Hepatoprotective Effect of Gamma-mangostin for Amelioration of Impaired Liver Structure and Function in Streptozotocin-induced Diabetic Mice

S A Husen1,2*, D Winarni1,2, Salamun1, A N M Ansori3, R J K Susilo1 and S Hayaza1

1Department of Biology, Faculty of Science and Technology Universitas Airlangga, Kampus C Universitas Airlangga, 60115, Surabaya, Indonesia
2Animal Histology Laboratory, Faculty of Science and Technology Universitas Airlangga, Kampus C Universitas Airlangga, 60115, Surabaya, Indonesia
3Faculty of Veterinary Medicine Universitas Airlangga, Kampus C Universitas Airlangga, 60115, Surabaya, Indonesia

*corresponding author: saikhu-a-h@fst.unair.ac.id

Abstract. The aim of this study was to investigate whether gamma-mangostin could reduce fasting blood glucose, cholesterol, SGOT, SGPT, and also ameliorate damaged hepatocytes in diabetic mice. In this study, we used male BALB/C mice. Mice were divided into two groups: normal control (KN) and streptozotocin-induced diabetic. Streptozotocin (STZ) induction was performed using multiple low doses of 30 mg/kg body weight injected for five consecutive days. The diabetic mice were separated into three subgroups: diabetic control (KD), diabetic mice treated with acarbose (KA), and diabetic mice treated with gamma-mangostin at either 0.5 mg/kg body weight (P1), 1 mg/kg body weight (P2), or 2 mg/kg body weight (P3). Before and after STZ injection, fasting blood glucose and cholesterol level would be observed. Fasting blood glucose and cholesterol level were also measured at the 1st, 7th, and 14th day of gamma-mangostin treatment. Treatment was given for 14 days. On the 15th day, SGOT and SGPT were measured using a Pentra C 200 Reader, while the liver was collected and processed onto the histological slides. Interestingly, we found that gamma-mangostin was able to reduce the fasting blood glucose, cholesterol, SGOT, SGPT, and ameliorate the damaged hepatocytes in significant diabetic mice. Therefore, we concluded that gamma-mangostin is a promising anti-diabetic agent due to its anti-hyperglycemic and antioxidant activities.

Keyword : Streptozotocin, Diabetic Mice, Gamma-mangostin, Amelioration, Liver Structure

1. Introduction
Diabetes mellitus (DM) is a hyperglycemic condition due to the reducing of insulin secretion, insulin sensitivity, or both. Chronic hyperglycemia in DM is related to the prolonged damage such as neuropathy, nephropathy, retinopathy, dysfunction, and organ damage, especially in the lungs, liver, and blood vessel.[1] Various symptoms were found in patients with DM such as polyuria, polydipsia, polyphagia along with weight loss. Long-term DM causes a series of metabolic disorders that cause macrovascular and microvascular pathological disorders.[2]

DM is divided into type-1 DM (insulin dependent DM) and type-2 DM (non-insulin dependent DM). Type-1 DM is an insulin deficiency condition due to the damage of β-cells in the islet of Langerhans and human leukocyte antigen (HLA). It triggers the immune system to attack pancreatic cells, thus damaging a person's ability to produce insulin. Type-2 DM is characterized by an insulin resistance, due to genetic factors, lifestyle, or the environment.[3-5] Type 2 DM can occur due to the lack of insulin receptors in muscle cells. Thus, even though the amount of insulin production is enough, cells cannot carry enough glucose in the blood so that the blood glucose levels remain high.[6,7]

Hyperglycemia in DM patients can lead to glucose autooxidation, increase the formation of advanced glycation end products (AGE), the activation of polyol pathways, and the activation of protein kinase C (PKC) which leads to high levels of reactive oxygen species (ROS).[5] Excessive presence of ROS causes oxidative stress due to the imbalance between free radicals and antioxidants produced by the body which damages to the cell membrane characterized by the decreased levels of body antioxidants.[8] Free radicals
can damage various body tissues such as hepatocytes, proximal renal tubules, β-cells in the islet of Langerhans, and etc.[9-14]

Antioxidants are substances that can prevent the negative effects of free radicals, by providing electrons so that the damages of lipids, cell membranes, blood vessels, DNA, and other damages caused by the reactive compounds; such as ROS and RNS; can be suppressed. To reduce the occurrence of the free radical’s effect, extra antioxidants from the outside (exogenous); such as vitamin E, vitamin C and other antioxidants which are obtained from consuming various types of fruits and vegetables that contain high antioxidants; are needed.[6,7] One type of antioxidants which still provide a chance to overcome free radicals up until today is xanthone from mangosteen pericarp extract. The antioxidant of Garcinia mangostana contributes its hydrogen atoms and stabilizes the free radicals by resonance, which is difficult to participate in the other radical reactions. In addition to the function of neutralizing free radicals, antioxidants are expected to reduce oxidative stress, mainly in various cells affected by the prolonged hyperglycemic conditions, such as β-cells in the islet of Langerhans.[12,13]

Indonesia has a high number of diversity of medicinal plants, which contains various natural potentials that can be utilized for the treatment of various diseases.[15] One of medicinal plants which currently has great potential to be developed as a medicinal raw material is mangosteen.[9] Mangosteen pericarp contains xanthone. Xanthone compounds are known as alpha-mangostin, beta-mangostin, and gamma-mangostin. The function of antioxidant is to overcome or neutralize free radicals. The expectation by giving these antioxidants is the oxidative stress can be reduced. Nowadays, the use of natural antioxidants is becoming a public concern.[16-20]

One of the functions of xanthone antioxidants is as a hepatoprotective agent. A damaged liver will release enzymes into the blood such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), gamma-glutamyl transpeptidase (γGT), and alkaline phosphatase (ALP). Serum glutamic oxaloacetic transaminase (SGOT) and SGPT enzymes are specific indicators of liver cell damage. Glutamic oxaloacetic serum transaminase is an enzyme located in the liver’s parenchyma cells, muscle cells, and heart cells. Serum glutamic pyruvic transaminase is an enzyme located in the liver cells. If there is any damage to the hepatocytes, SGOT and SGPT will come out of the liver cells to the blood circulation.[21] The aim of this study is to prove that gamma-mangostin can reduce blood plasma SGOT and SGPT levels, repair liver damage, and analyze the association between the decreased SGOT and SGPT levels and the decreased liver damage in diabetic mice.

2. Experimental Method

This experimental study was conducted at the Animal Laboratory, Animal Histology Laboratory, and Organic Chemistry Laboratory, in the Faculty of Science and Technology of Universitas Airlangga, as well as at Institute of Tropical Diseases (ITD) of Universitas Airlangga. The used sample was adult male mice, strain BALB/C, 3-4 months old, weight ranged from 25 to 40 g. The study materials consisted of the pure gamma-mangostin purchased from Sigma. Other materials consisted of streptozotocin (purchased from Sigma), buffer citrate solution with pH 4.5, and phosphate-buffered saline (PBS), solvent extract of carboxymethylcellulose (CMC), standard antidiabetic drugs (acarbose 100 mg/kg body weight), xylazine and ketamine, and glucose (10% D-glucose in distilled water).

The main tools used were mice cage made in plastic with lid of gauze wire, drinking bottle, feeding plate, husk, microscope, Petri dish, analytical scale, injection needles which have lead tackle at the end, 1 mL insulin injection needle, insulin syringe, On Call® Plus, oxidase-peroxidase reactive strips, micropipette, tips (white, blue, and yellow), freeze dryer, rotary vacuum evaporator, microplates, parafilm, micropipette, microtube, refrigerated centrifuge, paraffin bath, paraffin oven, microwave oven, microwaveable jar, microtome, object glasses, and cover glasses.

The procedure began with testing the gamma-mangostin compound in vitro with the DPPH (diphenyl picrylhydrazyl) method. The study samples consisted of 36 male mice, distributed to the normal control group (KN) and the diabetic group (induced by streptozotocin). The grouping of experimental animals was performed as follows; non-diabetic mice were used as normal control group (KN), diabetic mice which were induced by streptozotocin were divided into 2 control groups; they were diabetic control group (KD), diabetic control group which were given acarbose of dose 100 mg/kg body weight (KA), and, the last one was gamma-mangostin treatment group. Furthermore, the gamma-mangostin treatment group was divided into 3 subgroups. Group 1 (P1) was given 1 mg/kg body weight gamma-mangostin, group 2 (P2) was given 2 mg/kg body weight gamma-mangostin, and group 3 (P3) given was 4 mg/kg body weight gamma-mangostin. Each group consisted of 6 mice and those treatments were administered for 14 days. The dose was referred to the IC50 value of the results of the in vitro toxicity test and given
orally to the mice. Blood glucose of diabetic mice was measured on the 1st, 7th, and 14th day after gamma-
mangostin treatment. On the 15th day, blood plasma SGOT and SGPT were determined by Pentra C 200
Reader (Horiba Medical) using 510 nm wavelength. Damage on the liver structure was determined from
histological sections stained with hematoxylin-eosin (HE).[12,13]

Data with normal distribution and homogenous variation was analyzed using one-way variance
analysis continued with Duncan test. Data with normal distribution and non-homogenous variation was
analyzed using Brown-Forsythe test continued with a t-test. While the Pearson test was used to analyze
the relationship between the islet of Langerhans diameter and the fasting blood glucose level in mice.
All statistical test was conducted at α = 0.05.

3. Results and Discussion
The mean of mice’s fasting blood glucose level and cholesterol level data are presented in Figure 1. The
mean of SGOT and SGPT levels data is presented in Figure 2. While the mean data of swollen cells,
hydropic and necrotic cells is shown in Figure 3. Microscopic photo of liver tissue is presented in Figure
4.

![Figure 1](image1.png)

**Figure 1.** Fasting blood glucose and cholesterol levels (mg/dL) before and after STZ induction. The different letter indicated a significant difference.

![Figure 2](image2.png)

**Figure 2.** SGOT and SGPT level changes in each mice groups after treatments. The different letter indicated a significant difference.
Figure 3. The number of hepatocyte damage of each mice groups after treatments. The different letter indicated a significant difference.

Figure 4. Histological structure of liver’s mice after gamma-mangostin treatment. BV: blood vessel, N = normal cell, SC = swollen cell, HC = hydropic cell, Ne = necrotic cell. Bar: 500 µm.

The average of fasting blood glucose and cholesterol level measurement before and after STZ induction (Figure 1), showed that the STZ injection at 30 mg/kg body weight for 5 consecutive days was able to elevate the random blood glucose level significantly, from 133,125 ± 17,928 to 180,833 ± 29,620 mg/dL. This indicated that STZ was able to damage β-cells in the islet of Langerhans and reduce the insulin synthesis as well as elevate the fasting blood glucose level.[7] Mice’s blood cholesterol level was raising from 145,178 ± 21,639 pre-STZ to 166,714 ± 26,016 mg/dL after STZ induction. This condition was caused by hyperglycemia due to the β-cells in the islet of Langerhans damage and plasma insulin level reduction that both led to higher gluconeogenesis in liver and skeletal muscle. It was also caused by the mobilization of fat from adipose tissue. The breakdown of fatty tissue both within the striated muscle cells and the tissues of the body can lead to the increased levels of cholesterol in the blood.[22,23]

The glucose cannot be processed into energy because of the hyperglycemic condition. Therefore, the energy must be made from other sources such as fat and protein. Energy is obtained through the increased catabolism of protein and fat. Along with these conditions, there is a stimulation of lipolysis and the increased levels of free fatty acids and blood glycerol. This leads to the increased production of acetyl-CoA by the liver, which in turn is converted to acetoacetic acid and ultimately reduced to β-hydroxybutyric acid or decarboxylated to acetone. Due to the formation of energy from proteins and fats, the cholesterol levels formed in the chain of fat and protein metabolism increase. In patients with DM, hyperglycemic conditions lead to the increased production of ROS and RNS due to the increase of
NADPH oxidation in endothelial tissue. Reactive oxygen species (ROS) and RNS are highly reactive molecules that can directly oxidize and destroy DNA, proteins, and lipids and can cause an oxidative stress. An oxidative stress occurs when there is an imbalance between the number of highly reactive molecules (ROS and RNS) with the existing antioxidants.[6,7,9]

Streptozotocin (STZ) is highly reactive free radicals which are able to increase the hepatocytes of ROS and RNS. Serum glutamic oxaloacetic transaminase (SGOT) and SGPT were found to be increased in STZ-induced diabetic mice. Plasma SGOT on the KD was significantly different from the KN, KA, P1, P2, and P3. These phenomena were due to a diabetic condition triggered by ROS production using the glucose autooxidation pathway, advanced glycation end-products (AGEs), and polyol pathway mechanism.[5,9] Reactive oxygen species could also stimulate lipid peroxidation in the cell membrane, causing the cell damage. Free radicals could cause lipid peroxidation which will damage the structure of the membrane cell and cause damage to the liver structure, indicated by the raising SGOT level. It has been proved that hyperglycemia on diabetic control (KD) group played an important role in increasing the plasma SGOT level.[12,13]

Plasma SGPT level on the diabetic control (KD) was not significantly different from acarbose control (KA). The half-time of SGPT in circulation was higher compared to SGOT, therefore, even though damaged cells number of KA was lower, the SGPT level was higher. KD and KA were significantly different from KN, P1, P2, and P3. This indicated that gamma-mangostin administration was able to fix the damaged structure of the liver and its function, as well as affect the plasma SGPT level.[21]

The number of swollen hepatocytes in the KD was significantly different from KN, KA, P1, P2, dan P3. The hyperglycemia condition in the KD group had induced cellular damage, which later elevated the SGOT and SGPT level. The number of hydropic hepatocytes on the KD was also significantly different from KN, KA, P1, P2, and P3. This was due to the hyperglycemia condition caused by oxidative stress which led to the lipid peroxidation and the changes in cell structure, such as hydropic. Hyperglycemia condition in diabetic patients had a higher chance to induce lipid peroxidation that could cause oxidative damage on cellular structure.[6,7,12,13]

The number of necrotic hepatocytes on diabetic control was significantly different compared to KN, KA, P1, P2, and P3. Hyperglycemia on the KD group caused lipid peroxidation that induced cell membrane damage and inside the cell itself. Continuous lipid peroxidation would create a more extensively cellular damage and cause cell necrosis. In the current study, the percentage of a necrotic hepatocyte of KN, KA, P1, P2, and P3 were significantly different from the diabetic control (KD). This proved that the gamma-mangostin administration was capable of fixing the damage on liver structure and function, as well as reducing the percentage of necrotic cells. The antioxidative activity of gamma-mangostin suppressed free radicals’ action on lipid peroxidation in the cell membrane and prevented the cell damage from expanding [6,7,12,13].

During diabetic conditions, the increasing blood glucose levels contribute to the formation of AGEs and ROS production. Advanced glycation end products also produce ROS [24] and both are associated with cell death, tissue damage, and organ dysfunction. The hyperglycemic condition activates the polyol pathway and produces fructose from glucose. Fructose and its metabolites as well as glucose are involved in the non-enzymatic glycation of cellular proteins. The resulted Schiff base undergoes a rearrangement of the structure to form the Amadori product which later produces the α-dicarbonyl compound and AGEs by cross-linking with other proteins. On the other hand, Stevens et al. mentioned that in diabetic condition, the sorbitol pathway activity is increased in the tissues which do not require insulin to uptake its cellular glucose. Sorbitol cannot pass through the cell membranes easily, accumulated, and causes the osmotic damage to the cells (swelling). The accumulation of sorbitol decreases myo-inositol, which can interfere cell osmoregulation so that the cell is damaged.[25,26]

The antioxidant compound contained in the mangosteen pericarp was xanthone. Moongkarndi et al. and Weecharangsan et al. stated that xanthone of mangosteen pericarp plays a role as an antioxidant and able to lower free radicals.[16,17] Xanthone was a chemical substance belong to phenol or polyphenol group. Xanthone antioxidant could increase insulin secretion from the β-cells in the islet of Langerhans. Xanthone also neutralizes free radicals, which will prevent further damage of β-cells in the islet of Langerhans.[20]

4. Conclusion

Interestingly, this study has proved that the administration of gamma-mangostin was able to lower SGOT and SGPT levels and also ameliorate damaged hepatocytes in diabetic mice significantly, mainly on swollen, hydropic, and necrotic cells.
References

[1] American Diabetes Association. 2013 Standards of medical care in diabetes—2013. Diabetes Care 36 1 S11-S66.

[2] American Diabetes Association. 2011. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 34 1 562-569.

[3] Evans, J. L., Goldfine, I. D., Maddux, B. A., & Grodsky, G. M. 2003 Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction?. Diabetes 52 1 1-8.

[4] McClung, J. P., Roneker, C. A., Mu, W., Lisk, D. J., Langlais, P., Liu, F., & Lei, X. G. 2004 Development of insulin resistance and obesity in mice overexpressing cellular glutathione peroxidase PNAS 101 24 8852-8857.

[5] Mohora, M., Virgolici, B., Paveliu, F., Lixandru, D., & Greabu, M. 2006 Free Radical Activity in Development of insulin resistance and obesity in mice overexpressing cellular glutathione peroxidase. Naunyn Schmiedebergs Arch Pharmacol 366 1 137-37.

[6] Husen, S. A., Salamun, Khalayla, F., Ansori, A. N. M., & Ansori, A. N. M. 2017 Antioxidant and antidiabetic activity of Garcinia mangostana L. pericarp extract in streptozotocin-induced diabetic mice. BioMed Research International 2015 4 281-7.

[7] Husen, S. A., Khaleyla, F., Kalqutny, S. H., & Ansori, A. N. M. 2017 Antioxidant and antidiabetic activity of Garcinia mangostana L. pericarp extract fractions to decrease fasting blood cholesterol levels and lipid peroxidation activity in diabetic mice. Journal of Biological Researches 22 1 13-17.

[8] Novelli, M., Bonaamassa, B., Masini, M., Funel, N., Canistro, D., De Tata, V., Martano, M., Soleti, A., Campani, D., Paolini, M., & Masiello, P. Persistent correction of hyperglycemia in streptozotocin-nicotinamide-induced diabetic mice by a non-conventional radical scavenger. Naunyn Schmiedebergs Arch Pharmacol 362 2 127-37.

[9] Ansori, A. N. M., Susilo, R. J. K., Hayaza, S., Winarni, D., & Husen, S. A. 2018 Renoprotection by Garcinia mangostana L. pericarp extract in streptozotocin-induced diabetic mice. Iraqi Journal of Veterinary Sciences, article in press.

[10] Hosokawa, M., Dolci, W., & Thorens, B. 2001 Differential sensitivity of GLUT1- and GLUT2 expressing β cells to streptozotocin. Biochem. Biophys. Res. Commun 289 1114-1117.

[11] Park, J., Choe, S. S., Choi, A. H., Kim, K. H., Yoon, M. J., Suganami, T., Ogawa, Y., & Kim, J. B. 2006 Increase in glucose-6-phosphate dehydrogenase in adipocytes stimulates oxidative stress and inflammatory signals. Diabetes 55 11 2939-49.

[12] Park, J., Choe, S. S., Choi, A. H., Kim, K. H., Yoon, M. J., Suganami, T., Ogawa, Y., & Kim, J. B. 2006 Increase in glucose-6-phosphate dehydrogenase in adipocytes stimulates oxidative stress and inflammatory signals. Diabetes 55 11 2939-49.

[13] Husen, S. A., Khaleyla, F., Kalqutny, S. H., & Ansori, A. N. M. 2017 Antioxidant and antidiabetic activity of Garcinia mangostana L. pericarp extract in streptozotocin-induced diabetic mice. BioMed Research International 2015 4 281-7.

[14] Jung, H. A., Su, B. N., Keller, W. J., Mehta, R. G., & Kinghorn, A. D. 2006 Antioxidant xanthones from the pericarp of Garcinia mangostana (Mangosteen). J Agric Food Chem 54 6 2077-82.

[15] Jariyapongskul, A., Areebambud, C., Suksamrarn, S., & Mekseepralard, C. 2015 Alpha-mangostin attenuation of hyperglycemia-induced ocular hypoperfusion and blood retinal barrier leakage in the early stage of type 2 diabetes rats. BioMed Research International 2015 785826.

[16] Wahyuni, D. K., Ansori, A. N. M., & Vidiyanti, F. 2017 GC-MS analysis of phytocomponents in methanolic extracts of leaf-derived callus of Garcinia mangostana L. 2015 785826.

[17] Moongkarndi, P., Kosem, N., Kaslungka, S., Luanratana, O., Pongpan, N., & Neungton, N. 2004. Attenuation of hyperglycemia-induced ocular hypoperfusion and blood retinal barrier leakage in the early stage of type 2 diabetes rats. BioMed Research International 2015 785826.

[18] Chin, Y-W., & Kinghorn, A. D. 2008 Structural characterization, biological effects, and synthetic studies on xanthones from mangosteen (Garcinia mangostana), a popular botanical dietary supplement. Mini Rev Org Chem 5 4 355-364.

[19] Chen, L. G., Yang, L. L., & Wang, C. C. 2008 Anti-inflammatory activity of mangostins from Garcinia mangostana. Food Chem Toxicol 46 2 688-693.

[20] Cho, S. S., Choi, A. H., Kim, K. H., Yoon, M. J., Suganami, T., Ogawa, Y., & Kim, J. B. 2006 Increase in glucose-6-phosphate dehydrogenase in adipocytes stimulates oxidative stress and inflammatory signals. Diabetes 55 11 2939-49.
[21] Chang, H. F., Wu, C. H., & Yang, L. L. 2013 Antitumour and free radical scavenging effects of γ-mangostin isolated from *Garcinia mangostana* pericarps against hepatocellular carcinoma cell. *J Pharm Pharmacol* **65** 9 1419-1428.

[22] Karnieli, E., & Armoni, M. 2008 Transcriptional regulation of the insulin-responsive glucose transporter GLUT4 gene: from physiology to pathology. *Am J Physiol Endocrinol Metab* **295** 1 E38-45.

[23] Park, S-T., Kim, K., Yoon, J-H., & Lee, S. 2011 Effect of exercise on GLUT4 expression of skeletal muscle in streptozotocin-induced diabetic rats. *Journal of Exercise Physiology Online* **14** 3 113-122.

[24] Forbes, J. M., Coughlan, M. T., & Cooper, M. E. 2008 Oxidative stress as a major culprit in kidney disease in diabetes. *Diabetes* **57** 1446-1454.

[25] Schaffer, J., Azuma, J., & Mozaffari, M. 2009 Role of antioxidant activity of taurine in diabetes. *Canadian Journal of Physiology and Pharmacology* **87** 91-99.

[26] Palsamy, P., & Subramanian, S. 2011 Resveratrol protects diabetic kidney by attenuating hyperglycemia-mediated oxidative stress and renal inflammatory cytokines via Nrf2-Keap1 signaling. *Biochimica et Biophysica Acta* **1812** 719-731.

**Acknowledgement**

Authors would like to thank the Dean of Faculty of Science and Technology and the Head of Institute of Innovation and Research Universitas Airlangga for the given opportunity to conduct this research funded by the Directorate General of Higher Education, Ministry of Research, Technology, and Higher Education of the Republic of Indonesia which was granted to Saikhu Akhmad Husen (Associate Professor in Faculty of Science and Technology, Universitas Airlangga). Moreover, special thanks to PMDSU Scholarship - Batch III (Ministry of Research, Technology, and Higher Education of the Republic of Indonesia) which was awarded to Arif Nur Muhammad Ansori, Raden Joko Kuncoroningrat Susilo, and Suhailah Hayaza.