 DM.

8. Histopathology of the Pancreas

Based on microscopic observations in Figure 5 (a), it can be seen that the rat in the normal control group had a healthy and undamaged pancreas. The consistent distribution of endocrine cells throughout the broad islets of Langerhans and the absence of empty spaces in the middle of the islets of Langerhans (necrosis). The pancreas of the negative control group of rat (Figure 5b) experienced inflammation as indicated by the large number of empty spaces (necrosis) in the middle of the islets of Langerhans, the shape of the islets of Langerhans began to be damaged, in contrast to the other treatment groups, they were smaller and had an uneven cell layout. This necrosis is brought on by the induction of alloxan, which releases SH groups that can generate reactive oxygen and bind to pancreatic β cells selectively, preventing them from producing insulin (Magitasari et al., 2019). The islets of Langerhans are depicted in the positive control group which still have purple cell nuclei, unlike the negative control group where the cell nuclei are missing and there is no cell necrosis which shows emptiness in the Langerhans islets, much like in the negative control group. Because the big islets of Langerhans are visible and the number of cells increases, this demonstrates that insulin treatment can repair injured pancreatic tissue is large (Magitasari et al., 2019). Administration of 125 mg/kgBW of melinjo leaf ethanol extract in the dose I treatment group (Figure 5d) showed a histopathological picture In the pancreas, namely the existence of empty cells, that was not significantly different from the negative control group without nuclei indicating tissue necrosis, but there was an improvement in dose I seen from the start of the cell nucleus which was round even though it was not completely even.

When 250 mg/kgBW of ethanol extract of melinjo leaves was administered to the II dose treatment group (Figure 5e), the islets of Langerhans improved in shape and had round cell nuclei, though there were still some on the edges of the islets and no longer any cells without nuclei were visible. In comparison to the I and II dose treatments, the pancreas of the third dose treatment group (Figure 5f) showed improvement with nuclei in every cell and beginning to be more numerous up to half of the islets of Langerhans. This was due to the highest dose of 500 mg/kgBW of ethanol extract of melinjo leaves. This suggests that tissue necrosis overall may be improved by administering a higher dosage of melinjo leaf ethanol extract.



Note : (A) Normal
(B) Negative
(C) Positive
(D) Dose 125 mg/kgBW
(E) Dose 250 mg/kgBW
(F) Dose 500 mg/kgBW

Figure 5. Histopathology of the islets of langerhans

Active metabolites found in the ethanol extract of melinjo leaves were responsible for the improvement of pancreatic β cells in the treatment groups of dosage I, dose II, and dose III, namely tannins and flavonoids which are included in the polyphenol compound group that has an -OH group that can function as a free radical scavenger so that it can work as an antioxidant. By making up for the electrons that free radicals lack, antioxidants neutralize them and can prevent chain reactions that result from the production of free radicals (Widyantari and Armita, 2023). This antioxidant works by inhibiting damage to pancreatic β cells by counteracting free radicals caused by alloxan, in addition, pancreatic β cells that are still active can also be shielded by antioxidants so that they are able to regenerate and produce insulin. Statistical data analysis in this study using SPSS with the first analysis, namely a normality test was carried out on rat body weight and blood glucose levels. What the normalcy test seeks to determine whether the data is normally distributed or not (Uno et al., 2014). The normality test in this study used less than 50 samples, in this study 24 samples were used. The Shapiro-Wilk normality test revealed that the mice's blood glucose levels and each group's body weight were normally distributed($p>0.05$).

A paired t-test was used to compare the rat blood glucose levels before and after alloxan induction between groups which aims to see if there is any difference between the two variables. The positive, negative, dosage I, dose II, and dose III control groups all had significantly different blood glucose levels before and after alloxan induction, according to the results of the paired test across groups ($p <0.05$). This suggests that while there is no discernible difference in the normal group, giving mice alloxan can cause them to become hyperglycemic. This is because the test animals in the normal group received no treatment at all.

The results of the rat weight data before and after alloxan induction were also examined utilizing a paired t-test statistical test between groups and discovered a significant difference ($p < 0.05$) between the treatment groups dose I, dose II, and dose III, as well as the positive and negative control groups the administration of alloxan has an effect on the decrease in mouse weight.

The one-way ANOVA AUC0-20 parametric statistics and the %PKGD value indicated that the groups differed significantly ($p < 0.05$). The %PKGD value and the post hoc AUC0-20 test analysis findings indicated that the treatment groups dose I, dose II, and dose III differed significantly ($p < 0.05$). This demonstrates that administering varying dosages will result in varying blood glucose reduction outcomes.

CONCLUSION

It is possible to draw the conclusion from the research that the ethanol extract of melinjo leaves used has the characteristics of water-soluble essence content of 41.3 ± 0.0102%, ethanol-soluble essence content of 81.7 ± 0.0035%, water content of 6.071 ± 0.1420%, and total ash content of 2.34 ± 0.85%. At dosages of 125, 250, and 500 mg/kgBW, melinjo leaf ethanol extract can lower blood glucose levels with a percentage drop in blood glucose levels of 31.49%; 34.39%; and 42.90%, respectively, which have significant differences between dose groups ($p < 0.05$). The ethanol extract of melinjo leaves has an effective dosage (ED50) of 720.86 mg/kgBW. Histopathological features of the pancreas of rats showed improvement in the treatment groups' islets of Langerhans at dosages of 125, and 250 mg/kgBW, and the best improvement at the highest dose of 500 mg/kgBW.

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