ANTI DIABETIC ACTIVITY OF ETHANOL EXTRACT AND CHLOROFORM EXTRACT *Annona muricata* LEAF IN ALLOXAN INDUCED RATS

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Abstract

Background. *Annona muricata plant is a medicinal plant using by research and drug for human healthy including diabetes mellitus.*

Objective. *This research aimed to determine the effect of ethanol extract and chloroform extract of the sirsak leaf as antidiabetes mellitus.*

Methods. *This research was conducted in 9 groups of male Wistar rats consisting of 5 rats per group, consisting of normal control, alloxan control, glibenclamide control dose of 10 mg/Kg BW, group of ethanol extract sirsak leaf dose of 50 mg/Kg BW; 100 mg/Kg BW; 200 mg/Kg BW, and group of chloroform extract sirsak leaf dose of 50 mg/kg BW; 100 mg/Kg BW; 250 mg/Kg BW. Tests carried out for 2 weeks.*

Outcome measured. I*t also conducted assays of total flavonoids and histopathological tests of pancreatic* β *cells.*

Results. *Results of this research showed that the ethanol extract of sirsak leaves dose of 200 mg/Kg BW has activity in decreasing blood glucose levels better than any other group, with the percentage of total flavonoid 12.5%. Histopathological results of the ethanol extract of sirsak leaf has the same capabilities as compared with glibenclamide in reapair the damage of pancreatic* β *cells.*

Conclusion. *The conclusion of this research is the ethanol extract of sirsak leaf have activity antidiabetic mellitus.*

Keywords : *Annona muricata, antidiabetic, alloxan, blood glucose*

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INTRODUCTION

Diabetes mellitus (DM) is one of the most common endocrine and metabolic disorders in the 21st century, and a major threat to health worldwide. Many experimental and clinical observations have indicated that hyperglycemia may directly or indirectly contribute to the formation of excessive free radicals (Ceriello, 2003). Various epidemiological studies have shown a tendency for an increase in the incidence rate and prevalence of DM from year to year. For Indonesia, the WHO predicts rise in the number of patients from 8.4 million in 2000 to be approximately 21.3 million in 2030. Diabetes mellitus is a chronic disease that is characterized by the presence of abnormalities in the metabolism of carbohydrates, lipids, proteins and are associated with insulin deficiency (Suryawanshi *et al.*, 2006). Among the various diseases diabetes, more than 95% of people with diabetes is type 2 diabetes mellitus (T2DM) as well as the type at most disputed. The symptoms of type 2 diabetes mellitus, among others, due to pancreatic dysfunction and increased levels of lipids, fatty acids and cholesterol in the blood (lipemia) (K.A. Wadkar. *et al*, 2008). Annona muricata is a plant of the family Annonaceae. Medicinal plants have been used as a natural remedy for many diseases, one of which is for the treatment of diabetes mellitus (Adeyemi, *et al,* 2009). The bark, roots and leaves of Annona muricata has been reported to be used as an anti-diabetic. Therefore this study was designed to confirm the effects of the ethanol extract and chloroform extract of leaves of Annona muricata on glycemic control in diabetic rats in the alloxan induction, so the results of this study may help in the selection of treatment of diabetes mellitus even better.

METHODS

Materials and Instruments

Materials : the leaves of soursop (*Annona muricata*), ethanol 70%, chloroform, male Wistar rats aged 2-2.5 months with a weight of 190-210 grams, rat pellets, formalin 10%, glibenclamide, alloxan, Na CMC, NaCl 0,9%, aluminum chloride 10%, sodium acetate, coloring Gomori.

Instruments : analytical balance, maceration tools, grinding machines, sieve mesh 40 and mesh 30, separating funnel, rotary evaporator, oral syringes, syringes injection, On Call Plus Blood Glucose Test Strips, glucometer, Spectrophotometer Shimadzu UV-1800, centrifuges, glassware and tools that are commonly used.

Sample Preparation and Anti Diabetes Test

Leaves of soursop (*Annona muricata*) is obtained then do the sorting. Soursop leaves are dried in the powder and sieved to 30 mesh and 40. Extract prepared by maceration using ethanol 70% and chloroform. All maserat collected and evaporated with a rotary evaporator to obtain the viscous extract, weighed and recorded randemen obtained. TLC made to extract the ethanol and chloroform extracts. Stationary phase such as silica gel 254 nm, Butanol: Glacial Acetic: Water = 4: 1: 5 by comparison quercetin. Total flavonoid levels using a spectrophotometer Shimadzu UV -1800 where determination of total flavonoids in accordance with the method of Chang *et al* (2002).

Adaptation of the rats

Adult Wistar rats aged 2-2.5 months with 190-250 gram weight, male sex are maintained in wire cages with room temperature and experience 12-hour cycle of day and night. The rats were fed with pellets and given drink in moderation.

Rats were fasted overnight before intraperitoneal injection of alloxan created using new. Alloxan at a dose of 160mg/kg body weight dissolved in normal saline until dissolved. Fourth day after alloxan injection, blood was taken via the tail vein in the then measured serum fasting blood glucose when blood glucose levels 200-300mg/dl then the rat can be categorized as

diabetic rats. (Dhandapani, 2002; Chougale *et al.*, 2007).

Tests were carried out on 9 test groups each group consisted of 5 rats. Control group consisted of normal, alloxan control, glibenclamide control, ethanol extract group doses of 50mg/kg dose; 100mg/kg; 200mg/Kg, and chloroform extract group doses of 50mg/kg; 100mg/kg; 200mg/Kg. This treatment performed for 2 weeks.

Blood Glucose Measurement and Histopathology

Glucose test performed at baseline before induction of alloxan, after induction of alloxan, week 1 and week 2 to see the comparison. Testing is done by glukometer (On Call Plus Blood Glucose Test Strips). On day 14 control rats glibenclamide, pain control, normal, and ethanol extract group and the chloroform extracts were sacrificed by total anesthetized using inhaled chloroform. The pancreas from each group of rats collected by surgery. Pancreatic cleaned with normal saline and then stored in a tissues fluid-filled pot 10% formalin. Tissue homogenates and then stored for histopathological test-pancreatic β cells by using the method of Gomori.

Statistical analysis

Statistical analysis was done by using Mann-Whitney Results are expressed as the mean ± SD. Statistical significance was defined as $P < 0.05$.

RESULTS AND DISCUSSIONS

Total Flavonoid test

Determination of total flavonoid content using UV-Vis spectrophotometry. As standard used quercetin, a flavonoid compound commonly used identifier and quercetin is a flavonoid class of active substances which are biologically very strong. From the calculation of the percentage of total flavonoid levels of ethanol extract was 12.5% and the percentage of total flavonoid levels of chloroform extract was 5.06%. Flavonoids contain a conjugated aromatic system therefore shows a strong absorption band in the ultraviolet and visible area. Determination of flavonoids by using the method of Chang et al (2002), where the addition of a sliding AlCl3 reagent causes a bathochromic shift enables showed in the o-hydroxy group on ring A. While the addition of NaOAc shift reagent will cause bathochromic shift indicating a hydroxyl group (OH) at the C-7 position. Flavonoid easy to detect using UV light, it because of phenyl ring on flavonoid (Andersen and Markham, 2006).

Test of antidiabetic mellitus effects of ethanol and chloroform extract of soursop leaf to decrease blood glucose levels

Table I shows the data of blood glucose rats before and after induction of alloxan. From the the data shows that after alloxan administration for 4 days, blood glucose in mice induced increased.

Before the induced with alloxan, fasting blood glucose levels did not differ significantly ($p < 0.05$) between the eight groups of experimental animals. At 24 hours after alloxan administration, blood glucose levels were significantly ($p < 0.05$) higher in the group of animals ethanol extract 50 mg / kg bw and the negative control group which showed blood glucose levels 600 mg / dL. Increased glucose levels in rats that elevated levels 200 mg / dL were categorized as diabetic rats.

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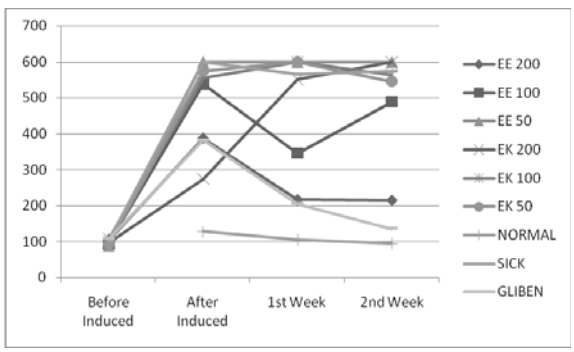
Table I. Blood Glucose Levels Average During the Treatment

Group	Before induced	After Induced	1st week	2nd week
NORMAL	-	127.6±9,83#	105.5±3,41#	95±9,59#
SICK	102.25±2,06	600±0*	565.4±28,31*	574.8±21,47*
GLIBEN	108.5±9,84	382±57,51*#	205.25±115,79#	135.25±13,69*#
EE 200	104±1,82	386.6±195,18*	216±94,08*#	214±85,14*#
EE 100	94±1,82#	538.25±71,36*	345.4±168,47*#	488.2±153,09*
EE 50	88.75±8,99#	600±0*	600±0*#	600±0*
EK 200	97.25±14,15	275±25*#	551.75±55,72*	600±0*
EK 100	107.25±3,5	556±50,91*	600±0*#	564.5±34,97*
EK 50	92.5±6,45#	575±28,87*	600±0*#	545.67±1,52*#

* p <0.05 significantly different compared to the normal group # p <0.05 significantly different compared with those sick



Picture I Blood Glucose Levels Average Graphic From Each Group

Description :

EE 200 : Ethanol Extract dose 200 mg/Kg BW

EE 100 : Ethanol Extract dose 100 mg/Kg BW

EE 50 : Ethanol Extract dose 50 mg/Kg BW

EK 200 : Choloform extract dose 200 mg/Kg BW

EK 100 : Choloform extract dose 100 mg/Kg BW

EK 50 : Choloform extract dose 50 mg/Kg BW

SICK : Negative controls, alloxan induced group dose 160 mg/Kg BW with blood glucose levels> 200 mg / dL

GLIBEN : Positive controls Glibenklamide dose10 mg/Kg BW

NORMAL : Group Without giving alloxan group, glibenclamide, ethanol extract and chloroform extract

Decrease in glucose levels of instability that occurred in the first week and the second week in which the first week of a decline in glucose levels but after the second week of increased glucose levels back can be seen from the above data. A steady decline in glucose levels only seen in the ethanol extract dose of 200 mg / kg bw.

Data homogeneity tested by ANOVA analysis and show that the data are not distributed homogenous. Data were tested for normality using one-sample analysis of the Kolmogorov-Smirnov test showed that the data were normally distributed for each group except the group after induced where the data is not normal. To see a significant difference from each treatment group can be analyzed using the non-parametric Mann-Whitney test. Said to be significantly different values when the value of significance p <0.05. Alloxan diabetes inducer is a compound commonly used in addition to streptozotocin.

Histopathology of Beta Cell Pancreas

Reading test result pancreatic beta cells can be seen in Table II and Picture II. the result showed that the number of pancreatic beta cells are still normal per surface area of the islets of Langerhans 400x400 micro. Pancreas were taken on day 14 one hour after administration of a preparation. Then the organ was cleaned in a solution of normal saline and immersed in 10% formalin solution to prevent organ damage. Organs are then taken to the Pathology Laboratory of the Faculty of Medicine, University of Gadjah Mada made preparations for pancreatic histology Chromalum Gomori coloring technique.

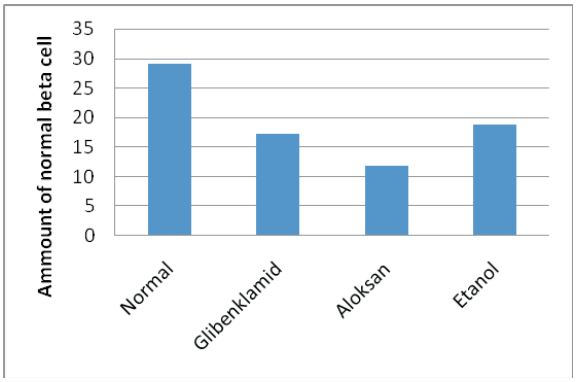
alloxan-induced damage than the islets of Langerhans of normal rats.

Picture III. Pancreas Histology With Gomori Chrome Alum Hematoxylin-Phloxine Staining. Whereas showed alpha cell (A); beta cell (B); vacuolization cell (C); hyperthropy cell (D).

From the picture is known that pancreatic beta cells undergo various damages including vacuolization, necrosis, and pancreatic beta cells hiperthropi. Sick group did not differ significantly with glibenclamide group and the group of ethanol extract dose of 200 mg / kg bw. However, glibenclamide group with a group of ethanol extract dose of 200 mg / kg bw no more

Table II. Histology test of beta cell pancreas with Gomori Chrome Alum Hematoxylin-Phloxine staining method

Group	Beta pankreas
Normal	29,00±7,09
Glibenklamid	17,25±6,95
Aloksan	11,78±3,34
Ethanol Extract 200 mg/Kg BW	18,75±6,27



Picture II. Histopathology Graphic of Beta Cell Pancreas

The data shows that the normal group had a number of healthy beta cells are still more than the other groups. While the alloxan-treated group had a number of less healthy beta cells, this is due to the islets of Langerhans of rats

different than the pain group. It can be concluded that the improvement of pancreatic beta cells by ethanol extract dose of 200 mg / kg bw comparable with glibenclamide administration.

On preparations seen a few different colors, where each color indicates a different cell. In the islets of Langerhans are three types of cells: alpha cells, beta cells and delta cells, and a few cells is not clear that granular (Permata, 2006).

CONCLUSION

Ethanol extract and chloroform extract of leaves of soursop (*Annona muricata*) various doses have not been able to lower blood glucose levels as well as the glibenclamide group.

Decrease in blood glucose levels can decrease and consisten tly better than the other treatment groups was 200mg/Kg BW ethanol extract dose with a significant value compared

with the normal group of post-induction, the first week and the second week in a row is 0.009; 0,020; 0,014. Repair of pancreatic beta cells by ethanol extract doses 200mg/KgBW comparable with glibenclamide administration.

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REFERENCES

Adewole S., Ojewole J A O., 2009, Protective Effects of Annona Muricata Linn. (Annonaceae) Leaf Aqueous Extract on Serum Lipid Profiles and Oxidative Stress in Hepatocytes of Streptozotocin-Treated Diabetic Rats. *African Journal of Traditional, Complementary and Alternative Medicines (AJTCAM)*., 6, 30-41.

Adeyemi O. D., Komolafe O. A., Adewole O. S., Oboutor E. M., Adenowo T. K., 2009, Anti Hyperglycemic Activities of Annona Muricata (Linn). *African Journal of Traditional, Complementary and Alternative Medicines (AJTCAM)*., 6, 62-69.

Andersen,Ø.M., and Markham,K.R., 2006, *Flavonoids : chemistry, biochemistry, and applications,* United State of America CRC Press, Taylor & Francis Group

Chang, Chia-Chi., Yang, Ming-Hua., Wen, Hwei-Mei., Chern, Jiing-Chuan., 2002, Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods, *Jounal of Food and Drug Analysis*, Mei, 10., 3, 178-182

Ceriello A., 2003, New insights on oxidative stress and diabetic complications may lead to a "causal" antioxidant therapy. Diabetes Care. 26, 1589–1596.

Chougale, A. D., Panaskar, S. N., Gurao, P. M., Arvindekar, A. U., 2007, Optimization of Alloxan Dose is Essential to Induce Stable Diabetes for Prolonged Period, *Asian Journal of Biochemistry*, 2 (6), 402-408

Dhandapani, S., Subramanian, V. R., Rajagopal, S., Namasivayam, N., 2002, Hypolipidemic Effect of *Cuminum cyminum* L. on Alloxan- Induced Diabetic Rats. *Pharmacological Research*, Juni., 46, 3, 251-255.

K.A. Wadkar, C.S Magdum, S.S.Patil and N.S.Naikwade., 2008, Anti-diabetic potential and indian medicinal plants. Journal of Herbal Medicine and Toxicology., 2(1), 45-50

Nugoho A.E., 2006, Hewan Percobaan Diabetes Melitus : Patologi dan Mekanisme Aksi Diabetogenik, 7(4), 378-382

Permata, D.A., 2006, . Potensi Rebusan Daun Sirih Merah *(Piper crocatum)* Terhadap Perbaikan Pankreas Tikus Putih Hiperglikemia, *Skripsi*, Fakultas Matematika dan Ilmu Pengetahuan Alam IPB, Bogor

Suryawanshi, N.P., Buthey, A.K., Nagdote, A.A., Manookar, G.S., 2006, Study of Lipid Peroxide and Lipid profile in Diabetes mellitus. Indian J. Biochem, 21 (1), 126-130.