

Antidiabetic Activity Test Of Ethanolic Seri Leave's (*Muntingia Calabura L.*) Extract In Male Rats Induced By Alloxan

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ABSTRACT

Antidiabetic activity test of ethanol extract of seri leave (*Muntingia calabura L.*) rats induced by alloxan has been done. Male wistar albino rats are used as animal models which divided into 6 groups, normal group (aquadest), negative control group (Na CMC 0,5%), positive control group (glibenclamide 0,43 mg/200 g of BW), and 1, 2, and 3 treatment groups (ethanol extract of seri leave 65, 130, dan 260 mg/kg of BW). Rats blood glucose level after induced intraperitoneally by alloxan 130 mg/kg of BW can be stated as diabetes when >200 mg/dL. Preprandial blood glucose levels are measured using DTN-410-K photometer, on day 0, 5, 10, and 15. The average result of AUC₀₋₁₅ and percentage of decreasing blood glucose level for positive control group are 2732,5 and 37,43%, and 3 treatment groups (65 mg/kg of BW, 130 mg/kg of BW, and 260 mg/kg of BW) 3105 and 28.90%; 2962.5 and 32.16%; 2810 and 35.66%. These points indicated that the ethanol extract of seri leave have an antidiabetic activity and there is no significant difference compared with glibenclamide (p<0.05). Percentage of blood glucose decrease level the third treatment group so there is no significant difference compared with positive control group. According to the relation between percentages of blood glucose decrease level with dose, value of ED₅₀ of ethanol extract of seri leave is 692.424 mg/kg of BW.

Keyword: ethanol extract, seri leaves, antidiabetic, alloxan

1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder accompanied by hyperglycemia that occurs when the pancreas is unable to produce enough insulin or when the body cannot use the insulin that is produced effectively (Price and Wilson, 2005). According to Anies (2006), type-1 diabetes is caused by pancreatic beta cell damage due to an autoimmune reaction in a long time so that the body can not produce insulin properly (Mrowicka M, 2005). The normal pancreas produces 31 units of insulin per day, whereas patients with type 1 diabetes produce only 0-4 units per day, and requires additional insulin from the outside (Pulungan and Herqutanto, 2009).

Based on data from the Health Research, the prevalence of diabetes in Indonesia aged 15 years and over 6.9% of the 176 million people, or about 12 million people in Indonesia suffer from diabetes (Riskesdas, 2013). Of these, 30.4% are already diagnosed with diabetes, and 69.6% of patients with diabetes but have not been diagnosed. Riskesdas estimated 2030 patients with DM in Indonesia will increase two-fold, or about 21.3 million people (Kemenkes RI, 2013).

According Wijayakusuma (2004), treatment of diabetes can be done medically with oral antidiabetic drugs or insulin injections. But because of the high costs, medical treatment is some-

times difficult to do. DM treatment can also be overcome by traditional medicine by using efficacious medicinal plants. One of the plants that can be used as antidiabetic drugs is the Seri plant.

Seri is a plant (*Muntingia calabura L.*) that belongs to genus *muntingiaceae* and already known by the public as a medicinal plant, including antidiabetic, gout, hypertension, laxative productive cough, flu, headache, fever, antiseptic, anti-seizure, gastroprotective activity, antioxidant and anti-inflammatory activities (Kaneeda et al., 1991; Wijoyo, 2004; Teak and Santoso, 2014; Balan T et al., 2015; Halim SZ et al., 2017). Empirically, the series leaves water extract has long been used by the public as antidiabetic drugs. The leaves of this plant contain chemicals known as flavonoids, triterpenoids, tannins, saponins, and glycosides (Amiruddin, 2007). Based on research conducted by Ramdhani (2008), the Seri leaves ethanol extract at a dose of 130 mg / kg body weight can lower blood glucose levels in mice due to DM type-2 induced streptozotocin. Research conducted by the Utama (2011), the group leaves the series ethyl acetate fraction dose of 240 mg / kg proven to lower blood glucose levels in mice induced by alloxan.

Compounds that have the potential to decrease blood glucose levels because in the ethanol extract of seri leaves contains flavonoids that act as antioxidants to inhibit damage to the islet cells of Langerhans in the pancreas by means of regenerating β cells of the pancreas and increases insulin secretion (Sondang et al., 2005). Seri leaves that used is the old leaves. Old seri leaf extract has antioxidant activity much stronger when compared to younger leaves (Mintowati et al., 2013).

Therefore, the research conducted in-vivo test blood glucose lowering alloxan-induced mice using seri leaves extract using

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70% ethanol. The method of diabetes induction in test animals uses alloxan. Diabetogenic substances are destructive selective pancreatic β cells. Furthermore, blood glucose levels will be measured by enzymatic methods GOD-PAP (glucose oxidase phenol 4-aminophenazone). This research is expected to provide information about the value of AUC (Area Under the Curve) as an important parameter that can be determined antidiabetic percentage reduction in blood glucose levels.

2. EXPERIMENTAL SECTION

2.1. Chemicals and Instrumentation

Materials used consisted of Seri leaves (*Muntingia calabura* L.), Wistar strain male rats, 70% ethanol (Brataco®), GOD-PAP kit (Dialab®), standard glucose (Dialab®), alloxan (Sigma Aldrich®), glibenclamide (Indofarma), distilled water (Brataco®), Na CMC 0.5% (Brataco®), and NaCl 0.9% (Merck®).

The tools used in this research, analytical balance (Ohaus®), rotary evaporator (Yamato®), tools glass (Pyrex and Iwaki), equipment maintenance test animals, sonde (MOH), the syringe injection (OneMed®) photometer DTN-410-K (Dialab®), non-EDTA vacutainer tubes (Vaculab®), micro pipette (Eppendorf®), Labopette®, pipette hematocrit (Nesco®), silica gel plate F₂₅₄ (Merck®), and centrifugator (IEC®).

2.2. Preparation of Extract

Seri dry leaves that has been mashed, then extracted by means of 500 grams of powder botanicals are macerated in a glass container protected against sunlight. The first maceration process is done by soaking the powder bulbs use 70% ethanol as much as 3.5 liters and stirred occasionally for 2 x 24 hours. The filtrate is collected and stored maceration results. Remaceration performed on the remaining pulp using 70% ethanol, 1.5 liters for 1 x 24 hours. The filtrate obtained was concentrated using a rotary evaporator to obtain a thick extract.

$$\text{Rendemen} = \frac{\text{weight of extract}}{\text{the weight of the extracted simplicia powder}} \times 100\%$$

2.3. Extract Characterization

2.3.1. Phytochemical Test Using Reagents

Identification of the flavonoids compound by reacting 1 mL of extract with ammonia solution in the ratio (1: 5) followed by the addition of hydrochloric acid. Positive results were marked by the color orange (Sibi et al., 2012).

Identification of the saponin compound by reacting 1 mL of extract by 2 mL of distilled water. The solution was boiled on a water bath accompanied by vigorous shaking. Positive result when the foam formed during one hour (Sibi et al., 2012).

Identification of the tannin compound by reacting with 1 mL of the extract with the addition of 5 mL of distilled water. The solution was boiled on a water bath. After the sample is cooled, it treated with 0.1% FeCl₃ solution until the resulting blue-black color which showed positive results (Sibi et al., 2012).

Identification of the terpenoid compound by reacting with the Liebermann-Burchard reagent in a way as much as 2 mL of the extract was treated with a solution of 0.5 mL of chloroform followed by the addition of 0.5 mL of glacial acetic acid and 2 mL of concentrated H₂SO₄. A positive result is indicated by the formation of a brownish or violet ring between two layers (Ciulei, 1984).

Identification of the glycoside compound by reacting 5 mL of the extract with 2 mL of glacial acetic acid followed by addition of 1 mL of concentrated H₂SO₄. Positive results were characterized by the formation of brown rings (Sibi et al., 2012).

Identification of the alkaloid compounds by reacting 5 mL of the extract with 5 mL of ammonia in chloroform followed by the addition of 2 N H₂SO₄ and will be formed of two layers. The top layer which acts as a water phase is taken and divided into three test tubes. Each test tube was tested using reagents Mayer, Dragendorff, and Wagner. The positive results of each tube characterized by the formation of a white precipitate, sediment deposition orange and brown (Astarina et al., 2013).

2.3.2. Flavonoids test with Thin Layer Chromatography

Flavonoid test of the ethanol extract of seri leaves using TLC with splattering seri extract solution on the TLC plate measuring 5 x 1 cm with an upper limit and a lower limit that has been created using a pencil. TLC plates and then eluted with an eluent mixture of ethanol and ethyl acetate (1: 1). TLC plate which was eluted was observed spots on UV lamps 254 and 366 nm, and then sprayed with cerium (IV) sulfate. Positive results contain flavonoids if the stain turns into brown or brownish-yellow color when observed with the eye (Pratiwi et al., 2013).

2.4. Preparation and Design of Animal Test

Rats were divided into 6 groups. Group 1 was only given food and drink as normal controls. Group 2, as a negative control, induced by alloxan 130 mg/kg of BW, and given 0.5% CMC Na solution. Group 3 as a positive control induced by alloxan 130 mg/kg of BW and given glibenclamide dose of 0.43 mg/200 gBW. Group 4, 5 and 6 induced alloxan 130 mg/kg of BW and given ethanol extract of seri leaves with consecutive doses of 65, 130, and 260 mg/kgBW.

2.4.1. Measurement of Glucose

Blood taken by retroorbitalis plexus of veins in the eye using a hematocrit pipette. Blood collected at the non-EDTA vacutainer tube and centrifuged at 2,500 rpm for 20 minutes to obtain serum. Measurement of glucose is done by adding 1 ml of GOD-PAP reagent in 10 mL of serum. Absorption was measured by using a photometer DTN-410-K at a wavelength of 505 nm (Purnamasari et al., 2014).

2.4.2. Determination of Value Area Under the Curve (AUC)

Changes in blood glucose levels from day 0 (after mice expressed DM) up to day 15 was calculated by the formula AUC₀₋₁₅. According Okta and Sofia (2013), AUC value of blood glucose levels of mice can be calculated using a trapezoid formula according to the following equation, with C as blood glucose levels (mg/dL) and t as time measurements.

$$AUC_{0-15} = \left[\frac{t_1 - t_0}{2} (C_0 + C_1) \right] + \left[\frac{t_2 - t_1}{2} (C_1 + C_2) \right] + \dots + \left[\frac{t_n - t_{n-1}}{2} (C_n + C_{n-1}) \right]$$

Calculation of decrease in blood glucose levels (DBGL), can be calculated using the formula in accordance with the following equation (Kurniawati et al., 2012).

Table 1. Phytochemical test of extract by using reagent

Secondary Metabolite	Extract
Flavonoid	+
Saponin	+
Tannin	+
Terpenoid	+
Glycoside	+
Alkaloid	-

$$\% \text{ DEGL} = \frac{\text{AUC}_{\text{negative control}} - \text{AUC}_{\text{treatment group}}}{\text{AUC}_{\text{negative control}}} \times 100\%$$

The calculation of the effective dose (ED₅₀) can be calculated according to the following equation which is based on the relationship between the percent of blood glucose lowering effects of the extract concentration was analyzed using linear regression.

$$y = a + bx$$

2.5. Data analysis

Normality that the reduction in blood glucose levels were analyzed using the Shapiro-Wilk normality test, normal distribution of data if the value of p > 0.05. The data analysis followed by One Way Anova test to examine differences in some groups sampled by a factor of concentration, if p < 0.05 indicates significant differences.

3. RESULT AND DISCUSSION

3.1. Extraction Plants

Simplicia of seri leaves that has powdered 500 g macerated with 70% ethanol. According to Voight (1984), 70% ethanol is used as an essence solvent for ethanol 70% very effective in generating an optimal active ingredient and can improve the stability of the drug substance is dissolved. After the extraction by maceration, the liquid extract is thickened by a rotary evaporator. Viscous extract obtained from the extraction of as much as 132.28 g with a rendement yield of 26.42%. From these results showed a large enough rendement indicating that that the most important substance extracted from botanicals that quite a lot. According Prasetyorini et al. (2011), the rendement may be affected by the extraction method used

3.2. Characterization Extract

Description: (+) positive and (-) negative

Based on the data in Table 1, the characterization of the extract performed using reagents shown positive results against flavonoids, saponins, tannins, terpenoids and glycosides, but showed a negative result of the alkaloid. This phytochemical test results together with the results of research that has been done Sibi et al. (2012) of the methanol extract of seri leaves that contains flavonoids, saponins, tannins, terpenoids and glycosides. The positive results of the characteristics of the extract by using reagents seen by the change in color, foam formation, and the formation of a layer or ring between two layers of the test solution.

Identification using Thin Layer Chromatography (TLC) is performed to further confirm the results obtained from using the

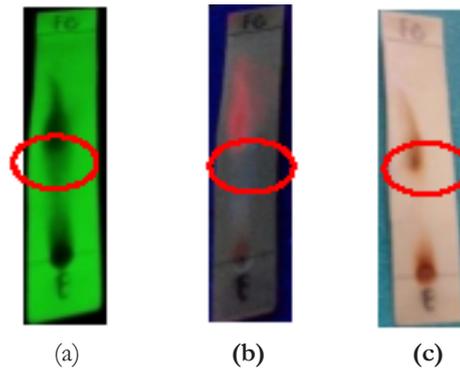


Figure 1. The chromatograms of flavonoid compound in the ethanol extract of seri leaf (a) under UV light 254 nm, (b) under UV light 366 nm and (c) sprayed with cerium (IV) sulfate

phytochemical test reagents. TLC method chosen because it only requires a little solvent, the amount of gear a bit, and convenient sample preparation (Gandjar and Rohman, 2007). The results of TLC flavonoid compounds can be seen in Figure 1.

Based on Figure 1, the TLC test results of flavonoid compounds contained in the ethanol extract of seri leaves, the TLC plate which was eluted using eluent ethanol and ethyl acetate in the ratio (1: 1) was sprayed with spotting visible cerium (IV) sulfate. Positive results contain flavonoids if the stain is brown or brownish yellow. The brown color is formed due to the cerium (IV) sulfate are H₂SO₄ that is a reductant in damaging the chromophore group of active substances so that the wavelength will be shifted toward the longer that stains become visible to the eye (Pratiwi et al., 2013)

3.3. Alloxan inducing

Alloxan used as an inducer in order to test animals used conditioned diabetes mellitus type 1. Alloxan can disrupt cell oxidation process due to the expenditure of the mitochondrial calcium ion homeostasis resulting in disturbance that causes the death of pancreatic cells (Sharma N and Garg V, 2009; Mohammed Fazil Ahmed et al., 2010; Verma L et al., 2010; Rotimi SO et al., 2011; Okey A. Ojiako et al., 2016; Attia ES et al., 2017).

Based on Figure 2, The normal group is not induced by alloxan, so that the blood glucose levels remain stable in the normal range is equal to 99 mg/dL. BGL value after induction of negative control group until the third treatment group experienced a significant improvement is 253-298 mg/dL. This indicates that the rats have experienced DM for BGL values > 200 mg/dL.

3.4. Antidiabetic Activity Test

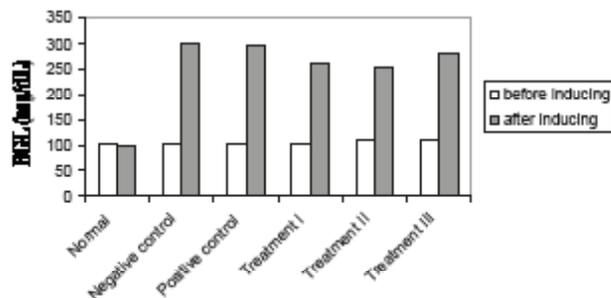


Figure 2. Effect of blood glucose levels of rats before and after inducing alloxan

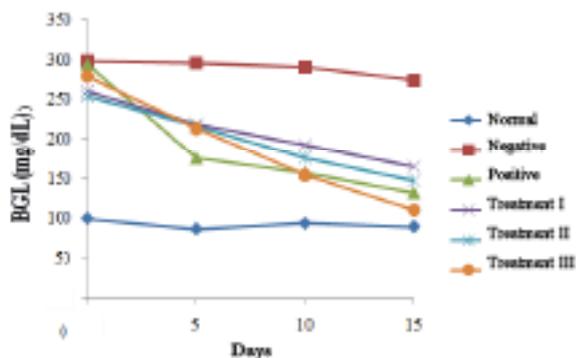


Figure 3. Effect of seri leaf extract on blood glucose levels for each group on days 0 to 15

After the test mice confirmed DM, hereinafter rats given the treatment for 15 days. Blood glucose levels were measured on day 5, 10 and 15 after being treated by using a photometer DTN-410-K at a wavelength of 505 nm and then calculated the average blood glucose levels from day 0 to day 15.

Based on Figure 3, it can be seen that the normal blood glucose levels stable group is in the range of normal blood glucose levels <126 mg/dL. This is because in normal group was not given any treatment other than the provision of feed and water.

The negative control group was given 0.5% CMC Na solution. CMC Na solution is used as a negative control, since Na CMC used as suspending the test preparation, so it can be ascertained that the Na CMC will not affect the decrease in blood glucose levels of mice (Illyani et al., 2015). In this group mouse blood glucose levels are still in a state of diabetic because giving a solution of Na CMC does not have an activity that can lower blood glucose levels,

Blood glucose levels in the positive control group decreased. This is because glibenclamide at a dose of 0.43 mg/200gBW capable of lowering blood glucose levels. According Sukandar et al. (2008) mechanism of action of glibenclamide, which stimulates the secretion of insulin from pancreatic Langerhans β cells. Membrane depolarization occurs due to the interaction glibenclamide with the ATP-sensitive K-channels in the cell membrane β. The effect is the further opening of calcium channels (Ca). The opening of the canal will lead to Ca²⁺ ions enter the cell β would then stimulate the β cells to secrete insulin.

Another reason glibenclamide election as a positive control that enable the sharing of route of administration is oral treatment, so that the time to achieve a therapeutic effect of the treatment groups can be close to or the same as the positive control group. It is not suitable when the insulin that is used as a positive control, and also in consideration of the price of insulin is relatively expensive. The working mechanism of a class of oral antidiabetic drugs (for diabetes mellitus type-2) the other is not suitable when used as a positive control for type-1 diabetes mellitus. The result is done on the research that has been done Ratimanjari (2011) using glibenclamide as a positive control in rats induced by alloxan.

The treatment group I, II and III (suspension of the ethanol extract of seri leaves of the dose 65 mg / kg of BW, 130 mg / kg of BW, and 260 mg / kg of BW) decreased blood glucose levels significantly. The treatment group which has the effect of lowering blood glucose levels are greatest III treatment at a dose of 260 mg / kg of BW, followed by treatment group II at a dose of 130 mg / kg of BW and the treatment group I at a dose of 65 mg / kg of BW. A decrease in blood glucose levels is caused by the ethanol extract of seri leaves of the flavonoids contains that have antidi-

Table 2. Data on average body weight (BW) rats

Group	Before alloxan induced	After alloxan induced	Day 5	Day 10	Day 15
Normal	215	219,3	222.3	226	230
Negative control	211	194.7	196	197	212.3
Positive control	202.3	186	207.7	205.7	215
Treatment I	193	173.7	179.7	187	196.7
Treatment II	220.3	194	199.7	204.7	221.3
Treatment III	209	192	204.7	216.3	227.3

abetic activity. According Sondang et al. (2005), flavonoids have antioxidant properties that can protect beta cell damage from free radicals. Antioxidants inhibit damage to the islet cells of Langerhans in the pancreas by means of regenerating beta cells of the pancreas and increases insulin secretion (Balan T et al., 2015). In addition, flavonoids can restore the sensitivity of insulin receptors on the cells so that the blood glucose down and return to normal (Ramdhani, 2008).

Weighing of the mice before and after treatment was also performed to see the differences. At the time prior to induced, average body weight of rats ranges from 193 to 220 grams (can be seen in Table 2). But after induced, the weight of negative control group mice up to the treatment group experienced a decline, except normal group. This is because the normal group was not induced by alloxan, so that the weight remains stable and after 15 days being treated, body weight of rats all test groups had increased, but not significantly.

This weight loss occurs due to the inability of the body to provide glucose due to lack of insulin to be burned into energy that the body more use of fatty acids and makes the protein as an energy source, while the normal group of mice were given food and drink plain that he was still able to secrete insulin properly (Lehninger, 1982).

3.5 Determination Value of Area Under Curve (AUC)

Having discovered that the average blood glucose levels, AUC₀₋₁₅ value is then calculated to determine changes in blood glucose levels from day 0 to day 15. AUC₀₋₁₅ data is presented in Table 3. Changes in blood glucose levels of each treatment group by calculating the area under the curve (AUC) at day 0 to day 15 (AUC₀₋₁₅). AUC₀₋₁₅ has value inversely with antidiabetic activity. According Chotimah et al. (2008), the lower the AUC value of the treatment group, the better the activity in the reduction of blood glucose levels. The greater the percentage decrease in blood glucose levels, then the better antidiabetes activity.

Based on data from Table 3, the normal group had AUC₀₋₁₅ value of 1380. This is due to the normal group was given no treatment is approved, but only given standard feed and drinking water. Negative control group had the highest AUC₀₋₁₅ value is 4367.5. This is due to the negative control group were given a 0.5% solution of Na CMC does not have the effect of lowering blood glucose levels. Positive control group had an average value of AUC₀₋₁₅ the lowest, followed by treatment group III, II and I.

Having obtained the AUC₀₋₁₅ value, then the calculated percent reduction in blood glucose levels. The greater the percentage decrease in blood glucose levels, the better the antidiabetic activity.

Based on data from Table 4, the normal group is not calculated the percentage reduction in blood glucose levels. This is because the normal group did not decrease blood glucose levels for

Table 3. Calculation of AUC₀₋₁₅

Group	Average of AUC ₀₋₁₅
Normal	1380 ± 7,50 *
Negative control	4367.5 ± 36.42 *
Positive control	2732.5 ± 237.39
Treatment I (65 mg/kgBW)	3105 ± 146.39
Treatment II (130 mg/kgBW)	2962.5 ± 183.86
Treatment III (260 mg/kgBW)	2810 ± 284.23

Table 4. Data percent decrease in blood glucose levels (%DBGL)

Group	%DBGL
Normal	-
Negative control	0
Positive control	37.43
Treatment I (65 mg/kgBW)	28.9
Treatment II (130 mg/kgBW)	32.16
Treatment III (260 mg/kgBW)	35.66

this group were not given treatment is approved, but only given standard feed and drinking water. Negative control group had a percentage decrease in blood glucose levels 0%. This is due to the negative control group were given a 0.5% solution of Na CMC does not have the effect of lowering blood glucose levels. The positive control group has a percentage decrease in blood glucose levels were highest, followed by treatment group III, II treatment, and treatment I.

Data reduction in blood glucose level each group has been obtained, then statistically analyzed using SPSS 23.0. Of Shapiro Wilk normality test results, note that the data reduction of blood glucose levels of each group normally distributed and did not differ significantly (p > 0.05). Subsequent analysis of parametric statistical analysis by ANOVA one way with a 95% confidence level. From the results of the parametric statistical analysis by ANOVA one way, it is known that there are significant differences between the groups (p < 0.05).

Data from one way ANOVA analysis a significant difference, then continued test post hoc LSD (Least Significant Differences). From the analysis of LSD post hoc test, it is known that there are no significant differences in blood glucose levels of data reduction the positive control group compared with each treatment group (p > 0.05). But there is a significant difference between the positive control group and the treatment of the normal group and negative control group (p < 0.05).

Based on the results of quantitative analysis using SPSS version 23.0 above, the effect of decreasing blood glucose levels of mice of glibenclamide (positive control) is better than ethanol extract of seri leaves doses of 65 mg/kgBW, 130 mg/kgBW, and 260 mg/kgBW, but there is no significant difference. All three doses of the extract treatment proven to have the potential to decrease blood glucose levels strain Wistar male rats induced by alloxan.

Decreased glucose levels is the case because in the ethanol extract of seri leaves of the contains flavonoid compounds that can act as an antioxidant that can inhibit damage to the islet cells of Langerhans in the pancreas by means of regenerating beta cells of

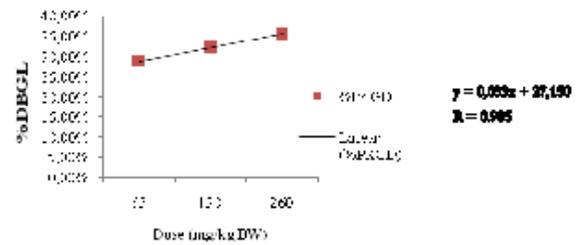


Figure 4. Graph of linear regression between the dose (mg/kgBW) and %DBGL ethanol extract of seri leaves

the pancreas and increases insulin secretion (Mrowicka M, 2005; Sondang et al., 2005; Balan T et al., 2015). Flavonoids are protective against damage to the insulin-producing beta cells as well as to improve insulin sensitivity (Panjuantiningrum, 2010). Flavonoids can also prevent diabetes by inhibiting the enzyme alpha glycosidase which serves for the breakdown of carbohydrates. Inhibition of this enzyme alpha glycosidase causes delays glucose absorption which in turn will lower blood glucose levels (Arjadi and Susatyo, 2010).

3.6 Effective Dose 50 (ED₅₀)

ED₅₀ value of the ethanol extract of leaves of the series is determined to find the dose that can cause blood glucose-lowering effect in 50% of individuals (animal studies). ED₅₀ value was calculated by linear regression between the dose and the percent reduction in blood glucose levels (%DBGL). Results of the linear regression between the dose and the percent reduction in blood glucose levels can be seen in Figure 4.

Linear equations obtained is $y = 0,033x + 27.150$ with a correlation coefficient (R) = 0.985. From the obtained equation, ED₅₀ of ethanol extract of seri leaves can be calculated, where y is the percent effective dose (50%) and x is the dose of ethanol extract of seri leaves that can decrease blood glucose levels of mice by 50%. From the calculation, the obtained ED₅₀ of ethanol extracts of seri leaves is 692.424 mg/kg. Thus, the dose required to achieve an effective dose for 50% in the reduction of blood glucose levels is 692.424 mg/kgBW.

4. CONCLUSION

Ethanol extract of seri leaves can give the effect of decreasing blood glucose levels dose 65 mg/kgBW, 130 mg/kgBW, and 260 mg/kgBW by percent decrease in blood glucose levels respectively by 28.90%; 32.16%; and 35.66% and has a value of AUC0-15 each group of 3105; 2962,5; and 2810. The effective dose (ED₅₀) of ethanol extracts of seri leaves is 692.424 mg/kgBW.

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