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Alpha Mangosteen Effect on MDA Level and the Pancreatic Morphology *Rattus norvegicus* (Berkenhout, 1769) Induced by Alloxan

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Abstract: The alpha mangosteen compound is a single compound isolated from rind extract *Garcinia mangostana* Linn, known to have antidiabetic bioactivity. This research aimed to determine the effect of the α -mangosteen compound on Malondialdehyde (MDA) and rats pancreatic tissue induced with type 2 *Diabetes mellitus*. Twenty-four Wistar male rats were divided into four groups, each group consisting of six rats. The first group was set as a positive control, rats injected with a single dose of 150 mg kg⁻¹ alloxan. The second group was the negative control, which means that no treatment. The third group was injected with a single dose of 150 mg kg⁻¹ alloxan and α -mangosteen compound 10 mg kg⁻¹, while the last group was injected with a single dose of 150 mg kg⁻¹ alloxan and 10 mg kg⁻¹ standard drug glibenclamide. All groups were monitored for 3 wk, and blood sugar levels were measured using a Glucose meter. Blood samples were taken for the measurement of MDA, and the pancreas organ was removed for histological study. This research showed that the difference of MDA level between α -mangosteen compound treatment and glibenclamide was only 0.4818. It means that the α -mangosteen compound can reduce MDA levels alike the standard drug glibenclamide capabilities. Islets of Langerhans on normal pancreatic mice (control (-)) looked filling full with the endocrine cells spread throughout the pancreas. While at the control group (+) there was a room-empty space (fewer the number of beta cells) in islets of Langerhans. On the treatment groups, those were giving α -mangosteen, and drug compounds standard (glibenclamide) showed improvement in Langerhans β cells compared with a treatment group of diabetic mice (control+). The administration of α -mangosteen compound 10 mg kg⁻¹ BW able to decrease MDA level 1.2047 nmol dl⁻¹. While based on histological observations of the pancreas showed that the alpha mangosteen treatment group showed improvement of Langerhans β cells compared to the diabetic rat group (positive control).

Keywords: Beta cells, *Diabetes mellitus*, *Garcinia mangostana* L., Lipid peroxidation

1. INTRODUCTION

Diabetes mellitus (DM) is a condition increasing of sugar levels were accompanied by a variety of metabolic disorders due to hormonal disorders

causing a variety of chronic complications in various target organs. International Diabetes Federation (IDF) confirmed that in 2005 there was 200 × 10⁶ (5.1 %) diabetic in the world and suspected 20 yr later in 2025 will increase to 333 × 10⁶ (6.3 %)

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people. Global status report on NCDs World Health Organization (WHO) in 2010 reported that 60 % of all ages death in the world is caused by Non-Communicable Diseases (NCDs). DM was ranked as the sixth leading cause of death. About 1.3×10^6 people died of diabetes, and 4 % die before the age of 70 yr. In 2030 DM is estimated as the 7th rank leading cause of death in the world. In Indonesia, it is estimated that in 2030, there will be 21.3×10^6 patients with diabetes [1–3]. Although diabetes is a chronic disease that does not cause death directly but can be fatal if not managed appropriately.

α -Mangosteen bioactive compounds in mangosteen rind shown to be effective decrease blood glucose levels tested in Wistar ras [4]. The molecule structure of α -mangosteen can be seen in Figure 2. It has many data from research results showing that MDA is a highly reactive compound and is the final product of the lipid peroxidation process used as an indicator of gastric cell damage due to the stress process oxidative. In diabetic patients, free radical production increases but the antioxidant system decreases; therefore it is recommended that diabetic patients need more antioxidants than healthy people. Since the influence of free radicals on diabetes is now widely discussed, it can be suggested that antioxidant agents can be used to block the formation of free radicals so that they can prevent the development of diabetes [5]. Therefore, to reinforce these studies, it will be further examined on the effects of α -mangosteen compound decrease MDA level and pancreatic tissue histology in rats induced *Diabetes mellitus* type 2.

2. METHODOLOGY

2.1. Plants Authentication and Isolate Preparation

Fresh fruits of *Garcinia mangostana* L. were collected in sufficient quantity from Jakarta. The pericarp of this fruit was separated from this pulp and carefully washed with tap water to remove other foreign materials. They were then air-dried in the open air, the dried pericarp was blended, then 1 kg of the pericarp powder was weighed and extracted using hexane at room temperature to obtain a solid sample. The alpha mangostin isolate was obtained from the fractionation process by column chromatography from the n-hexane extract of mangosteen rind. The fractionation process was carried out using a mixture of eluent n-hexane and ethyl acetate with a gradual increase in polarity gradient.

To achieve the objectives of this study, five steps will be carried out, as follows; First, establish technical standards in designing kitchen waste biogas digesters for urban households. This standard becomes a reference in subsequent calculations. Second, calculate the biogas potential from kitchen waste with the anaerobic digester. The composition of the typical waste organic matter is

2.2. Animal Handling

Healthy male Wistar rats [*Rattus norvegicus* (Berkenhout, 1769)] (150 g to 200 g) obtained from the Medical Faculty animal nursery,

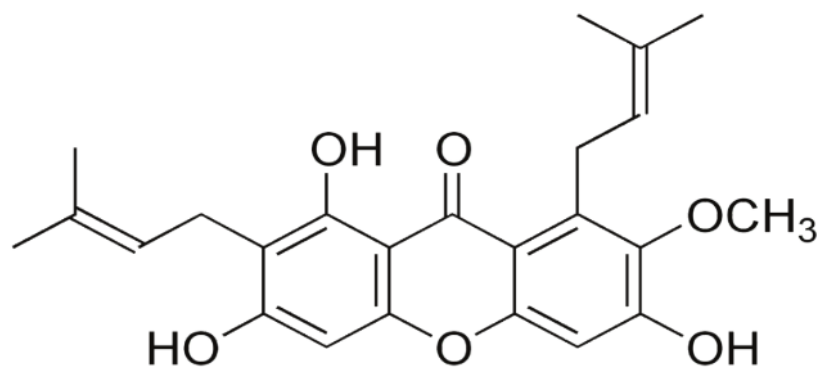


Fig. 1. Molecule structure of α -mangosteen

Airlangga University, Surabaya. They were housed under normal laboratory conditions of humidity, temperature, and lightening, and were allowed to access free drinking water and animal pellet. The animal treatment procedure was approved by The Ethical Committee of Faculty of Medicine, Universitas Wijaya Kusuma, Surabaya, Indonesia (Number, 10183/SLE/FK/UWKS/2016; Oct 19, 2016).

2.3. Alloxan Model of Diabetes mellitus

Adult male Wistar rats weighing 150 g to 200 g were induced with a single dose of 150 mg kg⁻¹ alloxan by intraperitoneal injection. Hyperglycemia was confirmed by the increase of blood glucose levels determined after 72 h. Rats with fasting blood glucose levels more than 126 mg dL⁻¹ were considered diabetic. Diabetic rat samples were taken from a rat that has a fasting blood glucose \geq of 150 mg dL⁻¹. Glibenclamide (10 mg kg⁻¹) was used as the standard drug.

2.4. Experimental Design

Animals were divided into four groups; each group was consisting of six rats.

- (i) Group A: Normal control rats saline administered (0.9 %, w/v);
- (ii) Group B: Diabetic control rats saline administered (0.9 %, w/v);
- (iii) Group C: Diabetic rats α -mangosteen administered (10 mg kg⁻¹);
- (iv) Group D: Diabetic rats glibenclamide administered (10 mg kg⁻¹);

All the drugs were administered orally for 3 wk. A blood sample was drawn from caudal vein of the rats 24 h after the last dose and blood glucose level was measured using On Call Plus Blood Glucose Monitoring System®. Pancreas organ removal for histological study.

2.5. Measurement of MDA Levels

Plasma MDA levels are measured according to the Wills method. A 200 μ L sample solution (plasma) added 1 mL of trichloroacetate (TCA) 20 % and 2 mL of thiobarbituric acid (TBA) 0.67 %. The solution is mixed homogeneously and heated over a water

bath for 10 min After being cooled centrifuged at 3 000 rpm (1 rpm = 1/60 Hz) for 10 min. Filtrate the pink color is measured at a wavelength of 532 nm using a UV-VIS spectrophotometer. MDA levels are calculated using MDA standard curve with a concentration of (0, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6) nmol mL⁻¹.

2.6. Pancreatic Histology Preparation

Organs fixed with 4 % PFA for 18 h to 24 h, put into distilled water for 1 h, then dehydrated using absolute alcohol 70 %, 80 %, 90 %, and 95 %, and put into xylol solution for 1 h. The next step is the infiltration process, which is done in liquid paraffin and embedding into blocks. The tissue on block paraffin is cut with a microtome 4 μ m to 5 μ m thick. Slices are placed on a glass object which was previously immersed in poly-L-lysine. After that, 24 h incubation is carried out. Furthermore, the deparaffination process is carried out using xylol (5 min), followed by the rehydration process using absolute alcohol 95 %, 90 %, 80 %, and 70 % for 5 min respectively. The tissue is then washed with distilled water was continued with PBS pH 7.4 m for 15 min. The tissue was later stained with Mayer's Hematoxylin-Eosin for 10 at room temperature and washed with distilled water for 15 min. The preparation is dried, entelled, and then closed with a glass cover. The prepared preparations are then observed under a microscope with 400x magnification.

3. RESULT AND DISCUSSION

3.1. Effect of α -mangosteen Compound on MDA Concentration

The MDA animal serum levels mean and standard deviation result is presented in Table 1 and Fig. 1. Based on Table 1 and Fig. 1. data, the serum MDA levels mean result showed that the average levels of MDA in the positive control group were significantly higher. This was due to alloxan injected could increase serum MDA levels. While, the average level of MDA in the treatment group with α -mangosteen, does not look significantly different from MDA levels in the negative control group. This indicates that α -mangosteen could reduce levels of MDA significantly.

Table 1. Effect of α -mangosteen on serum MDA levels

Groups n = 6	n	Mean \pm deviation standard
Possitive control	6	8.177 1 \pm 1.561 4
Negative control	6	6.490 6 \pm 0.646 4
Treatment group with 10 mg kg ⁻¹ α -mangostin	6	6.972 4 \pm 1.180 3
Treatment group with 10 mg kg ⁻¹ glibenclamid	6	6.611 1 \pm 0.710 6

Pancreatic tissue observations were done using the paraffin block Hematoxylin-eosin staining method. Observations of cells through HE staining showed a part of the endocrine (islets of Langerhans) from the pancreas. A Part of the islet of Langerhans appears more clearly than the serous acini (Fig. 3).

3.2. α -Mangosteen Effect on MDA Level

Alloxan has been widely used to induce diabetes as an experimental model that can be used to provide a hyperglycemic effect. In the process of the redox cycle and the formation of superoxide radicals, the product of reduction from alloxan is formed, namely dialuric acid. These radicals will decompose into hydrogen peroxide so that there is an increase in the concentration of calcium that is very high in the cytosol, resulting in beta damage to the pancreas and high hyperglycemia [6]. Therefore, alloxan can be used as a good diabetes-inducing agent. Glibenclamide is one of the well-known diabetes

drugs from the sulfonylurea group. The mechanism of action of sulfonylurea drugs binds to high-affinity receptors associated with potassium canals, which are sensitive to ATP in pancreatic beta cells. The binding of sulfonylureas will inhibit potassium ions, giving rise to depolarization, which can open potassium ion channels and give rise to potassium influx. The opening of the potassium ion channel can stimulate insulin release [7].

In this study, glibenclamide is used as a standard drug for *Diabetes mellitus*. In the development of diabetes and its complications, an increase in oxidative stress is very important. Oxidative stress is formed due to an increase in free radical production or/and increased antioxidant agents. *Diabetes mellitus* is a disease caused by free radicals derived from oxygen. The four routes passed in the production of free radicals caused by hyperglycemia include the following: automatic glucose oxidation, increasing glycolysis, activating intracellular sorbitol (polyol) pathways, and non-enzymatic protein glycation [8]. It has been recognized that lipid damage can be caused by the presence of high levels of free radicals or reactive oxygen species (ROS). Lipid damage occurs in lipid chains that contain double bonds, which are usually referred to as unsaturated fatty acids (PUFAs), will undergo insertion by oxygen to produce hydroperoxide and lipid peroxy radicals [9]. Among these, MDA has been documented as a primary biomarker of free radical-mediated lipid damage and oxidative stress [10].

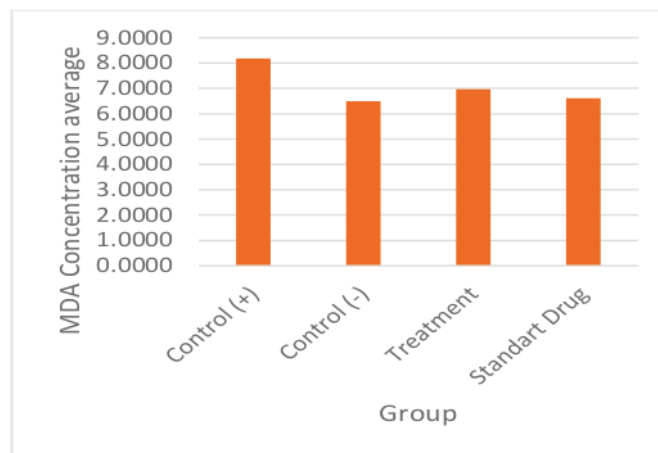


Fig. 2. Graphic of MDA concentration average for each group

Many of the compounds containing oxygen, especially aldehydes such as MDA, are produced when there is an attack of free radicals on the membrane lipoprotein and polyunsaturated fatty acids (PUFA). This reactive aldehyde compound is one of the electrophilic species, which can cause stress to the cells to form the end product of lipoxidation end products (ALE). The increased free radical formation can cause persistent hyperglycemia. Based on Ayaz K. Mallick's research shows that there is an increase in membrane peroxidation, which causes an increase in MDA levels [11].

The difference in the reduction in blood glucose levels from each group can be determined using one-way ANOVA statistical analysis, a significant value of $P > 0.05$ was obtained, which indicates that there was no significant difference between each treatment group. However, descriptively any difference. It is shown in Fig 2. the MDA levels in the positive control group are 8.177 2, the highest value of the other groups. It indicates that the injection of alloxan can increase serum MDA level. The negative control group, composed of normal mice without treatment showed MDA levels of 6.490 6. A level of 6.972 4 α -mangosteen MDA was obtained from the treatment group, not much different from the levels of MDA in the treatment group treated with standard drug glibenclamide (6.611) and with the negative control group. In the previous study, treatment with glibenclamide, MDA levels reduced significantly.

This information shows that the alpha mangosteen compound has an antiperoxidative effect. This can support the fact that it has a hypoglycemic effect and has an antioxidant activity such as research that has been done before [12]. This research results that the difference of MDA level between α -mangosteen compound treatment and glibenclamide was only 0.481 8 mean the α -mangosteen compound can reduce MDA levels alike the standard drug glibenclamide capabilities. The last research in 2011 found that α -mangosteen can reduce the area of infection as well as its ability to reduce cardiac ATP and phosphocreatine levels in myocardium reperfusion to maintain the mechanism of action of the cardiac [13]. The reduction of oxidative stress is very closely related to the particular effects of xanthone compounds.

Also, treatment with α -mangosteen can prevent lipid peroxidation, glutathione reduction (GSH), and reduce protein oxidation induced by reperfusion injury.

In this herbal treatment, phytoconstituents derived from *G. mangostana* fruit hulls are important and include α -mangosteen. The phenolic group of α -mangosteen compounds plays a big role in antioxidant properties. These act on lipid free radicals and break the chain. This is the reason that the α -mangosteen compound reduces the MDA serum level.

3.3. Pancreas Histology Study

This research is a follow-up study from Devyana's research in 2012 which explains that alpha mangosteen compounds can reduce blood glucose levels. This study wants to examine to the molecular level how alpha mangosteen compounds can reduce blood glucose levels, namely through measurement of malondialdehyde levels and pancreatic histology. In this study, pancreatic tissue of diabetic rats was observed after 21 d of treatment with administration of an α -mangosteen compound and standard drug glibenclamide. Histological preparations were made with the paraffin block and staining method. Following is the pancreatic tissue picture in each treatment:

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Langerhans Island in the pancreas consists of five different types of endocrine cells, alpha, beta, 10 gamma, delta, and epsilon cells. This hormone is secreted in response to metabolic cues and together regulates the maintenance of blood glucose homeostasis [14]. Biologically the production of insulin by beta cells on the island of Langerhans can occur under control as the loss of pancreatic beta-cell function causes loss of insulin, which is then referred to as the condition of diabetes. This condition is often associated with type 1 diabetes characterized by loss of beta-cell mass due to autoimmune attacks, but the loss of beta-cell mass function is currently also associated with type 2 diabetes [15]. In Fig. 3 shows the results in pancreatic tissue, the data obtained in the positive control group (A) contained necrotic cell, while in the negative control group (B), a treatment group of α -mangosteen (C), and standard drug group (glibenclamide) (D) showed that necrotic cell is

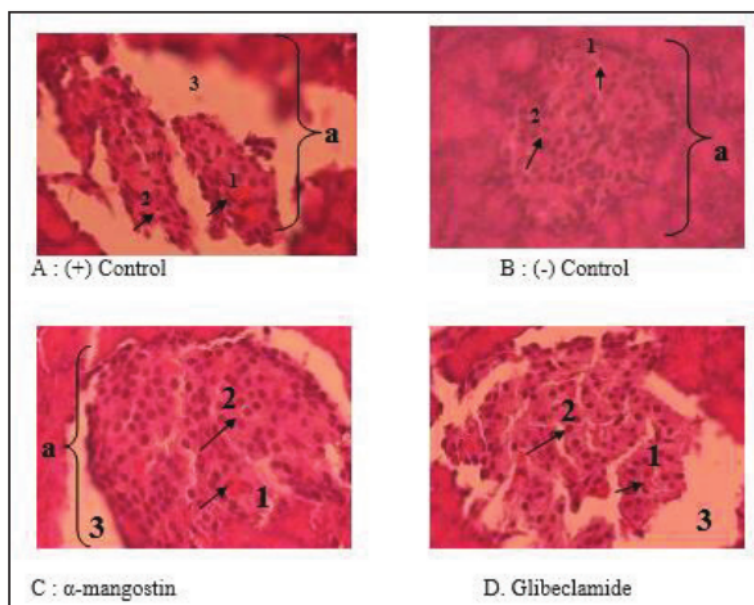


Fig. 3. Islet of Langerhans (40 × 10 magnification).

not often found when compared with the control (+). Necrosis is cell death occurring unnaturally. [5] effect is cell swelling; then the cell becomes damaged; the damaged cell is not destroyed by phagocytes, which can damage neighboring cells (inflammato[5]). While apoptosis is programmed cell death, damaged cells are directly ingested by phagocytes and do not disturb or damage neighboring cells [16].

The Islets of Langerhans of the pancreas in the rat [control (-)] looked filled by endocrine cells scattered throughout all areas of the islet of Langerhans. The control group (+) presented a space (a fewer number of beta cells) in the area of the islet of Langerhans. These empty spaces due to the beta cell lysis manifest as decreased insulin release that causes hyperglycemia or *Diabetes mellitus* onset. The treatment group administered with alpha mangosteen compound and standard drug (glibenclamide) showed improvement of Langerhans β cells compared to diabetic rat group with positive control. Based on Tjahjani's research in 2014, it was suggested that alpha mangostin isolate was a phenolic compound that was a strong antioxidant. Increased reactive oxygen species (ROS) such as mitochondrial superoxide

in endothelial cells and endoplasmic reticulum stress followed by a decrease in antioxidant defense mechanisms can trigger cell and enzyme damage, resulting in lipid peroxidation which then leads to insulin resistance and hyperglycemia. The occurrence of hyperglycemia and insulin resistance due to the formation of oxidative stress results in impaired insulin action. The results showed that hyperlipidemia works to produce oxidative stress in mitochondria through the same route as hyperglycemia. So, it can be concluded that the antioxidant compounds can improve the action of insulin [17].

4. CONCLUSIONS

Blood glucose levels were raised in rats induced by alloxan (positive control) and can be decreased by alpha mangosteen isolate compound treatment. The pancreatic histopathology investigation showed that the alpha mangosteen treatment group showed improvement of Langerhans β cells compared to the diabetic rat group (positive control). It can be concluded that alpha mangosteen compound has hypoglycemic potential because it has phenol groups known as a potent free radical scavenger, which has been widely used as a therapy against

free radical-mediated diseases, especially *Diabetes mellitus*.

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