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EFFECT OF ETHANOLIC EXTRACT OF *Annona muricata* L SEEDS POWDER TO DECREASE BLOOD GLUCOSE LEVEL OF WISTAR MALE RATS WITH GLUCOSE PRELOAD

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BACKGROUND

Based on data from World Health Organization (WHO) diabetes mellitus was the direct cause of death of 1.5 million people, more than 80% of deaths due to diabetes occurs in middle-income countries and low (WHO, 2014). According to the Basic Health Research (Riskesdas) in 2013, the incidence of diabetes mellitus in Indonesia increased by two times greater than in 2007.

Annona muricata L is used as a traditional medicine to lower blood glucose levels with contain tannin, phytosterols, calcium oxalate, alkaloids murisin (Arief and Hariana, 2006), flavonoids and essential oils (Surbakti, 1994). Flavonoid compounds have an important role in the prevention of diabetes and its complications (Jack, 2012). Previous research showed that bark of plants soursop has a most excellent efficacy compared with the roots and leaves of the *Annona muricata* L in lowering blood glucose levels in male rats induced streptozozin (Rahmawati, 2014).

This study aimed to examine the effect of ethanolic extract of *Annona muricata* L. seeds powder on decreasing blood glucose level in wistar strain male rat with glucose loading.

MATERIALS AND METHODS

Materials

The materials used in this study consisted of *Annona muricata* L seeds, animals used were male rats Wistar strain obtained Laboratory of Pharmacology Muhammadiyah University of Purwokerto. The chemicals used in this study was 96% ethanol, GOD-PAP reagent, distilled water, 1% NaCMC, glibenclamide, glucose monohydrat, sitroborat reagents, reagent FeCl3 reagent dragendroff, aquabidest.

Sample Preparation and Animal Testing

Annona muricata L seeds determination tested, cleaned, and dried. 100 grams of *Annona muricata* L seed powder was extracted by maceration using 96% ethanol for 5 days. The ethanolic extract is separated from the solvent with a rotary vacuum evaporator to obtain viscous ethanolic extract. Before being used for the trial test animals adapted for 7 days for male Wistar rats can adapt to the environment and allowed free access to standard pellet diet and water ad libtum

Preliminary phytochemical screening

The ethanolic extract of *Annona muricata* L seeds was screened for the presence of alkaloids, flavonoids, and tannins according to established testing protocols.

Experimental animals

The rats were divided at random into six groups. Then all the rats have blood drawn separately determine blood glucose levels early. After that in each group were treated as follows :

Group I : Negative control (1% CMC-Na)
Group II : Positive control (glibenclamide 0.6 mg / KgBW)
Group III : Mice untreated (as normal controls)
Group IV : Diabetic rats given ethanolic extract 100 mg / kgBW
Group V : Diabetic rats given ethanolic extract 200 mg / kgBW
Goup VI : Diabetic rats given ethanolic extract 400 mg / kgBW

Thirty minutes later the rats were given glucose at a dose of 1.35 grams / 200 gramBB. Blood was taken 30 minutes after administration of glucose from the rats. Blood sampling performed at the minute -60, -30, 0, 30, 60, 90, and 120. Blood glucose levels are determined enzymatically with the GOD-PAP reagent and absorbance read with UV-Vis spectrophotometer at a wavelength of 500 nm.

Sampling and Blood Glucose Determination

Animal test blood taken from the tail section as 0,5-1mL, accommodated in Eppendorf tubes. Then blood and serum separated by centrifuge for 15 minutes at a speed of 2500 rpm. Separate plasma glucose levels were taken and determined. For absorbance measurement with spectrophotometer.

Data Analysis

Analysis of blood glucose level is obtained treated statistically using One Way *ANOVA* statistical analysis using SPSS with 95% confidence level and *Post Hoc LSD* test.

RESULTS AND DISCUSSION

Ethanolic extract of *Annona muricata* L seeds obtained by maceration method, because this method is easier in the process and the equipment used is simple, in addition maceration method is also advantageous because the compound of natural materials will be submerged so that the breaking of walls and the cell membrane due to the pressure difference between the inside and outside cells, so that the secondary metabolites present in the cytoplasm will be soluble in organic solvents and extraction of compounds will be perfect. The solvent used is 96% ethanol for polar so that the compounds contained in the seeds of *Annona muricata* L be interested (Sharon, 2013).

The 96 % ethanolic seed extract of *Annona muricata* L gave a greenish brown semi-solid product with a percentage yield of 7.6%. Preliminary phytochemical screening indicated that the extract contains flavonoids, alkaloids, and tannins.

Hypoglycemic effect of soursop seeds are determined by a decrease in blood glucose levels using the oral glucose tolerance test (UTGO). The principle works are burdening with glucose test animals up to a state of hyperglycemia without damaging the pancreas of experimental animals. Animals were fasted but still given to drink ad libitum before being treated. It aims to avoid the influence of food that can affect even enhance blood glucose levels when the mice weighed glucose (Asmonie, 2013).

Determination of blood glucose levels is done by enzymatic methods, namely by adding a reagent GOD-PAP. There was a reaction between glucose and H_2O_2. Hydrogen peroxide is formed (H_2O_2) will react with a 4-aminoantipirin and then form kuinoimin phenol red violet. The intensity of the red color produced kuinoimin shows blood glucose levels (Dias, 1999).

From the data shown in Table 1 indicate that the glucose loading at a dose of 1.35g/ KgBW can cause a hyperglycemic condition in test animals. Blood glucose levels are negative control group started up in minute 30 which had an average 163,23mg / dL but at 120 minutes to start to decline by an average of 164.33 mg / dL. This increase resulted mostly glucose is absorbed by the gastrointestinal tract and into the body on a half-hour after glucose loading.

This is according to research conducted by Kurniawan (2011) when blood glucose levels will rise at minute 30 after administration of glucose orally group negative control begins to experience an increase in blood glucose levels at minute 30 and continued right up to the 90th minute, it occurs due to the negative control only treated with a solution of Na CMC so that blood glucose levels tend to remain high. The treatment group were given a soursop seed extract dose of 100 mg / dL, 200 mg / dL and 400 mg/dL experienced elevated levels of glucose in minute 30 to minute 90, this happens because of the negative control only treated with a solution of Na CMC that blood glucose levels tend to remain high.

The treatment group were given extracts of the seeds of soursop dose of 100 mg/ dL, 200 mg / dL and a dose of 400 mg / dL increased levels of glucose in minute 30 to minute to minute 60, but in the 90th minute began to decrease blood glucose levels. Decreased levels of glucose in the test group proved that soursop seed extract has a hypoglycemic effect after 90 minutes of administration of glucose.

Table 1. Average blood glucose levels for each treatment and the mean AUC

The average blood glucose levels ± SD (mg / dl) in minutes						
Time (minute)	**Positve control**	**Negative control**	**Normal control**	**Dose 100 mg/KgBB**	**Dose 200 mg/KgBB**	**Dose 400 mg/KgBB**
T-60	97,32±15,29	109,33±13,64	108,38±3,53	113,74±11,61	103,00±9,68	104,58±6,95
T-30	84,10±13,31	104,73±6,59	104,76±3,65	105,76±23,11	98,03±14,31	98,29±5,57
T0	90,02±8,77	110,14±3.62	110,47±2,28	119,29±1.55	105,26±14,61	107,99±9,89
T30	124,35±15,58	163,23±11,92	100,51±4,67	133,75±8,91	123,23±11,34	138,39±15,92
T60	138,74±17,70	168,69±9,10	98,04±7,06	161,34±21,05	134,60±6,31	144,13±9,79
T90	121,44±15,97	174,71±9,10	95,41±4,63	159,00±4,63	128,97±4,45	130,68±4,08
T120	108,16±17,50	164,33±7,12	102,92±5,21	149,38±4,48	123,18±11,40	109,37±6,71
AUC	19840,31±2329,33	24792,15±1975.54	18445,76±257,21	24321,75±1259,03	21024,07±1961,73	20750,97±2728,91

Data blood glucose levels in each group were statistically analyzed using the Kolmogorov-Smirnov test is then followed by analysis of One Way ANOVA and Post Hoc Test with a confidence level of 95%. Furthermore, to determine blood glucose levels on a minute specific for each test animals in all treatment groups using the calculation Area Under the Curve (AUC0.n). The significance of the statistical analysis of the *Kolmogorov-Smirnov* and *Levene* statistics for the main test showed normal distribution of data (P = 0.410; p> 0.05). This shows that the AUC (-60) -120 of each test is normally distributed and had homogeneous variant, then performed the test using One-Way ANOVA.

Results One-Way ANOVA test of the six treatment obtained significance value less than 0.05. So that there is a difference in lowering blood glucose levels between the positive control, negative control, the normal controls, a dose of 100 mg / KgBW, a dose of 200 mg / KgBW, and dosis 400 mg / KgBW. Subsequent analysis that LSD test to determine and compare the real differences between the treatment groups with each other in lowering blood glucose levels. Based on the results of LSD test data is known that there is a significant difference between the positive control and a negative control (p=0.001;<0.05).

Positive controls had a nonsignificant difference with the ethanolic extract doses of *Annona muricata* L seeds 200 mg / KgBW and 400 mg / KgBW, it indicates that have an effect in reducing blood glucose levels were similar to controls positive form of suspension glibenclamide. Furthermore, to determine blood glucose levels on a minute specific for each test animals in all treatment groups using the calculation Area Under the Curve (AUC0.n). AUC value (-60) -120 is inversely proportional to the effect of a decrease in blood glucose levels. The smaller the value AUC maka the greater the effect of decreasing blood glucose levels (Baroroh, 2011).

Based on AUC values histogram (-60) -120 (min mg / dL) of blood glucose-lowering effect is greatest soursop seed ethanolic extract treatment dose of 400 mg / KgBW with a value amounting to 20750.97 AUC because it has the lowest AUC compared nilia AUC with a dose of 100 mg / KgBW and a dose of 200 mg / KgBW. AUC value (-60) -120 is inversely proportional to the effect of a decrease in blood glucose levels. The smaller the AUC value, the greater the effect of a decrease in blood glucose levels (Baroroh, 2011). AUC value (-60) -120 all smaller than the test group AUC value (-60) -120 negative control. This shows that all three doses of ethanol extract of soursop seeds can lower blood glucose levels.

A decrease in blood glucose levels occurs presumably because their secondary metabolites contained in the ethanol extract of the seeds of soursop are flavonoids, tannins and alkaloids. Alkaloid compounds can inhibit the enzyme alpha-glucosidase in duodenal mucosa so that the decomposition reaction of polysaccharides into monosaccharides can be inhibited. Thus the glucose is released more slowly and less absorbsinya into the blood faster, lower and evenly, so that the peak blood sugar levels can be avoided (Tjay and Rajardja, 2007). This research is in accordance with Amudhan (2012) which states that the alkaloid found in betel nut can lower blood glucose levels.

Flavonoid compounds have a hypoglycemic effect by several mechanisms by inhibiting the absorption of glucose, improve glucose tolerance, stimulates the release of insulin, increasing glucose uptake by peripheral tissues and regulate enzymes involved in carbohydrate metabolism (Brachmachari, 2011). Besides alkaloids and flavonoids, tannin in the *Annona muricata* L seed has the ability as an astringent, which can precipitate proteins on the surface of the mucous membrane of the small intestine and form a layer that protects the intestine, thus inhibiting the absorption of glucose (Monica, 2006).

This study showed that the ethanolic extract *Annona muricata* L seeds has potential as an alternative treatment of diabetes, but further research is needed to determine the content in the *Annona muricata* L seed specific role in decreasing blood glucose levels.

CONCLUSION

The crude extract *Annona muricata* L seeds has shown significant hypoglyceamic and oral glucose tolerance improving effects. The effective dose of the extract was found to be 400 mg/kg. Moreover, it supports the traditional use of this plant to help decrease of blood glucose level.

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