Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2017 14(4): 1238-1245. **OPEN ACCESS**

Antioxidant and antidiabetic activity of *Garcinia mangostana* L. pericarp extract in streptozotocin-induced diabetic mice

Saikhu Akhmad Husen*[1,2], Septian Hary Kalqutny[1], Arif Nur Muhammad Ansori[3], Raden Joko Kuncoroningrat Susilo[1], Achmad Dary Alymahdy[1], Dwi Winarni[1,2]

[1]Department of Biology, Faculty of Science and Technology Universitas Airlangga, Kampus C Universitas Airlangga, 60115, Surabaya, **Indonesia**
[2]Animal Histology Laboratory, Faculty of Science and Technology Universitas Airlangga, Kampus C Universitas Airlangga, 60115, Surabaya, **Indonesia**
[3]Faculty of Veterinary Medicine Universitas Airlangga, Kampus C Universitas Airlangga, 60115, Surabaya, **Indonesia**
.
***Correspondence:** saikhuakhmad1408@gmail.com **Accepted:** 18 Nov. 2017 **Published online:** 30 Dec. 2017

This study aimed to explore the antioxidant effects of pericarp extract from mangosteen (*Garcinia mangostana* L.) used for the improvement of β-cells of the islets of Langerhans and pancreatic glands damaged by the induction of streptozotocin (STZ), thereby increasing the production of insulin and improving the sensitivity of striated muscle cells to decreasing insulin in diabetic mice. In this study, we used male mice (*Mus musculus*) of the BALB/C strain which were divided into 2 groups: the control group (without treatment of mangosteen pericarp extract) and the treatment group. The control group was divided into three: normal control (KN), diabetic control (KD), diabetic control-Metformin HCl (KM). The treatment group (with mangosteen pericarp extract) was divided into 3 groups (P1, P2, and P3) with the dose of 50 mg/kg body weight, 100 mg/kg body weight, and 200 mg/kg body weight, respectively. The induction of diabetes was done with the injection of multiple low-doses of STZ (30 mg/kg of body weight) for 5 consecutive days. Interestingly, we found that mangosteen pericarp extract was able to increase the body weight of mice, reduce fasting blood glucose, improve cellular β-cells of the islets of Langerhans by increasing the diameter of the islets of Langerhans, and increase fasting blood plasma insulin level in mice significantly. In conclusion, mangosteen pericarp extract is a promising antidiabetic agent due to its anti-hyperglycemic and antioxidant properties.

Keywords: antidiabetic - blood glucose - diabetic mice - *Garcinia mangostana* L. - insulin plasma - islets of Langerhans.

INTRODUCTION

Diabetes mellitus (DM) is a multisystem metabolic disorder suffered by about 6% of the world population. Diabetes mellitus (DM) is characterized by hyperglycemic conditions, because the pancreas cannot produce enough insulin or because there is a disturbance in the utilization of insulin, resulting in hyperglycemic conditions (WHO, 2017). Hyperglycemia conditions can also decrease insulin secretion and increase insulin resistance, causing more severe hyperglycemia and the decrease in the insulin production in β-cells of the islets of Langerhans. One of the factors causing diabetes mellitus (DM) is obesity due to the elevated levels of fat in the body, which are caused by hyperlipidemia and elevated blood cholesterol levels (American Diabetes Association, 2011). Increased blood cholesterol levels can be followed by elevated levels of free fatty acids, resulting in increased

production of mitochondrial superoxide anions, which increases the risk of exposure of cells by reactive oxygen species (ROS) (Evans et al., 2003). The increase of superoxide anion production will result in the increase of nitric oxide (NO) caused by the induction of the NO synthase enzyme. This condition causes the production of reactive nitrogen species (RNS) that will oxidize the sulfhydryl group of proteins, increase lipid peroxidation and cause DNA damage that may harm the cells. (Novelli et al., 2010; Husen et al., 2017; Poolsil et al., 2017).

The condition of hyperlipidemia in obese people, may increase the oxidative stress in the body, which can lead to various complications. Obese people also suffer from increased levels of cholesterol in the body (hypercholesterolemia) caused by excessive accumulation of fat in the body. One of the negative effects of obesity is the occurrence of insulin resistance, which is the inability of insulin to produce normal biological functions, resulting in decreased tissue sensitivity to insulin (McClung et al., 2004; Husen et al., 2016b). From the results of study that was conducted by Husen et al (2017), it is revealed that obese people develop cell resistance to the cellular action of insulin, which is characterized by reduced ability of insulin to support the removal of glucose in fat and muscle resulting in hyperglycemic conditions. The condition of hyperglycemia directly results in increased levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS can directly oxidize and destroy DNA, proteins, and lipids. High levels of ROS and RNS also can indirectly damage the macromolecules, which causes oxidative stress. Oxidative stress occurs when there is an imbalance between reactive molecules (ROS and RNS) and antioxidants (Evans et al., 2003; Novelli, 2010; Husen et al., 2016a). Antioxidants are substances that can inhibit the negative effects of free radicals by acting as an electron donor. To reduce the negative effects of the free radicals, extra antioxidants from the outside (exogenous), such as vitamin E, vitamin C and other antioxidants obtained from consuming various kinds of fruit and vegetables that contain high antioxidants are needed. One type of antioxidant that has the potential to overcome free radicals is xanthone found in the extract of mangosteen pericarp. Antioxidant compounds from *Garcinia mangostana* L. are able to donate hydrogen atoms and stabilize free radicals. In addition to neutralizing free radicals, antioxidants are expected to reduce oxidative stress, especially in various cells exposed to deteriorating effect due to prolonged hyperglycemic conditions, such as β-cells of the islets of Langerhans (Moongkarndi et al., 2004; Jung et al., 2006; Husen et al., 2017). The previous research, conducted by El-Bana et al. (2017) found that anti-hyperglycemic, antioxidant, and anti-inflammatory properties from *Myrtus communis* could work in diabetic rats.

This research was designed to answer the problems: What is the value of LD_{50} and IC_{50} of crude extracts from mangosteen pericarp? Can the administration of crude pericarp extract from mangosteen increase the weight and decrease the fasting blood glucose levels in diabetic mice? Is it possible to repair the β-cells of the islets of Langerhans and pancreatic glands damaged by the induction of STZ? And whether the crude extract of mangosteen pericarp can increase the production of insulin in type-2 diabetic mice? This study aimed to find the values of LD_{50} and IC_{50} of the mangosteen pericarp extract and determine the effects of mangosteen pericarp extract to increase the body weight, decrease the fasting blood glucose levels, repair the β-cells of the islets of Langerhans and pancreatic glands damaged by the induction of STZ, and increase the production of insulin in type-2 diabetic mice.

MATERIALS AND METHODS

This study was conducted at the Animal Histology Laboratory of Faculty of Science and Technology and the Institute of Tropical Diseases (ITD), Universitas Airlangga. Male rats (*Mus musculus*) of BALB/C strain (3-4 months), weighing 30-40 grams, were used in this study. Mangosteen fruit (*Garcinia mangostana* L.), ethanol 96%, STZ (streptozotocin, purchased from SIGMA) to induce diabetic mice, citrate buffer solution pH 4.5, CMC (carboxymethylcellulose), standard antidiabetic drug (Metformin HCl 100 mg/kg), 10% D-glucose for glucose tolerant test, On Call Plus^{TM} glucometers, Easy Touch^{TM} blood cholesterol meters, ELISA kit anti-mouse insulin, and anesthesia (ketamine and xylazine) were used in this study. The main tools in this research are a cage, drinking bottles, feeding containers, husks, microscopes, petri dish, an analytical scale, Eppendorf tubes, surgical instruments, surgical tables, injection syringes, ELISA reader, micropipettes, tips (white, blue, and yellow), a freeze dryer, glassware, a rotary vacuum evaporator, microplates, parafilms, refrigerated

centrifuge, paraffin bath, paraffin oven, microwave ovens, microwaveable jars, and microtomes.

The extraction of the mangosteen pericarp

The pericarp of the mangosteen fruit was used as the extraction material, which was scraped, dried, and made into powder. Dried mangosteen pericarp powder was weighed, extracted with 96% ethanol, and filtered using filter paper, repeatedly until the solvent was clear. The solvent was evaporated with a rotary vacuum evaporator at 50 °C temperature. Furthermore, the extracts were dried using a freeze dryer at -45 °C.

The determination of LD_{50} and IC_{50}

In this study, the toxicity test was performed orally, using male mice aged 3-4 months and weighed between 30 to 40 grams. The test was conducted following the research by Bhardwaj and Gupta (2012). The tested doses were 50 mg, 100 mg, 200 mg 400 mg, 600 mg and 800 mg/kg body weight. The determination of IC_{50} antioxidant activity test of crude extract of mangosteen in vitro was conducted by DPPH method (2,2-diphenyl-1-picrylhydrazyl). In this method, the ability of the compound to reduce 2,2-diphenyl-1-picrylhydrazyl was measured in accordance to research by Garcia et al. (2012).

The induction of type-2 diabetic mice

The procedure of the induction of diabetic mice was based on Novelli et al. (2010). The induction was initiated by injecting STZ in citrate buffer pH 4,5 with a dose of 30 mg/kg body weight in mice for 5 consecutive days. Blood glucose levels were measured 7 and 14 days after the STZ induction. The fasting blood glucose was measured using a glucometer. Only mice with fasting blood sugar levels higher than 200 mg/dl were used as diabetic mice in this study.

The administration of mangosteen pericarp extract

The mice were divided into six groups. Non-diabetic mice were used as a normal control group (KN). Diabetic mice were divided into 2 control groups: the diabetic control group that was not treated by either mangosteen pericarp extract or metformin HCl (KD), diabetic control group that was treated by metformin HCl (100 mg/kg BW) (KM) and treatment groups that were treated by mangosteen pericarp extract. P1, P2, and P3 as the treatment groups were treated by using three different concentrations of the mangosteen pericarp extracts of 50 mg/kg body weight, 100 mg/kg body weight, 200 mg/kg body weight, respectively. Each group consists of 6 mice. The treatment was given for 14 days.

The measurement of body weight and fasting blood glucose levels of mice

Measurements of the weight and fasting blood glucose levels were performed on all groups of mice before the STZ induction (14 days before treatment), at the initial treatment (day 1), at mid-treatment (day 7), and at the end of treatment (day 14). The fasting blood glucose levels were measured by using a glucometer. Measurements of the fasting blood glucose were done on blood samples taken from the vein in the tail area of the mice that had been fastened for 8 to 10 hours. Fasting blood glucose levels are expressed in mg/dL. It was done to determine the diabetic condition of mice. Only mice with fasting blood glucose levels higher than 200 mg/dL were used as diabetic mice in this study.

Measurement of plasma insulin level and the diameter of the islets of Langerhans

Measurement of the diameter of the islets of Langerhans was measured by using an ocular micrometer and an objective micrometer on a light microscope, with 400x magnification. The histologic preparations of the pancreas were observed by measuring the longest and widest sides of the islets of Langerhans, then the results of the measurements were added and divided by two. Plasma insulin levels were measured using ELISA (enzyme linked immune sorbent assay) method.

Data Analysis

The data of body weights, fasting blood glucose levels, diameters of the islets of Langerhans, and plasma insulin levels were obtained and statistically analyzed. Data with normal distribution and homogeneous variance were analyzed using variance analysis followed by Duncan test. Data with normal distribution and non-homogeneous variance were analyzed using Brown-Forsythe test followed by t-test. Pearson correlation test was used to determine the correlation between the diameters of the islets of Langerhans and the fasting blood plasma insulin levels.

RESULTS

Data of body weights and random blood glucose levels, before and after the STZ induction, can be seen in Figure 1. The mean results of weight changes and fasting blood glucose levels

during the treatment can be observed in Figure 2. Figure 3 shows the results of the measurement on plasma blood insulin obtained from intra cardiac blood on day 15. The mean diameter of the islets of Langerhans of mice is presented in Figure 4.

Figure 1. Diagrams showing LD_{50} values of acute toxicity test result, and IC50 values of antioxidant test result in vitro with DPPH method on mangosteen pericarp extract.

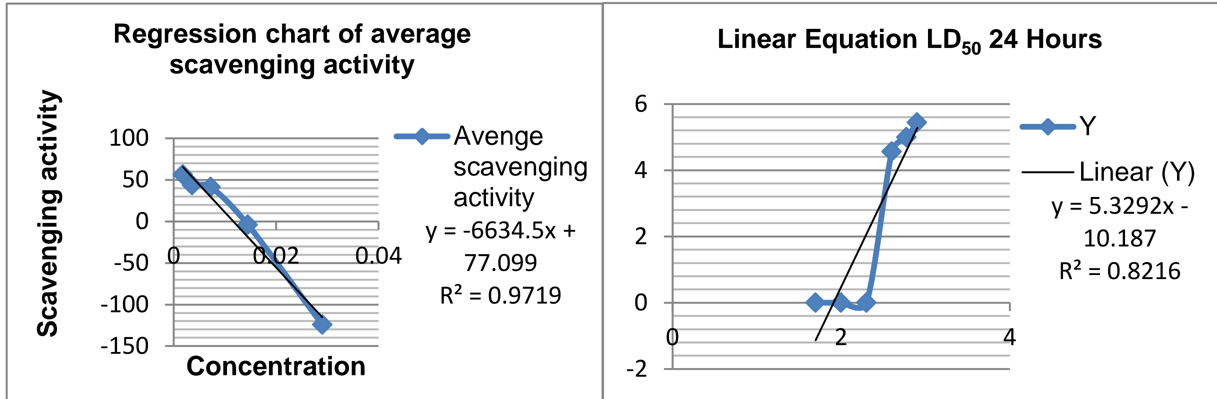
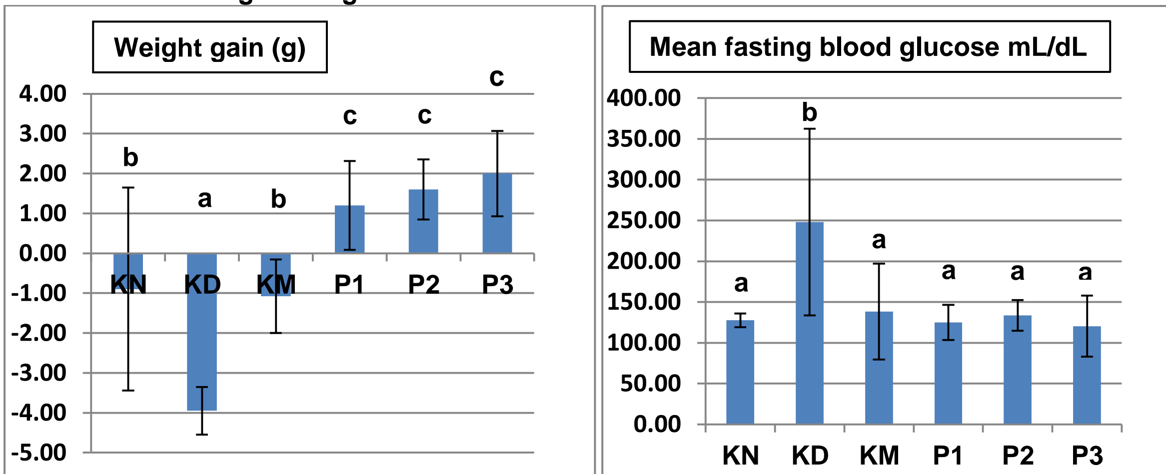


Figure 2. Diagrams showing effects of mangosteen pericarp extract on average weight gain of mice and mean fasting blood glucose level in DM mice.



KN = normal control group, KD = untreated diabetic control, KM = diabetic control treated with 100 mg/kg body weight metformin-HCl, P1 = diabetic mice treated with 50 mg/kg body weight mangosteen pericarp extract. P2 = diabetic mice treated with 100 mg/kg body weight mangosteen pericarp extract. P3 = diabetic mice treated with 200 mg/kg body weight mangosteen pericarp extract. Treatment of mangosteen pericarp extract were given for 14 days. The letters above the diagrams of each group show the Duncan test results at α = 0.05. The same letter shows no significant difference.

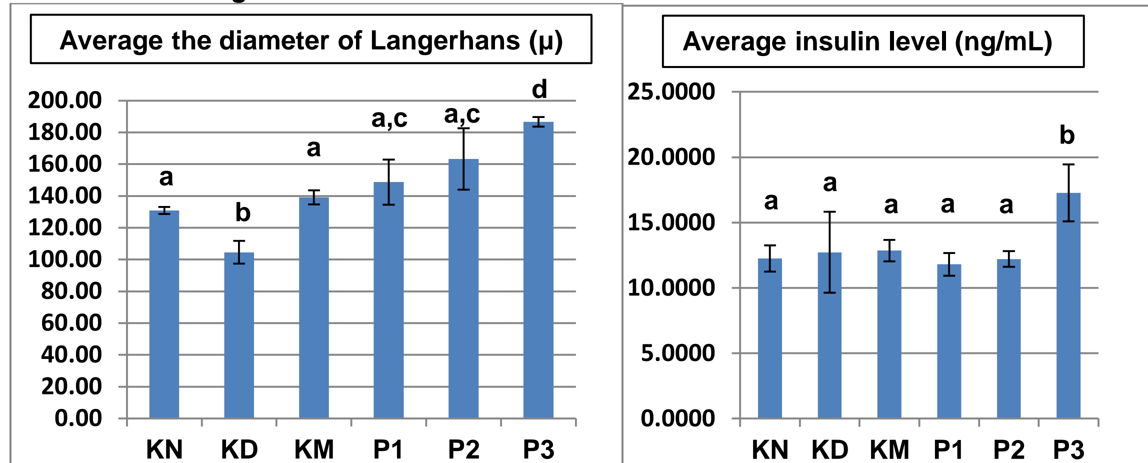
From the figure 1, it can be seen that the LD_{50} value of acute toxicity test results in mice was 630.95 mg/kg body weight, while the IC_{50} of pericarp extract of mangosteen, using the test of in vitro activity with DPPH was 0.0040835 mg/mL, equivalent to 40.835 mg/kg body weight. LD_{50} value can be used as the reference of the dosage treatment of mangosteen pericarp extract. The dose should not be given above 630 mg/kg body weight. In this study, the doses of mangosteen pericarp extract used were 50, 100, and 200 mg/kg body weight because the doses were considered safe during the *in vivo* toxicity treatment, using the mangosteen pericarp extract. From the result of measurement of the average percentage of scavenging activity to the test compound, it showed that the IC_{50} value of mangosteen pericarp extract was at concentration of 0.0040835 mg/mL. It was used as a benchmark that the effective dose of antioxidant compounds found in mangosteen pericarp extract test was in the dose range of 50 mg/kg body weight. As the value of IC_{50} is less than 50 mg/kg body weight, it can be said that the antioxidant of mangosteen pericarp extract is classified as a powerful antioxidant and has an effect on free radical decline, so it can repair various cells damaged by free radicals such as β-cells of the islets of Langerhans.

From the Figure 2, it can be seen that hyperglycemic conditions can cause weight loss in all diabetic mice group (KD) and diabetic-metformin HCl (KM) group, which means that for 14 days the weights of mice decreased on an average of about 3.95 grams in the diabetic group, and a decrease of about 1.08 grams in the metformin HCl group. This is because the effect of STZ which is a free radical compound, capable of destroying β-cells of the islets of Langerhans and the experimental animals were not been able to perform metabolic improvement in the body. In the normal group (KN), without induction of STZ and only given CMC as a placebo, for 14 days of treatment the weight loss also occurred. This was presumably caused due to environmental conditions of the cage, or due to the effects of CMC which can suppress the increase of weight during treatment. From the results of body weight measurements in all treatment groups P1, P2, and P3, with the doses of 50, 100 and 200 mg/kg body weight, respectively, it showed that the treatments were able to significantly reduce the occurrence of weight loss in the experimental animals having DM conditions due to STZ induction. This is because the extract of the mangosteen pericarp contains xanthone compounds that plays a role in antioxidant activities and have been able to repair damage to β-cells the islets of Langerhans. For the mean of fasting blood glucose levels receiving the treatment of mangosteen pericarp extract, it showed that the treatment was able to decrease the fasting blood glucose levels significantly, compared to that of the diabetic control group (DM). The results showed that the antioxidant compounds from mangosteen pericarp extract were able to repair β-cells of the islets of Langerhansmaking the insulin production increase again. Increasing insulin may increase the absorption of glucose from outside the cells into the muscles and liver cells, which made the decrease in the glucose levels of the treatment group P1, P2, and P3 equivalent to the glucose levels of the normal control group. From Figure 2 above it can be seen that the administration of mangosteen pericarp extract with the doses of P1, P2, and P3, for 14 day treatment could significantly increase the diameters of the islets of Langerhans, compared to that of the diabetic control group (KD) that showed a shrinkage.

Figure 3 showed that the diabetic control group treated with metformin HCl (KM), P1, P2, and P3 group, for 14 days treatment had a significant effect, when compared to the fasting blood glucose level in the diabetic control group. Likewise, the normal control group (KN) differed significantly with the diabetic group (KD). This suggests that STZ induction, may damage the pancreas gland, indicated by the small islets of Langerhans and the fragility of pancreatic tissue when the preparation with HE staining was done. The decreased mean diameter of the islets of Langerhans has a significant effect on the secretion of insulin secreted by the pancreas gland. These conditions resulted in increased fasting blood glucose levels in the diabetic group. Whereas in the group of KM, P1, P2 and P3 showed a significant difference compared to the diabetic control group, whose mean diameter of the islets of Langerhans increased significantly. The mean fasting insulin plasma levels showed that the diabetic group did not differ significantly compared to KM, P1 and P2 groups. Only group

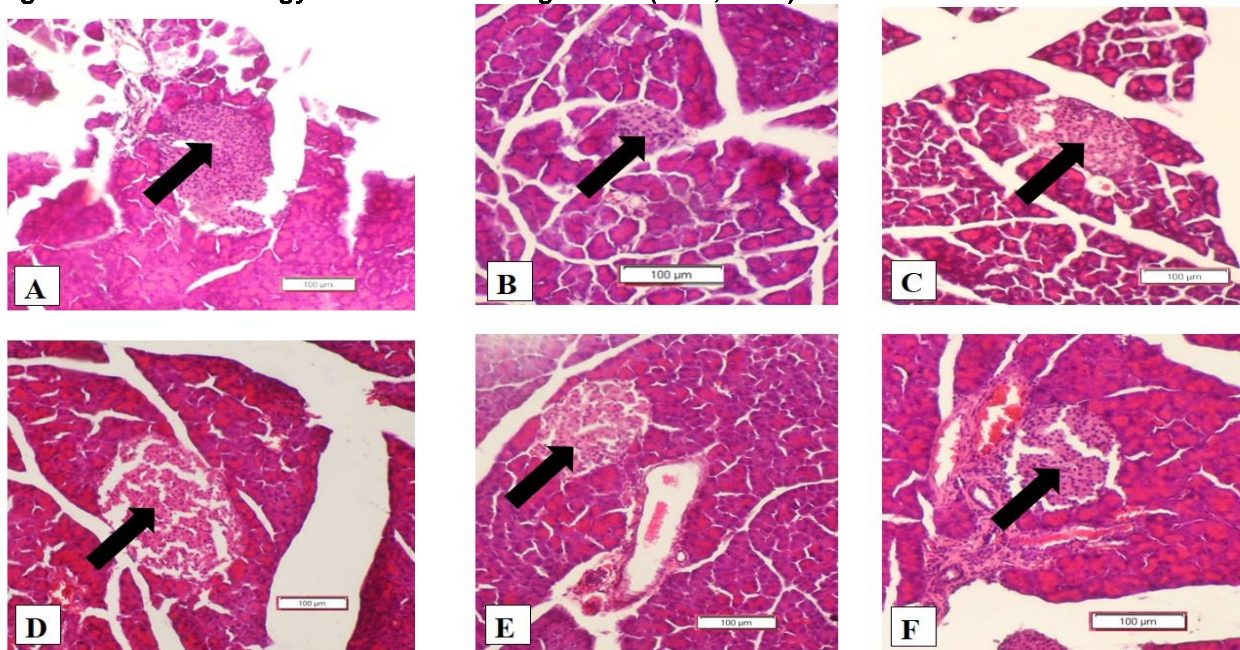
P3 differed significantly compared to KN, KD, KM P1 and P2. From the analysis of Pearson correlation, there is a positive correlation between the increase in diameter of the islets of Langerhans with the increase in plasma insulin levels of fasting blood of mice, which means that the larger the diameter of the island of Langerhans was measured, the higher levels of insulin secreted by pancreatic glands were, with a correlation value of 0.474 at significance level α = 0.05.

Figure 3. Diagrams showing the effects of mangosteen pericarp extract on the average diameter of the islet of Langerhan and insulin content level in DM mice.



KN = normal control group, KD = untreated diabetic control, KM = diabetic control treated with 100 mg/kg body weight metformin-HCl, P1 = diabetic mice treated with 50 mg/kg body weight mangosteen pericarp extract. P2 = diabetic mice treated with 100 mg/kg body weight mangosteen pericarp extract. P3 = diabetic mice treated with 200 mg/kg body weight mangosteen pericarp extract. Treatment of mangosteen pericarp extract was given for 14 days. The letters above the diagrams of each group show the Duncan test results at α = 0.05. The same letter shows no significant difference.

Figure 4. Histology of the islet of Langerhans (H&E, 200x).



KN (A) = normal control group, KD (B) = untreated diabetic control, KM (C) = diabetic control treated with

100 mg/kg body weight of metformin-HCl, P1 (D) = diabetic mice treated with 50 mg/kg body weight of mangosteen pericarp extract. P2 (E) = diabetic mice treated with 100 mg/kg body weight of mangosteen pericarp extract. P3 (F) = diabetic mice treated with 200 mg/kg body weight of mangosteen pericarp extract. Treatment of mangosteen pericarp extract was given for 14 days. Size of pancreatic islets of Langerhans (black arrow).

CONCLUSION

Interestingly, our research found that mangosteen pericarp extract was able to increase the body weight of mice, reduce fasting blood glucose, improve β-cells of the islets of Langerhans by increasing the diameter of the islets of Langerhans, and increase fasting blood plasma insulin level in mice, significantly. On the other hand, we revealed that the values of LD_{50} and IC_{50} of mangosteen pericarp extract were 630.95 mg/kg body weight and 0.0040835 mg/kg body weight, respectively. Also, there was a positive correlation between the increasing of diameter of the islets of Langerhans and the increasing of plasma insulin levels. In conclusion, pericarp extract of mangosteen is a promising anti-diabetic agent due to its anti-hyperglycemic and antioxidant properties.

CONFLICT OF INTEREST

We found no conflict of interest in this work.

ACKNOWLEGEMENT

Authors would like to thank Universitas Airlangga and Ministry of Research, Technology, and Higher Education of the Republic of Indonesia for supporting this work. Furthermore, special thanks to PMDSU Scholarship - Batch III (awarded to ANMA and RJKS).

AUTHOR CONTRIBUTIONS

SAH, SHK, ANMA, RJKS, ADA designed and performed the experiments and also wrote the manuscript. SAH, DW reviewed, corrected, and edited the manuscript. All authors read and approved the final version.

Copyrights: © 2017 @ author (s).

This is an open access article distributed under the terms of the **Creative Commons Attribution License (CC BY 4.0)**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author(s) and source are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

REFERENCES

American Diabetes Association, 2011. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 34(Suppl 1): 562-569.

Bhardwaj S, Gupta D, 2012. Study of Acute, Sub Acute and Chronic Toxicity Test. International Journal of Advanced Research in Pharmaceutical & Bio Sciences 2(2): 103-129.

El-Bana MA, Medhat D, Ashour MN, Diab Y, Hussein J, 2017. *Myrtus communis* Extract Attenuates Atherosclerosis in Streptozotocin-induced Diabetic Rats. Bioscience Research 14(2): 257-264.

Evans JL, Goldfine ID, Maddux BA, Grodsky GM, 2003. Are Oxidative Stress-activated Signaling Pathways Mediators of Insulin Resistance and Beta-cell Dysfunction?. Diabetes 52(1): 1-8.

Garcia EJ, Oldoni TLC, de Alencar SM, Reis A, Loguercio AD, Grande RHM, 2012. Antioxidant Activity by DPPH Assay of Potential Solutions to be Applied on Bleached Teeth. Braz. Dent. J. 23(1): 22-27.

Husen SA, Winarni D, Ansori ANM, Susilo RJK, 2016a. Potensi Ekstrak Kasar Kulit Buah Manggis (*Garcinia Mangostana* L.) Terhadap Kadar Kolesterol dan Kadar Glukosa Darah Puasa Mencit Diabetik. Proceeding Seminar Nasional Biodiversitas VI, Universitas Airlangga, Surabaya, Indonesia, pp 841-850.

Husen SA, Winarni D, Khaleyla F, Kalqutny SH, 2016b. Activity Test of Various Mangosteen (*Garcinia mangostana*) Pericarp Extract Fractions to Decrease Fasting Blood Cholesterol Levels and Lipid Peroxidation Activity in Diabetic Mice. Journal of Biological Researches 22(1): 13-17.

Husen SA, Winarni D, Khaleyla F, Kalqutny SH, Ansori ANM, 2017. Activity Assay of Mangosteen (*Garcinia mangostana* L.) Pericarp Extract for Decreasing Fasting Blood Cholesterol Level and Lipid Peroxidation in Type-2 Diabetic Mice. AIP Conference Proceedings 1888(1): 020026-1-6.

Jung HA, Su BN, Keller WJ, Mehta RG, Kinghorn AD, 2006. Antioxidant xanthones from the

pericarp of *Garcinia mangostana* (Mangosteen). J Agric Food Chem. 2006 Mar 22;54(6):2077-82.

McClung JP, Roneker CA, Mu W, Lisk DJ, Langlais P, Liu F, Lei XG, 2004. Development of Insulin Resistance and Obesity in Mice Overexpressing Cellular Glutathione Peroxidase. PNAS 101(24): 8852-8857.

Moongkarndi P, Kosem N, Kaslungka S, Luanratana O, Pongpan N, Neungton N, 2004. Antiplorifetion, Antioxidation and Induction of Apoptotosis by *Garcinia mangostana* (mangosteen) on SKBR3 Human Breast Cancer Cell Line. J. Ethnopharmacol, 90(1): 161-166.

Novelli M, Bonamassa B, Masini M, Funel N, Canistro D, De Tata V, Martano M, Soleti A, Campani D, Paolini M, Masiello P, 2010. Persistent Correction of Hyperglycemia in Streptozotocin-Nicotinamide-Induced Diabetic Mice by A Non-Conventional Radical Scavenger.Naunyn Schmiedebergs Arch Pharmacol 382(2): 127-137.

Poolsil P, Promprom W, Talubmook C, 2017. Anti-hyperglicemic and Anti-hyperlipidemic Effects of Extract from Houttuynia cordata Thumb. In Streptozotocin-Induced Diabetic Rats. Pharmacognosy Journal 9(3): 382-387.

World Health Organization, 2017. Diabetes Mellitus. http://who.int/topics/diabetes-mellitus/en/. Accessed 29 October 2017.