Renal Protective Effects of Gamma-Mangostin in Streptozotocin-Induced Diabetic Mice

Saikhu Akhmad Husen[1,2], Salamun[1], Arif Nur Muhammad Ansori[3], Suhailah Hayaza[4], Raden Joko Kuncoroningrat Susilo[4], Dwi Winarni[1,2], Win Darmanto[5]

[1]Associate Professor, Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, 60115, Indonesia, [2]Researcher, Animal Histology Laboratory, Faculty of Science and Technology, Universitas Airlangga, Surabaya, 60115, Indonesia, [3]Doctoral Student, Doctoral Program in Veterinary Science, Faculty of Veterinary Medicine, Universitas, Airlangga, Surabaya, 60115, Indonesia. [4]Doctoral Student, Doctoral Program in Mathematics and Natural Sciences, Faculty of Science and Technology, Universitas Airlangga, Surabaya, 60115, Indonesia, [5]Professor, Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, 60115, Indonesia

Abstract

This study was aimed to investigate the ability of gamma-mangostin to reduce plasma blood urea nitrogen (BUN) and creatinine and ameliorates the impaired renal proximal tubular cells in diabetic mice. Antioxidant assay was conducted by using male BALB/c mice. Mice were divided into two groups, they were normal control (KN) and streptozotocin-induced diabetic mice. Streptozotocin (STZ) induction was performed using multiple low-dose of 30 mg/kg body weight injected for five consecutive days. Diabetic mice have divided into three subgroups; diabetic control (KD), diabetic mice treated with acarbose (KA), and diabetic mice treated with gamma-mangostin. The gamma-mangostin treatment group was categorized based on the dose given; P1 (1 mg/kg BW), P2 (2 mg/kg BW), and P3 (4 mg/kg BW). Interestingly, gamma-mangostin administration was found to be able to lower plasma BUN and creatinine and ameliorate the impaired renal proximal tubular cells in diabetic mice significantly. Therefore, gamma-mangostin has demonstrated high antioxidant activity. The proof suggests that gamma-mangostin is a lead compound candidate for clinical management or prevent diabetes mellitus.

Keywords: *antioxidant activity, diabetes mellitus, gamma-mangostin.*

Introduction

Diabetes mellitus (DM) is a multifactorial disease characterized by a chronic hyperglycemia syndrome and an impaired metabolism of carbohydrates, fats, and proteins caused by insulin secretion insufficiency and endogenous insulin activity. Insulin insufficiency can occur due to a body's cells irresponsiveness to insulin caused by the impaired insulin production in Langerhans beta cells of the pancreatic gland[1,2]. Indonesia is currently the sixth biggest DM patients in the world, which is 5.8% of its population or around 10 million people. The number of DM patients at the national and international levels from year to year continues to increase[3]. Type-2 DM is the most common type of DM in Indonesia. Type-2 DM is caused by a decrease in the insulin sensitivity or an increase in the insulin resistance[2, 4].

Hyperglycemia is a condition of the increasing blood glucose levels above normal due to insulin deficiency, damage of beta cells, and the presence of insulin resistance in the liver and muscles. Chronic hyperglycemia in DM has an important role in various organs damages including the heart, eyes, bones, kidneys, liver, nerves, and vascular system, which can cause complications in the body system. Complications

Corresponding Author:
Win Darmanto
Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, 60115, Indonesia, Email: windarmanto@fst.unair.ac.id

of DM are associated with non-enzymatic glycation reactions in the hyperglycemia called glycosylation. Glycosylation is a reaction that occurs between protein and glucose at high concentrations, this reaction is also called the Maillard reaction. Maillard reactions form an advanced glycation end products (AGEs) and advanced oxidation protein products (AOPP) which show an oxidative stress that disrupts the balance of oxidants and antioxidants in the body resulting in an increase in free radicals[5,6,7].

Antioxidants are substances that inhibit the negative effects of free radicals by giving the electrons so that the damage of lipids, cell membranes, blood vessels, DNA, and others caused by the reactive compounds such as ROS could be prevented[8]. To reduce the adverse effects of these free radicals, exogenous antioxidants are needed, such as vitamin E, vitamin C, and other antioxidants obtained from consuming various types of fruits and vegetables that contain high antioxidants. One of them is gamma-mangostin[1]. The gamma-mangostin compound is a pigment from *Garcinia mangostana* which is able to donate hydrogen atoms and stabilize free radicals in resonance. In addition to neutralizing free radicals, these antioxidants are expected to reduce the oxidative stress, especially in various affected cells due to the prolonged hyperglycemic conditions, such as hepatocytes and renal proximal tubular cells[4,6,7,8]. Thus, this study was designed to answer the problem of whether the administration of gamma-mangostin can reduce BUN levels and blood plasma creatinine as well as repair the renal proximal tubular cells damage in diabetic mice.

Materials and Method

This experimental study was conducted at the Animal Laboratory and Animal Histology Laboratory, Faculty of Science and Technology, Universitas Airlangga and also at the Institute of Tropical Diseases (ITD), Universitas Airlangga. The used sample was adult male mice, strain BALB/C, 3-4 months old, weight ranged from 25-40 g. The study materials consisted of gamma-mangostin (purchased from Sigma). Other materials consisted of streptozotocin (purchased from Sigma), buffer citrate solution pH 4.5, and phosphate-buffered saline (PBS), solvent extract of carboxymethylcellulose (CMC), standard antidiabetic drug (Acarbose, 100 mg/kg body weight), ketamine hydrochloride/xylazine hydrochloride (purchased from Sigma), and D-glucose (purchased from Sigma)[7].

The study samples consisted of 24 male mice, distributed to the normal control group (KN) and the diabetic group which was induced by STZ. 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 STZ were divided into two 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 in which the treatment 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 4 mice and those treatments were administered for 14 days[7].

Blood glucose of diabetic mice was measured on 1st, 7th, and 14th day after gamma-mangostin treatment to make sure the mice were successfully in the hyperglycemic condition. On the 15th day, blood was taken from the intracardial and the measurement of BUN and blood plasma creatinine were done using Pentra C200 (Horiba Medical) in 510 nm wavelength. The damage on the kidney structure was determined from the histological sections stained with hematoxylin-eosin (HE). Furthermore, data with normal distribution and homogenous variation was analyzed using one-way variance analysis continued by Duncan test. Data with normal distribution and non-homogenous variation was analyzed using Brown Forsythe test continued with a t-test. All statistical test was conducted at $\alpha = 0.05$.

Results

The mean of mice's blood glucose level before and after the STZ induction are presented in Figure 1. The data of BUN and creatinine level is presented in Figure 2. The mean data of swollen cells and necrotic cells in renal proximal tubules is shown in Figure 3. Photomicrographs of renal proximal tubular cells are presented in Figure 4.

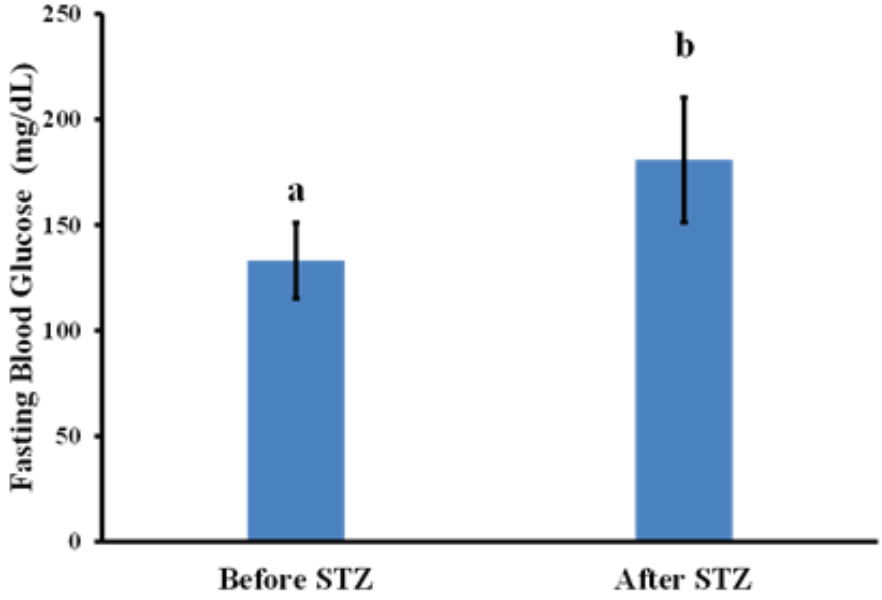

Figure 1. fasting blood glucose (mg/dL) before and after STZ induction. The different letter indicated a significant difference.

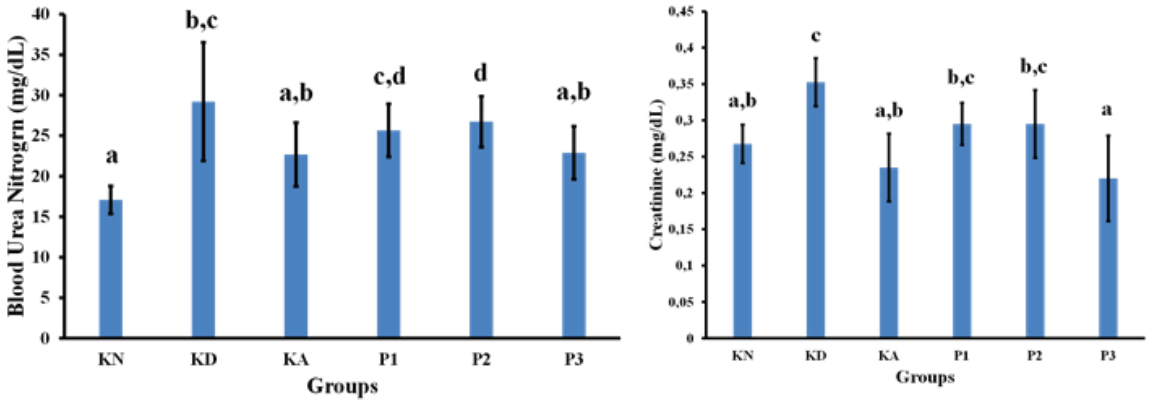

Figure 2. BUN and creatinine level changes in each mice group after treatments. The different letters indicated a significant difference.

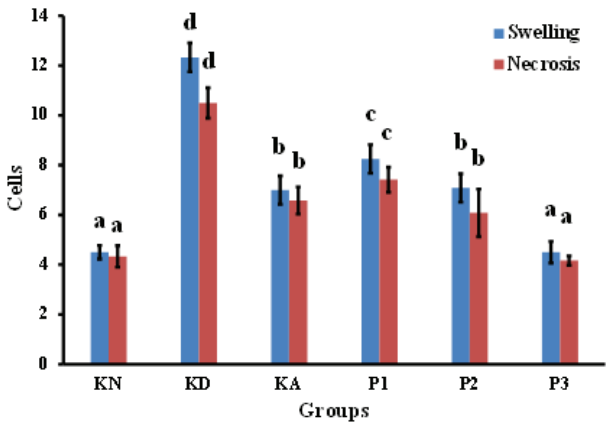

Figure 3. the renal proximal tubular cells damage of each mice groups after treatments. The different

letters indicated a significant difference.

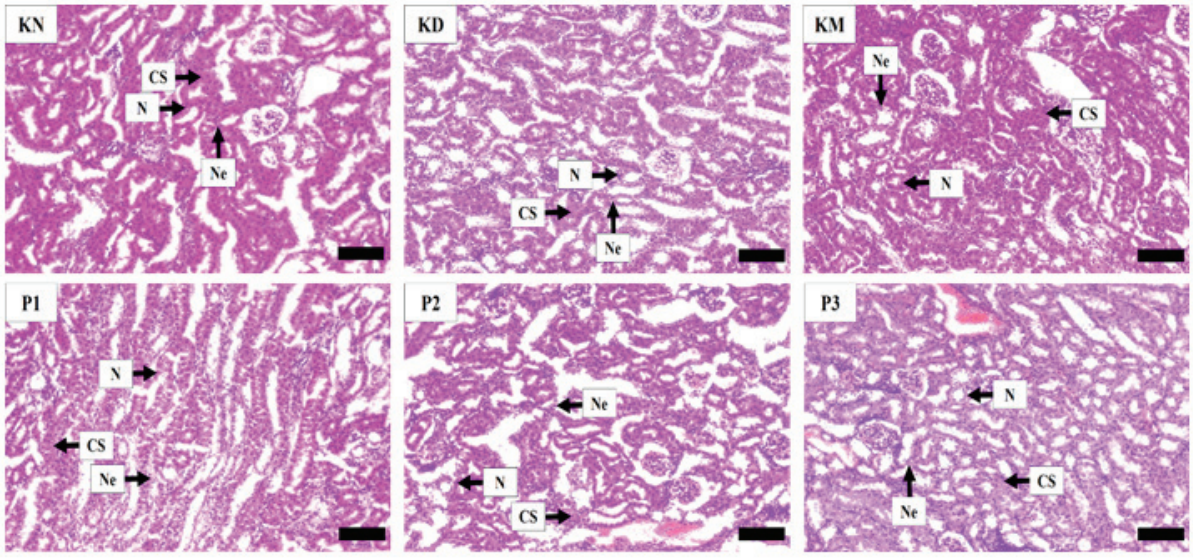

Figure 4. histological structure of renal proximal tubular cells of mice after gamma-mangostin treatment.

N = normal cell, CS = swollen cell, Ne = necrotic cell. Bar: 100 µm.

Discussion

A condition of hyperglycemia in patients with DM causes a glucose autoxidation, resulting in the activation of protein kinase C, protein glycation, and the activation of the polyol metabolic pathway which further accelerates the formation of ROS or the oxidative stress conditions. The presence of ROS causes free radicals in the body to increase. Free radicals can damage the structure and function of various body tissues, one of them is the kidney tissue[9,10]. In patients with DM, the condition of hyperglycemia causes an increase in the production of ROS and RNS due to the increased oxidation of NADPH on endothelial tissue. ROS and RNS are highly reactive molecules that can directly oxidize and damage DNA, proteins, lipids, and cause an oxidative stress. An oxidative stress occurs when there is an imbalance between the number of highly reactive molecules (ROS and RNS) and the existing antioxidants[4, 6].

STZ is a free radical that can increase ROS and RNS, very reactive, especially for hepatocytes and renal tubule cells. In the group of mice induced by STZ, it showed an increase in BUN levels and plasma creatinine in the diabetic control group (KD). KD was significantly different from the normal control group (KN), KM, P1, P2 or P3. This is because the diabetic condition triggers the formation of ROS through the glucose autoxidation pathway, the formation of advanced glycation end products (AGEs), and the polyol pathway mechanism. ROS can trigger lipid peroxidation in the cell membranes and cause damage to these cells. Free radicals can cause lipid peroxidation, which can damage the structure of cell membranes, and damage to the structure and function of renal proximal tubular cells which is characterized by the increased BUN and plasma creatinine levels in the diabetic control group compared to the KN, KM, P1, P2, and P3. The data analysis on BUN and creatinine levels showed that the average BUN in the diabetic group was significantly different compared to KN, KM, P1, P2, and P3. This indicated that the injection of multiple low-dose STZ was able to significantly increase the plasma BUN levels and creatinine plasma levels. The plasma creatinine levels of diabetic group also showed the significant differences compared to KN, KM, P1, P2, and P3.

The kidneys are organs that play a role in regulating the body's balance, maintaining body fluids, and regulating the disposal of metabolic waste and toxic substances such as urea, uric acid, ammonia, creatinine, inorganic salts, as well as the drugs that are not needed by the body[8]. BUN and creatinine are urea protein and creatine metabolites which are excreted through glomerular filtration and are actively secreted by the proximal renal tubules[11]. A damage to the kidney's proximal tubular cells of the kidney is an indicator of disease progression. BUN and creatinine excretion

are the results of two physiological processes, namely glomerular filtration and proximal renal tubular secretion. If there is a disruption in BUN and creatinine secretion by the proximal renal tubules, then BUN and plasma creatinine levels will increase[12].

Creatinine excretion in the kidneys is relatively constant and is not affected by outside factors. Creatinine is an effective indicator of kidney damage because creatinine levels in the blood are more stable[8]. The increased creatinine levels in the blood can be caused by kidney damage mainly due to glomerular filtration disorders, acute tubular necrosis, glomerulonephritis or the damage to glomeruli and tubular apoptosis[13,14]. Normal plasma creatinine levels in mice (*Mus musculus*) are 0.2 to 0.9 mg/dL[8]. The results of research conducted by Husen *et al*.[15] showed that diabetic mice that had been injected with STZ experiencing an increase in the serum creatinine levels compared to the normal group. This is due to the result of kidney's histology structural damage that occurs in the diabetic mice which causes the kidney's work in eliminating creatinine is disrupted.

STZ is capable of generating a reactive oxygen which has an important role in cell damage[16,17]. ROS and RNS can interfere the physiological function of a tissue and then cause a kidney damage[6,8]. Lee *et al*.[18] stated that an increase in ROS and proinflammatory cytokines play an important role in the damage of glomeruli, tubules and blood vessels. The results of research from Zafar *et al*.[19] stated that diabetic *Rattus norvegicus* mice were injected with single dose STZ at a dose of 45 mg/kg of body weight can cause tubular necrosis, glomerulosclerosis, tubular atrophy, and thickening of the glomerular membrane. Other studies conducted by Hou *et al*.[20] found that an increase in BUN and creatinine levels in the diabetic group indicates the damage to kidneys. The biochemical parameters correlate with the renal histology studies. STZ causes a significant damage of kidney histological structure, including glomerulus and tubules. Thus, STZ injection is able to interfere in the structure and function of renal tubular epithelial cells.

The kidneys are not dependent on insulin for glucose absorption, so an increase in blood glucose levels in a diabetic condition will produce a high glucose at the intracellular level and could cause a severe and sustained hyperglycemia. Increasing the amount of glucose filtered by the glomeruli under hyperglycemia conditions will increase the workload of renal proximal tubular cells. In addition, the proximal tubular cells cannot reduce the glucose transport levels to prevent excess intracellular glucose in hyperglycemia. Excessive glucose uptake to the proximal tubule can inhibit the reabsorption and secretion in proximal tubular, thus, the creatinine levels increase[21]. Mohora *et al*.[5] states that hyperglycemia conditions can cause glucose metabolism disorders, as well as the direct reactions to other molecules in the cell which lead to the high formation of body's oxidants. This condition heads to an oxidative stress, which is a condition where there is an increase in the production of oxidants in the body, while the endogenous antioxidants which play a role in neutralizing the oxidant's performance are disrupted. This is supported by Winiarska *et al*.[22] which states that hyperglycemia is associated with the increased ROS production and oxidative stress condition which perform a key role in the pathogenesis of this disorder. In addition, study conducted by Li *et al*.[23] stated that PKC activation can induce the renal tubular epithelial cell damage.

Conclusion

Interestingly, gamma-mangostin administration was found to be able to lower plasma BUN and creatinine and ameliorate the impaired renal proximal tubular cells in diabetic mice significantly. Therefore, gamma-mangostin has demonstrated high antioxidant activity. The proof suggests that gamma-mangostin is a lead compound candidate for clinical management or prevent DM.

Conflict of Interest : The authors declare that they have no conflict of interest.

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Ethical Approval: This study was approved by the Animal Care and Use Committee, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

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