Journal of Physics: Conference Series **1445** (2020) 012018 doi:10.1088/1742-6596/1445/1/012018

Hepato-Renal Protective Effects of Mangosteen (*Garcinia mangostana* L.) Pericarp Extract in Streptozotocin-induced Diabetic Mice

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Abstract. This study aimed to explore the effect of mangosteen pericarp extract used for reduce random blood glucose, total cholesterol, SGOT, SGPT, plasma creatinine levels, also ameliorates damaged liver hepatocytes and renal proximal tubular cells 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. Before and after STZ injection, random blood glucose and total cholesterol were measured at 1st, 7th, and 14th day of mangosteen pericarp extract treatments. Treatments were given for 14 days. At 15th day, SGOT, SGPT and plasma creatinine levels were measured using Pentra C200, while liver and kidney were collected and then processed into histological slides. Interestingly, we found that mangosteen pericarp extract administration was able to reduce random blood glucose, total cholesterol, SGOT, SGPT, plasma creatinine levels, also ameliorates damaged liver hepatocytes and renal proximal tubular cells in diabetic mice significantly. In conclusion, mangosteen pericarp extract is a promising antidiabetic agent due to its anti-hyperglycemic and antioxidant properties.

1. Introduction

Diabetes mellitus (DM) is a common endocrine disease and affects more than 380 million people worldwide (6% of the population). By 2025 it is estimated that the number of patients with DM wil be increased by fivefold [1]. According to Wild et al. [2], Indonesia ranks fourth in the world with the number of people with DM of 8.4 million people in 2000 and this figure is expected to increase to 21.3

million people in 2030. Diabetes mellitus is divided into type-1 diabetes (insulin dependent DM) and type-2 DM (non-insulin dependent DM). Type-1 DM is an autoimmune disorder that causes the immune system to attack pancreatic cells, therefore damaging one's ability to produce insulin. Type-2 DM is characterized by the occurrence of insulin deficiency or decreased sensitivity of the insulin sensitive target cells due to changes in receptors (insulin resistance) [3-4].

Insulin resistance is a condition associated to target organ failure to respond insulin hormone activity normally. Insulin resistance leads to decreased expression of glucose transporter 4 (GLUT-4) in cells in muscle tissue [5]. Glucose transporter 4 is a glucose transporter that requires insulin to transport glucose from the blood into cells. The decrease in GLUT-4 density causes a decrease in the use of glucose in tissues that are insulin-sensitive resulting in elevated blood sugar or hyperglycemia. The condition of chronic hyperglycemia leads to various complications of DM, including abnormal renal function (nephropathy), retinal dysfunction (retinopathy), neuropathy, and damage to both micro and macro blood vessels [6-7].

Increased blood glucose levels is usually followed by the elevated levels of free fatty acids, leading to the increased superoxide production by mitochondria that may oxidizing sulfhydryl groups of proteins, amino nitrate acids such as tyrosine, increasing lipid peroxidation and causing harmful DNA damage to cells [8]. This condition worsens both the action and the secretion of insulin, thereby increasing the risk of complications due to type-2 DM. The use of antioxidants such as vitamins E and C, lipoic acid, and glutathione, may improve the insulin sensitivity of cells in type-2 DM [9]. This study was designed to determine the effects of mangosteen pericarp extract to lower the blood glucose, blood cholesterol, SGOT, SGPT, and plasma creatinine levels also to repair liver and kidney damage in diabetic mice.

2. Materials and Methods

This study was an experimental research conducted at Animal Laboratory and Histology Laboratory, Faculty of Science and Technology Universitas Airlangga. Male 3-4 months old mice strain BALB/c, weighted 30-40 g were used in this study as sample. Materials used included crude mangosteen (*Garcinia mangostana*) pericarp extract, 90% ethanol, phosphate-buffered saline (PBS), STZ (streptozotocin, SIGMA) to induce diabetes in mice, citrate buffer solution pH 4.5, CMC (carboxymethylcellulose), standard antidiabetic drugs (Metformin HCl 100 mg/kg), lard, 10% D-glucose for glucose tolerant test, anesthesia (ketamine and xylasin) were used in this study. The main tools in this study were: plastic cage, drinking bottle, feeding container, husk, light microscope, petridish, analytical scales, injection syringe, On Call Plus^{TM} glucometer, Easy Touch^{TM} blood cholesterol meter, and rotary vacuum evaporator.

The procedure began with preparation of mangosteen pericarp extract. The extracted material was pericarp of mangosteen fruit. The determination of 50% lethal dose (LD_{50}) was performed. The determination of antioxidant activity from mangosteen pericarp extract (ethanol) in vitro was performed using DPPH (diphenylpicrylhydrazyl) method. Twenty-four mice were divided into normal control group (KN) and diabetes group. The body weight, fasting blood cholesterol level (before and after lard induction) of mice were then measured. The diabetic group was then induced using STZ. Blood glucose levels were measured at days 7 and 14 after STZ induction. Fasting blood glucose was measured using glucometer to determine diabetic condition. Only mice with fasting blood sugar levels higher than 170 mg/dl were used as diabetic mice. Model animal was grouped as following: non-diabetic mice used as normal control group (KN) and diabetic mice. The diabetic mice were later divided into 2 control groups: untreated diabetic control (KD), diabetic control treated with metformin (100 mg/kg body weight) (KM) and treatment groups given mangosteen pericarp extract. P1, P2, and P3 as the treatment groups were treated using three different concentrations of pericarp extracts; 200 mg/kg body weight, 100 mg/kg body weight, 50 mg/kg body weight, respectively. Each group consisted of 4 mice. Measurements of random blood glucose and blood cholesterol levels were performed before and after STZ injection. Treatment of mangosteen pericarp extract was given for 14 days. On the 15th day after treatment, intra cardiac blood was sampled to measure the levels of SGOT,

SGPT, and blood plasma creatinine levels. The liver and kidney were collected as well. Blood cholesterol levels were measured using Easy Touch™ blood cholesterol meter, and random blood glucose levels were measured using On Call Plus™ glucometer. SGOT, SGPT, and plasma creatinine levels were measured by its absorbances by using Pentra C200 (Horiba Medical) at 510 nm wavelength [4,10]. Histological sections of liver and kidney were stained with Haematoxylin-Eosin and then evaluated. 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. All statistical test was performed at α=0.05.

3. Results and Discussion

Result of random blood glucose and cholesterol level measurement before and after STZ injection was presented in Figure 1, measurement of SGOT and SGPT after extract treatment in Figure 2, evaluation of hepatocytes damage in Figure 3, measurement of creatinine level after extract treatment in Figure 4, and evaluation of damage in renal proximal tubules in Figure 5. Histological section of liver and renal proximal tubules presented respectively in Figure 6 and Figure 7.

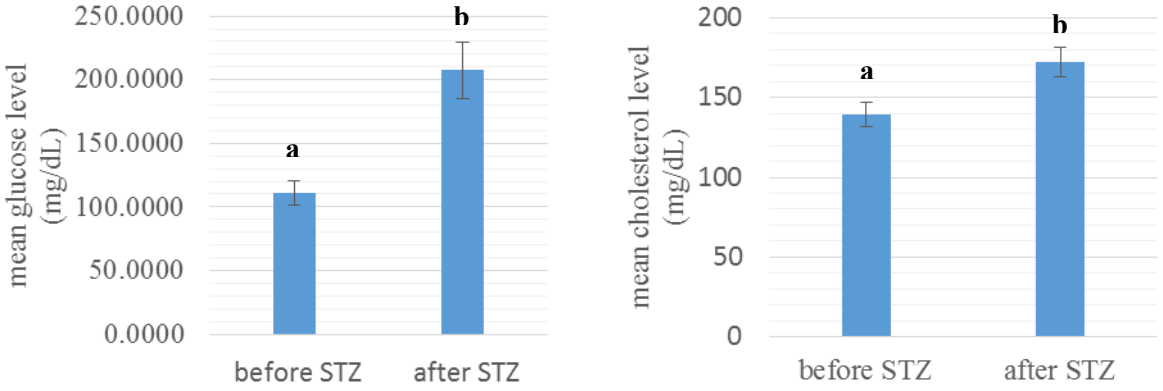

Figure 1. Graphics showing effect of STZ injection on mean random blood glucose and cholesterol level (mg/dL) of mice. Letters above each bar indicated result of t-test (α = 0.05). Same letter indicated insignificant difference, while different letter indicated significant difference.

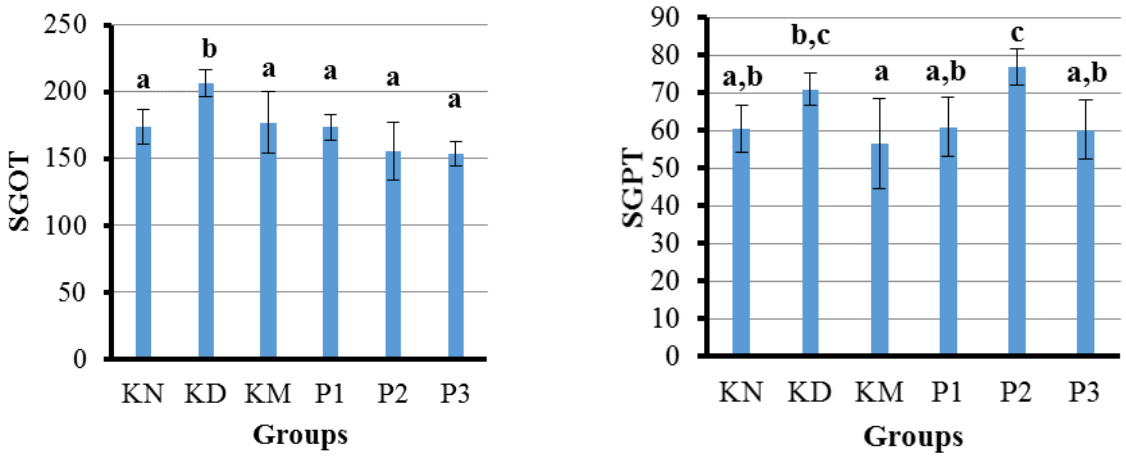

Figure 2. Graphics showing effect of mangosteen pericarp extract on serum SGOT and SGPT of diabetic mice. KN: normal control, KD: untreated diabetic control, KM: diabetic mice treated with metformin HCl (100 mg/kg body weight), P1: diabetic mice treated with 200 mg/kg body weight mangosteen extract, P2: diabetic mice treated with 100 mg/kg body weight mangosteen extract, P3: diabetic mice treated with 50 mg/kg body weight mangosteen extract. Letters above each bar indicated result of Duncan test (α = 0,05). Same letter indicated insignificant difference, while different letter indicated significant difference.

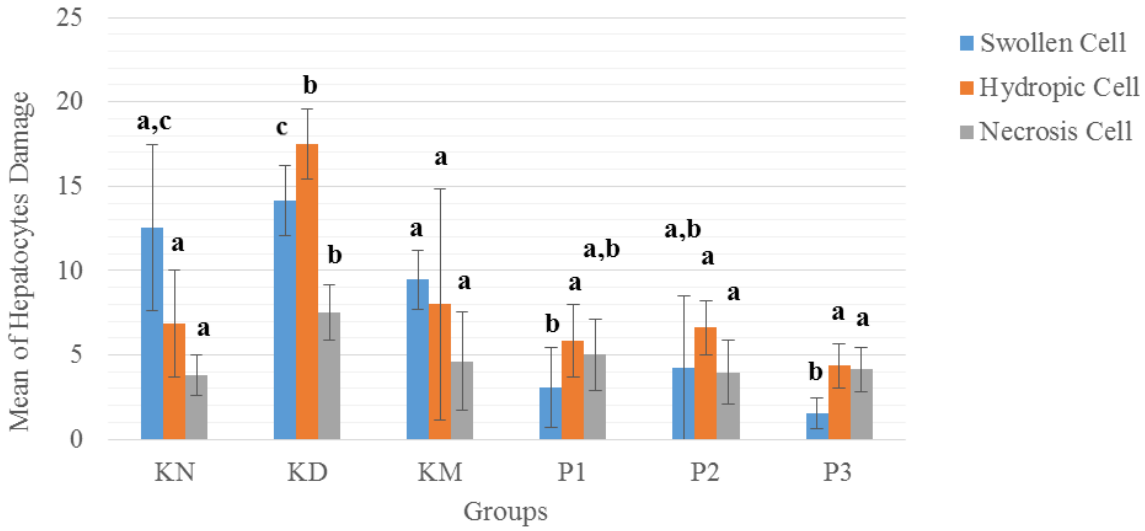

Figure 3. Graphic showing the effect of pericarp extract on hepatocyte damage of diabetic mice. KN: normal control, KD: untreated diabetic control, KM: diabetic mice treated with metformin HCl (100 mg/kg body weight), P1: diabetic mice treated with 200 mg/kg body weight mangosteen extract, P2: diabetic mice treated with 100 mg/kg body weight mangosteen extract, P3: diabetic mice treated with 50 mg/kg body weight mangosteen extract. Letters above each bar indicated result of Duncan test (α = 0,05). Same letter indicated insignificant difference, while different letter indicated significant difference.

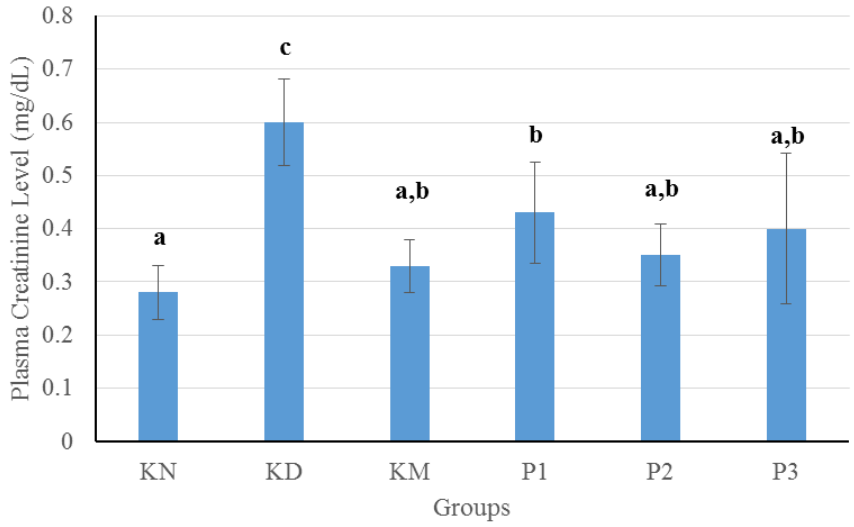

Figure 4. Graphic showing the effect of pericarp extract on plasma creatinine level of diabetic mice. KN: normal control, KD: untreated diabetic control, KM: diabetic mice treated with metformin HCl (100 mg/kg body weight), P1: diabetic mice treated with 200 mg/kg body weight mangosteen extract, P2: diabetic mice treated with 100 mg/kg body weight mangosteen extract, P3: diabetic mice treated with 50 mg/kg body weight mangosteen extract. Letters above each bar indicated result of Duncan test (α = 0,05). Same letter indicated insignificant difference, while different letter indicated significant difference.

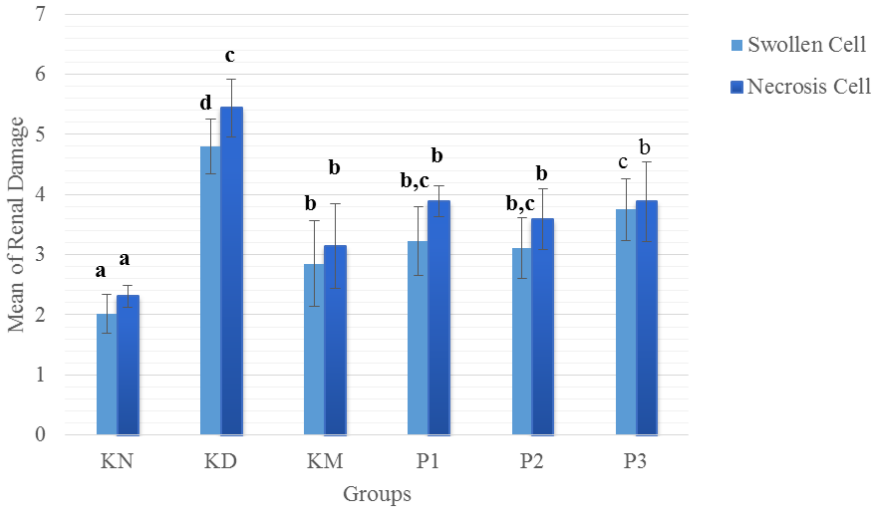

Figure 5. Graphic showing the effect of pericarp extract on histological structure of renal proximal tubular cells of diabetic mice. KN: normal control, KD: untreated diabetic control, KM: diabetic mice treated with metformin HCl (100 mg/kg body weight), P1: diabetic mice treated with 200 mg/kg body weight mangosteen extract, P2: diabetic mice treated with 100 mg/kg body weight mangosteen extract, P3: diabetic mice treated with 50 mg/kg body weight mangosteen extract. Letters above each bar indicated result of Duncan test (α = 0,05). Same letter indicated insignificant difference, while different letter indicated significant difference.

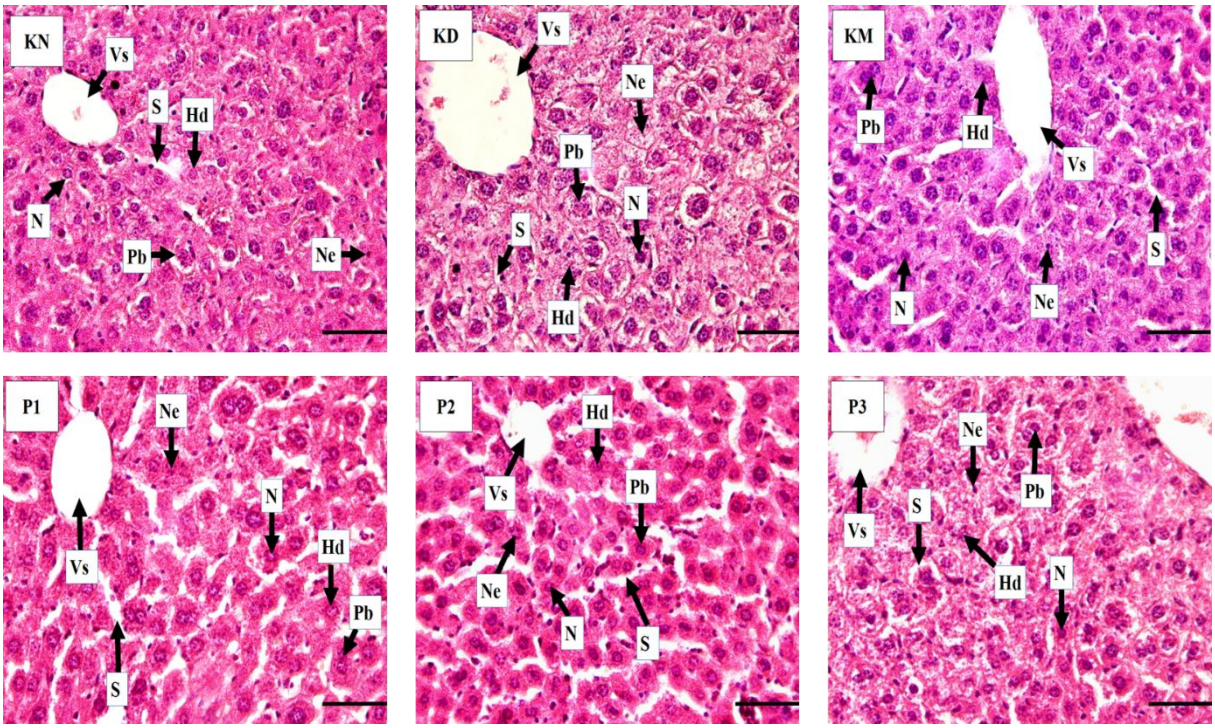

Figure 6. Histological section of liver of diabetic mice after mangosteen pericarp extract administration. KN: normal control, KD: untreated diabetic control, KM: diabetic control treated with 100 mg/kg body weight Metformin HCl, P1: diabetic mice given 200 mg/kg body weight mangosteen extract, P2: diabetic mice given 100 mg/kg body weight mangosteen extract, P3: diabetic mice given 50 mg/kg body weight mangosteen extract. Vs = central vena, S = sinusoid, N = normal cell, Pb = swollen cell, Hd = hydropic cell, Ne = necrotic cell. Bar = 60 µm.

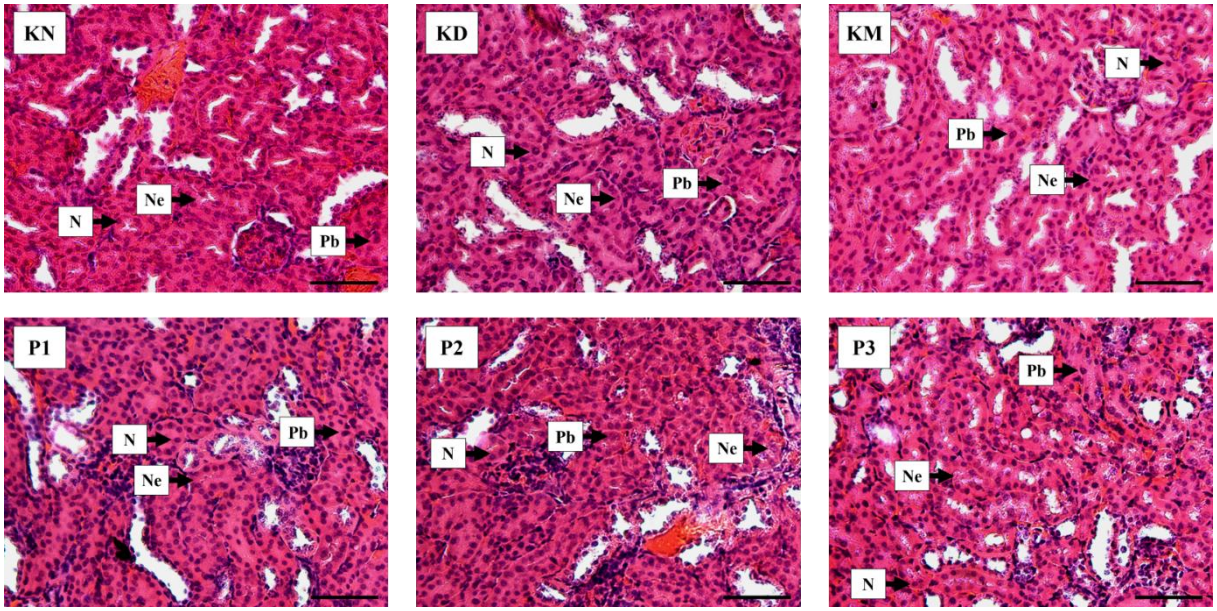

Figure 7. Histological section of kidney of diabetic mice after mangosteen pericarp extract administration. KN: normal control, KD: untreated diabetic control, KM: diabetic control treated with 100 mg/kg body weight Metformin HCl, P1: diabetic mice given 200 mg/kg body weight mangosteen extract, P2: diabetic mice given 100 mg/kg body weight mangosteen extract, P3: diabetic mice given 50 mg/kg body weight mangosteen extract. N: normal cell, Pb: swollen cell, Ne: necrotic cell. Bar = 60 µm.

Based on result of mean random blood glucose and cholesterol level before and after STZ induction (Fig. 1), it was shown that STZ injection at 30 mg/Kg bw for 5 consecutive days was found to be able to elevate random blood glucose level significantly, from mean of 111,391 ± 9,618 to 207,261 ± 21,810 mg/dL. This indicated that STZ was able to impair β-cells of the islets of Langerhans, leading to reduced insulin synthesis and elevated blood glucose level [7]. While cholesterol level was also found to be increased after STZ induction, from mean of 139,695 ± 7,636 to 172,043 ± 9,083 mg/dL after STZ induction. Increasing cholesterol level was caused by hyperglycemia due to damaged β-cells of the islets of Langerhans and reduced plasma insulin level, leading to increased gluconeogenesis both in liver and skeletal muscle and mobilization of fat from adipose tissue, causing blood cholesterol level to rise. Breakdown of fat storage, both on skeletal muscle and liver would cause significant rise of blood cholesterol level [10-11].

Extended hyperglicemia also lead to glucose could not be processed into energy, thus energy needed to be produced from other source. Because of that, energy was produced from increasing catabolism of fat and protein [10]. Along with such condition, lipolysis was stimulated and both free fatty acid and glycerol level in the blood were elevated. In this case, acetyl-KoA production in liver was increased, which in turn would be converted into acetoacetic acid and finally reduced into β-hidroxybutiric acid or decarboxylated into aceton [12]. Due to energy produced from protein and fat, cholesterol level produced on metabolism chain of fat and protein also increased. On DM patients, hyperglycemia condition would cause elevation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) production due to increasing NADPH oxidation in endothelium tissue. Reactive oxygen species (ROS) and Reactive nitrogen species (RNS) were highly reactive molecules able to directly oxidized and damaged DNA, protein, and lipid, also elevated oxidative stress. Oxidative stress occurred when imbalance happened between highly reactive molecules (ROS and RNS) and existing antioxidant.

Rising ROS and RNS level in current study after STZ injection for five consecutive days also affecting decrease of the islets of Langerhans diameter in diabetic control group [6-7].
Streptozotocin was nitric oxide (NO) donor and also able to induce reactive oxygen that could cause cellular damage [13]. Based on Pedraza-Chaverrí et al. [14], free radicals ROS and RNS disturbed physiological function of tissue and caused damage to liver and kidney. Kim et al. [15] mentioned that elevation of ROS and proinflammatory cytokines also played role in various organ damage. In STZ-induced mice, level of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and plasma creatinine were found to be elevated. This was due diabetic condition in mice, which induced ROS production. Biochemical changes activated by hyperlipidemic oxidative stress including rising glucose metabolism via polyol pathway, increasing level of advanced glycation end products (AGEs), activation of protein kinase C (PKC), and elevation of excessive glucose transport via heksoamine pathway. These biochemical changes would further increase ROS production, leading to lipid peroxidation that could damage cell membrane and finally the cell itself. Cellular damage occurred in liver and kidney were indicated by rising level of serum SGOT, SGPT, and creatinine. In short, diabetic condition played role in elevation of serum SGOT, SGPT, and creatinine [16].
The number of swollen, hydropic, and necrotic hepatocytes in diabetic control (KD) significantly different compared to other treatment groups KN, KM, P1, P2, and P3. Hyperlipidemia in KD caused cellular damage that lead to increasing level of serum SGOT, SGPT, and creatinine. The same circumstance also occurred in kidney; the number of hydropic and necrotic tubule cells in diabetic control mice kidney were significantly different to other groups KN, KM, P1, P2, and P3. Alteration of cell structure was also induced by hyperglycemia, as hiperglicemia-induced oxidative stress would lead to lipid peroxidation and structural damage of various cells [8].
Kidney had important role in homeostasis, as it maintained bodily fluid, and organized dispersal of metabolism waste and toxic materials, such as urea, uric acid, ammonium, creatinine, inorganic salts, and various unneeded medicinal substances. BUN and creatinine were metabolites of urea protein and creatine excreted via glomerular filtration and actively secreted by renal proximal tubules [17]. Damage on renal proximal tubular cells could indicate progressivity of disease better than gramerular damage. Excretion of BUN and creatinine were result of two physiological processes; glomerular filtration and secretion of proximal tubules. If disturbance was to be found in both processes, serum BUN, and creatinine level could rise [18].
Creatinine excretion via kidney was relatively constant and not affected by external factors. Elevating serum creatinine level might be due to damage in kidney disrupting glomerulus filtration, acute tubules necrosis, glomerulonephritis (glomerular damage), and tubules apoptosis [19]. Normal creatinine serum in mice was found in range of 0.2-0.9 mg/dL (Hall, 2007) [20]. Result of Parvizi et al. [21] indicated that STZ-induced diabetic rat had higher serum creatinine level compared to its normal counterpart. This was due damage in renal histological structure of rat disrupted kidney activity in eliminating creatinine. Guyton and Hall [22] mentioned that serum creatinine level rose to two-fold of its normal level if renal function decreased 50%.

4. Conclusion

From result of current study, it could be concluded that STZ injection could elevate random blood glucose, cholesterol, SGOT, SGPT, and creatinine level while administration of mangosteen pericarp extract was able to significantly lower plasma SGOT, SGPT, and plasma creatinine levels, also ameliorates damaged hepatocytes and renal proximal tubules of diabetic mice.

Acknowledgement

The authors would like to thank Universitas Airlangga and Ministry of Research, Technology and Higher Education of the Republic of Indonesia for supporting this work (granted to SAH). Also,

PMDSU Scholarship (Batch III) from Ministry of Research, Technology and Higher Education of the Republic of Indonesia to ANMA and RJKS.

References

[1] Sharma B, Satapathi SK and Roy P 2007 *International Journal of Pharmacology* **3(6)** 444
[2] Wild S, Roglic G, Green A, Secree R and King H 2004 *Diabetes Care* **27** 1047
[3] Husen SA and Winarni D 2014 *Proceeding Seminar Nasional Biodiversitas V*, Universitas Airlangga, Surabaya, Indonesia
[4] Husen SA, Winarni D, Khaleyla F, Kalqutny SH and Ansori ANM 2017 *AIP Conference Proceedings* **1888(1)** 020026-1
[5] Park J 2006 *Diabetes* **55** 2939
[6] Husen SA, Winarni D, Ansori ANM and Susilo RJK 2016 *Proceeding Seminar Nasional Biodiversitas VI*, Universitas Airlangga, Surabaya, Indonesia
[7] Husen SA, Winarni D, Khaleyla F and Kalqutny SH 2016 *Journal of Biological Researches* **22(1)** 13
[8] Novelli M, Bonamassa B, Masini M, Funel N, Canistro D, De Tata V, Martano M, Soleti A, Campani A, Paolini M and Masiello P 2010 *Naunyn Schmiedebergs Arch Pharmacol* **382(2)** 127
[9] Evans JL, Goldfine ID, Maddux BA and Grodsky GM 2003 *Diabetes* **52** 1
[10] Husen SA, Kalqutny SH, Ansori ANM, Susilo RJK, Alymahdy AD and Winarni D 2017 *Bioscience Research* **14(4)**
[11] Husen SA and Winarni D 2015 *Proceeding 5th International Conference and Workshop on Basic and Applied Sciences*, Universitas Airlangga, Surabaya, Indonesia
[12] Powers SK and Jackson MJ 2008 *Physiol Rev* **88(4)** 1243
[13] Szkudelski T 2001 *Physiol Res* **50** 537
[14] Pedraza-Chaverrí J, Barrera D, Maldonado PD, Chirino YI, Macías-Ruvalcaba NA, Medina-Campos ON, Castro L, Salcedo MI and Hernández-Pando R 2004 *BMC Clin Pharmacol* **4**
[15] Kim EY, Kim CS, Choi JS, Bae EH, Ma SK, Kim KK, Lee JU, Kim, SW and Lee KE, 2013 *Biochem Biophys Res Commun* **434** 527
[16] Ceriello A 2011 *Medicographia* **33** 29
[17] Breyer MD and Qi Z 2010 *Kidney Int* **77** 487
[18] Musso CG, Michelángelo H, Vilas M, Reynaldi J, Martinez B, Algranati L and Macías NJF 2009 *Int Urol Nephrol* **41** 727
[19] Yuan PST, Dunn SR, Miyaji T, Yasuda H, Sharma K and Star RA 2004 *Am J Physiol* **286** F1116
[20] Hall RL 2007 *Clinical pathology of laboratory animals in Animal Model in Toxicology*, 2nd Edition. (USA: CRC Press)
[21] Parvizi MR, Parviz M, Tavagar SM, Soltani N, Kadkhodaee M, Seifi B, Azizi Y and Keshavarz M 2014 *Journal of Diabetes & Metabolic Disorders* **13** 84.
[22] Guyton AC and Hall JE 2013 *Buku Ajar Fisiologi Kedokteran*, Edisi 12 (Jakarta: EGC)