

The Effect of Juice Mangosteen Rind (*Garcinia Mangostana L.*) to Blood Sugar Levels and Histological of Pancreatic Rats With The Induction of Streptozotocin

Maris Kurniawati,^{1*} Chanif Mahdi,² and Aulanni'am Aulanni'am²

¹Education Faculty of Kanjuruhan University, Jl. Slamet Supriadi, Malang, East Java 65148, Indonesia

²Mathematic and Science Faculty of Brawijaya University, Jl. Veteran 65145, Malang Indonesia

*Corresponding email : mrskurniawati@gmail.com

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ABSTRACT

Diabetes Mellitus (DM) is a chronic metabolic disorder characterized by high blood sugar levels as a result of insufficiency of insulin function. Xanton, a bioactive compound in the mangosteen rind is convinced to possess antidiabetic effects. There for, the effect of juice mangosteen rind (*Garcinia mangostana L.*) on blood sugar levels, and histological features of pancreatic rats with the induction of streptozotocin was investigated. This research using experimental rats species of *Rattus norvegicus* Wistar strain male. Rats was divided into three groups, the first group was the control without any treatments, group II was sick rat group, and group III was therapy rat treated with juice mangosteen rind (a dose of 110 mg/kg body weight) by *sonde* for 2 consecutive weeks. Then, blood sugar levels of all rat groups were measured along with observation of their pancreatic histological. The results showed that the blood sugar level in therapy rat group (104.7 ± 10.9 mg/dL) is not significantly different with that of rat control group (108.5 ± 19.5 mg/dL), revealing effectiveness of therapy. Conversely, blood sugar level remained high in sick rat group (163.8 ± 16.2 mg/dL). Juice mangosteen rind can improve conditions of the pancreatic histology rat with the induction of streptozotocin.

Keywords: Mangosteen, blood sugar levels, pancreatic histology, streptozotocin

INTRODUCTION

The World Health Organization (WHO) estimates that 300 million people worldwide will suffer diabetes mellitus in 2025. According to the survey conducted by WHO in 2005, Indonesia ranks fourth with the largest number of diabetics in the world after India, China and the United States [1]. The magnitude of the prevalence of diabetes mellitus is an important issue that deserves serious attention and treatment.

The main goal of diabetes treatment is to keep blood sugar levels within a normal range. Hypoglycemic drugs can restore blood sugar levels within the normal range [2]. Xanton is a bioactive compounds belonging to polyketide in the mangosteen rind were thought to have anti-diabetic effect that can lower blood sugar levels of the condition of hyperglycemia in patients with diabetes mellitus.

Hyperglycemia in diabetes mellitus can cause auto-oxidation of glucose, protein glycation and polyol pathway activation metabolism, thus increasing the formation of reactive oxygen species (ROS). Excessive ROS production will lead to oxidative stress. Oxidative stress was ROS production exceeds the antioxidant capacity. It has a negative

effect chain reactions on cell membranes namely lipid peroxidation, DNA and proteins in different tissues so it would appear from diabetic complications such as retinopathy, nepropathy, neuropathy, microvascular and macrovascular problems [1].

To reduce the effects of oxidative damage caused by hyperglycemia required exogenous antioxidants. Xanton from mangosteen rind potent antioxidant has been tested by using reagent 2,2-diphenyl-1-pikrilhidrazil (DPPH) in vitro. Increased supply of antioxidants will help prevent clinical complications of diabetes mellitus. Compounds xanton group also has pharmacological activity such as anti-inflammation [3]. Its antioxidant and anti-inflammatory of xanton very useful to improve a pancreatic beta cell histology of insulinitic conditions that occur in the state of hyperglycemia.

EXPERIMENT

Chemicals and instrumentation

This study used animal rat *Rattus norvegicus* type Winstar strain males aged 2 months with an average body weight 100-180 grams. Rats were divided into 3 groups, group I was the control without treatment, group II was sick rat group with induction streptozotocin, and group III was rat therapy group that given juice mangosteen rind. The use of animals in this research has received an ethical clearance from animal care and use committee of Brawijaya University No.133-KEP-UB.

Materials used in the study were streptozotocin purchased from Sigma Chemical Co. The apparatus used in the study include glukotest tools (one touch) and microscope (Shimadzu).

Procedure reaction

MLD-STZ Injection At Rat

Injection of streptozotocin (STZ) in rats groups II and III performed intraperitoneal that is the abdominal peritoneal cavity with multi-low dose, i.e. 20 mg/kg of bw/day for 5 times for 5 consecutive days [4]. Subsequently incubation for 2 weeks (14 days) after the injection and measurement of blood glucose levels to determine the condition of diabetic rats.

Determination of Blood Glucose Levels

Blood glucose levels were determined by the glucometer digital. Blood of the rat tail dripped to the wells located on the stick glucometer and the results appear on screen digital glucometer. The process of a glucose checking level were conducted i.e after MLD-STZ injection and after treatment with juice mangosteen rind to rats otherwise recovered.

Preparation of Juice Mangosteen Rind

Juice was made by mangosteen rind obtain from Malang (Indonesia). Taxonomi identification was conducted at Biology Department of Brawijaya University No.0081/Takso. Identifikasi/03/2013. Mangosteen fruit is washed, cooled and stored at 4°C, then separated from the rind. Mangosteen rind and water with the ratio of 1:1 (w/w) was mashed in a blender to produce a juice mangosteen rind, filtered and was stored in the refrigerator.

Therapy with Juice Mangosteen Rind

Rats in group III were treated with juice mangosteen rind. Therapy conducted for 2 weeks at a dose that is 110 mg/kg body weight given by *sonde* for 2 consecutive weeks using a syringe (Terumo syringe).

Embedding Pancreas

The first step embedding (planting) is the pancreas organ immersed in a fixative solution. Then, it was soaked in 70% ethanol for 24 hours, then transferred and immersed in 80% ethanol for 2 hours; ethanol 90% for 20 minutes; 95% ethanol for 20 min, and absolute ethanol for 20 minutes. Each step is repeated for 3 times. The next treatment is removal of the pancreas in organ xylol solution 1 and 2 respectively for 20 minutes. Xilol 3 done at a temperature of 60-63°C for 30 min. Further, organ pancreas dipped in liquid paraffin was poured into the container. After a while paraffin would solidify and pancreas was put in a paraffin block.

Making Preparations Pancreas

The first step is the creation of pancreatic preparations to enter the pancreas in paraffin blocks before embedding the clip results (block holder) mitokrom and arranged parallel to the blade mitokrom. The pancreas was cut to a size of 5 µm. Sliced drawn with a brush and put water at room temperature to unfold that may occur in preparations. Results slices removed with a brush in warm water 38-40°C for rectifying existing wrinkles. Slices were stretched perfectly taken with a glass object. Selected pieces were dried and placed on a hot plate at 38-40°C until further dried and the preparations stored in an incubator at a temperature of 38-40°C for 24 hours.

Hematoxylen-Eosin Staining (HE)

Staining begins with deparafinisasi phase. Deparafinisasi phase was the preparations included in the multilevel xilol 1-3 respectively for 5 minutes. Furthermore, stage of rehydration preparations included in the ethanol-rise started from absolute ethanol 1-3, ethanol 95%, 90%, 80%, and 70% respectively for 5 minutes. Furthermore, it was soaked in distilled water for 5 minutes. The next stage is the coloring, dye preparations included in hemotoxylen to obtain the best color results, ± 10 minutes is enough for penetration of the color of the preparations. Then washed with running water for 30 minutes, then rinsed with distilled water before colored with eosin. After rinsing with distilled water in the dye eosin preparations included alcohol for 5 minutes. Preparations then soaked in distilled water to remove excess eosin. The next stage is to include preparations dehydration in graded ethanol series of 80%, 90%, and 95% to absolute ethanol 1-3. Further clearing is done by inserting xilol preparations at 1, 2, and dried. Further mounting (adhesion) with entellan and ready observed under light microscope with a magnification of 400 times.

Data Analysis

Data obtained blood sugar levels in control group rat, groups of sick rat with induction streptozotocin, and group of rat therapy that given juice mangosteen rind. Data were analyzed by F test using completely randomized design.

RESULT AND DISCUSSION

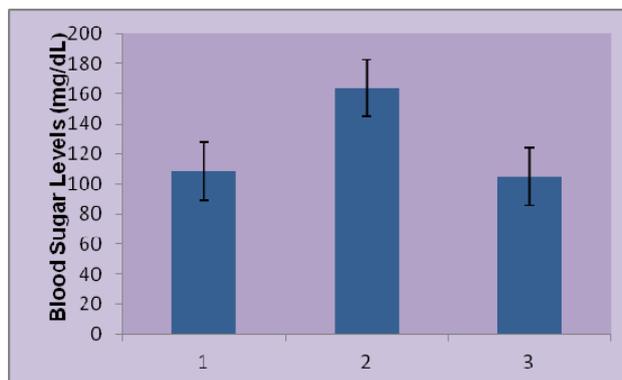
Effect of Juice Mangosteen Rind for Blood Sugar Levels Rats With The Induction of Streptozotocin

After 2 weeks of therapy blood glucose data obtained from each group of rat. Group of healthy rat as a control group obtained blood sugar average of 108.5±19.5 mg/dL, a group of sick rat blood sugar levels on average 163.8±16.2 mg/dL and a group of therapy rats

receiving juice mangosteen rind blood sugar average of 104.7 ± 10.9 mg/dL as showed in figure 1.

The above data showed that the supply of juice mangosteen rind give an effect to control blood sugar levels of the sick rat. Statistical analysis using the F test 1% with a completely randomized design, showed that there were differences in outcomes between each treatment groups.

Figure 1 (Right): The Curve Blood Sugar Levels. (Blood sugar levels average group of rat control (1); Blood sugar levels average group of sick rat (2); Blood sugar levels average group of therapy rat (3))



By consuming juice of mangosteen rind in the third group of rat is able to control blood sugar levels to the normal range. That is juice mangosteen rind therapy has been able to normalize blood sugar levels of rat.

Effect of Juice Mangosteen Rind for Pancreatic Histology Rats With The Induction of Streptozotocin

Beta cells in the island of Langerhans (Islet of Langerhans) produce the hormone insulin that have role in regulating blood glucose levels. Insulin plays a role in glucose transport from the blood into the cells through insulin receptors on the surface of target cells. Insulin also affects the change of glucose into glycogen, reducing glycogenolysis and gluconeogenesis, stimulates changes in glucose or other nutrients into fatty acids (lipogenesis), and helps stimulate protein synthesis [5].

In type 1 diabetes mellitus occur abnormalities in insulin secretion by pancreatic beta cells. People with this type of diabetes have a genetic susceptibility predispose to autoimmune destruction of pancreatic beta cells. Autoimmune response is driven by the activity of lymphocytes which are antibodies to islet cells of Langerhans and the insulin itself [6].

Changes of islet indicate the presence of insulinitis, i.e mononuclear cells (macrophages and dendritic cells) accumulate in the islet. This resulted in decreased pancreatic beta cells in imunoreaktiviti so the destruction of insulin-producing progressive experience. The destruction of the pancreatic beta cells can occur after one week to several months, even years, where a decline in the number of pancreatic beta cells, but the cells are not affected [7].

In this study, pancreatic histology observed experimental animals were taken from group of rat control, group of sick rat with induction of streptozotocin and group of therapy rat treated with juice mangosteen rind. Histological preparations were made by the method of paraffin blocks with Hematoxylen-eosin staining is presented in Figure 2.

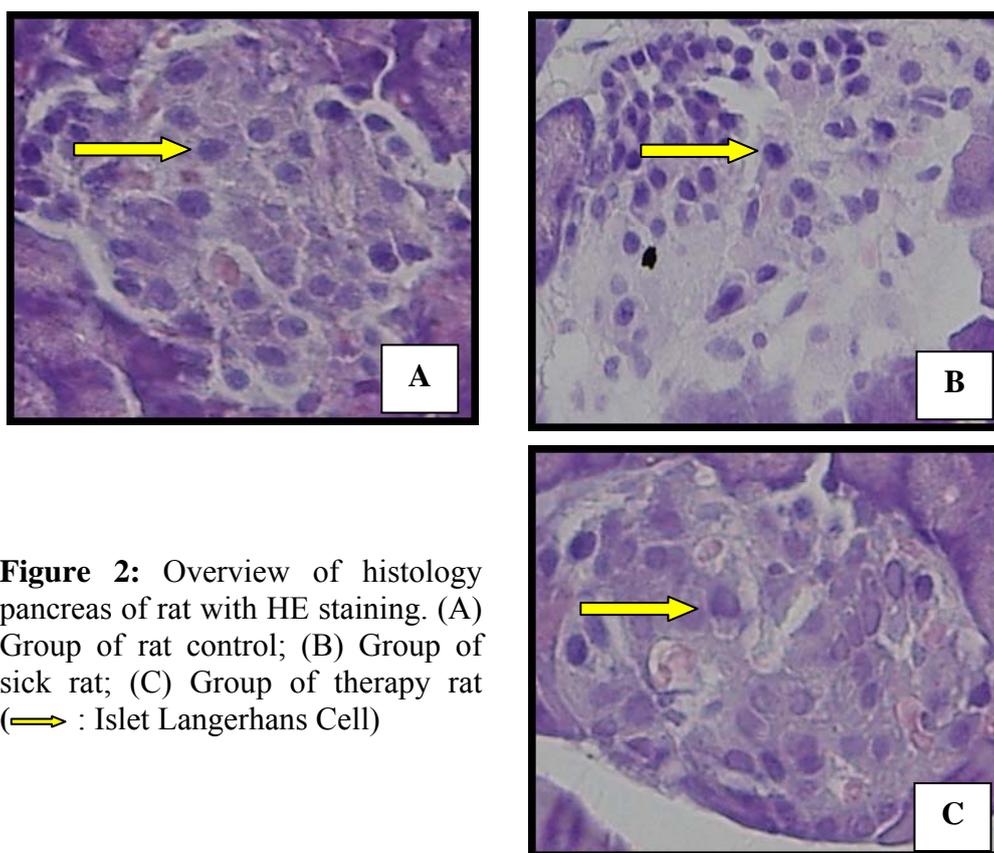


Figure 2: Overview of histology pancreas of rat with HE staining. (A) Group of rat control; (B) Group of sick rat; (C) Group of therapy rat (→ : Islet Langerhans Cell)

Islet Langerhans cells in the group of rat sick visible reduction in the amount of cell mass, the size becomes smaller and some even disappeared. Islet Langerhans cells damage resulting in decreased insulin production that increases blood sugar levels. High blood sugar levels can exacerbate the destruction of islet Langerhans cells because it can increase the formation of ROS such as glucose metabolism through autooxidasi glucose, oxidative phosphorylation, and increased oxidative stress in pancreatic beta cells.

In the group of rats therapy where the islets of Langerhans shape, size and mass of the cell can still be maintained. This is presumably due to the influence of xanton contained in the rind of mangosteen juices which is able to neutralize free radicals that inhibit islet Langerhans cell damage.

The above results support the data measurement of blood sugar levels group of sick rat levels of blood sugar remains high, while rat receiving juice mangosteen rind therapy was able to achieve a state of blood sugar levels back to normal. Normal blood sugar levels in the group of rats who received treatment related to the state of islet Langerhans cells that undergo repairs. Although the overall pancreatic islet yet back to normal circumstances, but the improvement of pancreatic islet has been able to overcome the condition of hyperglycemia in animal models. This proves that the juice mangosteen rind has the potential farmologis good effect.

The potential pharmacological owned juice mangosteen rind is due xanton group containing compounds that act as antioxidants. Chemicals that contain antioxidants can decrease the activity of free radicals that can protect the islet of Langerhans of the cytotoxic effect. The content of antioxidant of xanton compounds in mangosteen rind inhibits the

formation of Reactive Oxygen Species (ROS) that induce cytokines in enhancing cell apoptosis. Diabetic with high blood glucose levels become catalysts of lipid peroxidation and the formation of Advanced Glycation End Products (AGEs), which induces the formation of free radicals. Inhibition of the formation of AGEs and ROS production prevent insulin facilitated migration of neutrophils, thereby inhibiting the inflammation of the pancreatic beta cells (insulinitis) [8].

Xanton is also known to have anti-inflammatory effects so it is possible to stop the autoimmune reaction attacking inflator cells (mononuclear lymphocytes) and enhance cell so as to hold the healing process due to infection. These conditions favor the occurrence of tissue repair and formation of beta cells is hence reproducible insulin to maintain blood glucose levels within the normal range.

CONCLUSION

The give of juice mangosteen rind with a dose of 110 mg/kg body weight rat everyday for 2 weeks to the group of therapy rat reduce blood sugar levels normal. Observations on the histology pancreatic of rats showed that juice mangosteen rind can improve conditions of the pancreatic histology rat with the induction of streptozotocin.

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