THE EFFECT OF TOMATO SKIN EXTRACT *(Solanum lycopersicum)* ON FASTING BLOOD GLUCOSE LEVEL IN RAT MODEL OF DIABETES MELLITUS TYPE 2

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ABSTRACT

Diabetes Mellitus (DM) is a metabolic syndrome identified by hyperglycemia. DM Type 2 is caused by abnormalities of insulin resistance therefore the cells and membranes fail to utilize glucose and end up with hyperglycemia as the result. The state of hyperglycemia induces increasing superoxide radicals that caused disruptions in pancreatic β cells. Tomato (*Solanum lycopersicum*) on the skin has a higher level of antioxidant contents compared to the flesh of the fruit or a whole intact tomato such as lycopene, β-carotene, vitamin A, vitamin C, vitamin E, and quercetin. These antioxidants can hold up any free radicals so they were potential in lowering fasting blood glucose level. This research aims to prove the effects of tomato skin extract in lowering fasting blood glucose level by using rat *Rattus norvegicus* DM type 2 model. Total sample of 20 were divided into five groups which were negative control, positive control, 3 treatment groups that were given a dose of tomato skin extract 50mg/BW, 100 mg/BW, and 150 mg/BW for 4 weeks. Blood glucose level was measured before and after treatment. Data in fasting blood glucose level tested with *Paired T test* and *Wilcoxon Signed test* with 0.05 significations. The results showed that dose of 50 mg/BW tomato skin extract decreased the level of fasting blood glucose (FBG) significantly, while the dose of 100mg/BW and 150 mg/BW tomato skin extract decreased level of FBG less significant. The conclusion of this research is that the tomato skin extract at a dose of 50 mg/BW tomato skin extract can lower fasting blood glucose level in a rat model of DM type 2.

Keyword: diabetes mellitus, tomato skin extract, fasting blood glucose

INTRODUCTION

World Health Organization (2006) stated that Diabetes Melitus (DM) is one of the health problems. Number of diabetes sufferers in the world was rising allegedly from 171 million in 2000 to 366 million in 2030.Those numbers showed that the phenomenom of diabetes in the world is still quite high and will continue to increase. The diabetes type 2 is more prevalent in developing countries, triggered by environmental factors and lifestyle [1].

Diabetes mellitus is a metabolic syndrome marked by hyperglycemia caused by abnormalities in insulin secretion, insulin performance or both. The effects of diabetes mellitus include a long-term damage, dysfunction and failure of various organ functions. DM has the characteristic symptoms such as polyuria, polidipsi, polifagi, blurred vision, and weight loss [2].

Diabetes mellitus type 2 (DM type 2) is an abnormality in the production of insulin or decrement in target body network sensitivity to insulin (insulin resistance).In the early stage, the most important abnormality is the decrement of insulin sensitivity

characterized by increasinglevel of insulin in the blood. However, the severity of diabetes type 2 that occurs will lead to a failure in the balance and the production of insulin, therefore the blood sugar level can not be controlled properly [3].

Diabetes mellitus type 2 associated with the insulin hormone secreted by pancreatic β cells.This hormone is responsible for the maintenance of glucose level in the blood that help the body's cells to use glucose as the main energy source. Yet, in patients with diabetes type 2, cells and network can not utilize the glucose due to abnormal insulin metabolism, thus causing hyperglycemia. Chronic hyperglycemia may lead to decreased insulin production, body network damage and organ dysfunction in the long term as in the eyes, kidneys, nerve and vascular system [4,5].

Diabetes mellitus type 2 can be overcomed by adjusting the balance body's nutrient metabolism, and assisted with the use of medications. However, the medications used in the treatment of diabetes have side effects, so it requires another therapeutic alternative. Diabetes type 2 is generally come along with escalation of free radicals and reduction of antioxidant activities [6].

. The increasing oxidative stress might cause complications in diabetes type 2 based on the state of hyperglycemia. Oxidative stress is a pathological condition resulted from increased production of free radicals or decreased antioxidant level. Oxidative stress can be overcomed by increasing the antioxidant [7].

Indonesia's natural ingredients contain a lot of antioxidants along with other different active substances, one of them is a natural material, which is tomato. Tomatois included in fruit that iswidely available and consumed and contains nutrients such as carotenoids, β-carotene (which contains vitamin A for the activity in the body), flavonoids (quercetin), vitamin C, and vitamin E, which acts as an antioxidant, whereas the dominant type of carotenoid is lycopene [8,9]. The tomato skin has higher antioxidant content than in the seed (79.2 ± 0.2%) and the flesh (94.5 ± 0.3%) that is equal to 97.4 ± 0.2% [10]. Based on the elaboration of the content of the tomatoes, the tomato skin can be functioned as an antioxidant for inhibiting free radicals, so that the tomato skin extract (Solanum lycipersicum) has potential in lowering blood glucose level in diabetes type 2. Based on the data that has been elaborated above it is necessary to do research on the effect of giving tomato skin extract (Solanum lycopersicum) on blood glucose level in rat (Rattus norvegicus) male wistar with diabetes type 2 models.

RESEARCH METHOD

Research Design

The research design used was the pure experimental research design (true experimental design) in vivo. The design applied was Randomized Controlled Pre and Post Test Group. The type of sampling technique used was Simple Randomized study Sampling [11].

Research Subject

The research subjects were rat Rattus norvergicus male wistar 6-8 weeks old with the average body weight of 150-200 grams [12]. Subjects were divided into 5 groups after adaptation in the 1st week, which are the group KN (negative control: normal diet and not given STZ); KP (positive control:

normal diet, induced STZ, untreated); KP1 (treatment group 1: a high-fat diet, induced STZ, given tomato skin extract 50mg/BW); KP2 (treatment group 2: high fat diet, induced STZ, given tomato skin extract 100 mg/BW); KP3 (treatment group 3: high fat diet, induced STZ, given tomato skin extract 150 mg/BW) (corresponding ethical treatment of experimental animals "Ethical Clearance" No. 142/EC/KEPK/ 05/2016.

Normal Feed Production

Normal feed was made from a mixture BR1, flour, and water that is printed and then dried [13]. Normal feed was given as much as 25 grams and refilled each day. Normal feed was given to all groups at the 1st week adaptation period and given until the end of the research only in the negative control group.

Animal Experiment of DM Type 2

High Fat Feed Production

High fat feed was made from a mixture of BR1, flour,cholate acid, coleterol, water, oil and dried pork printed [13]. Normal feed were given as much as 25 grams and refilled each day. High fat feed given to the KP group, KP, KP2, and KP3 from 2nd week until the end of the research.

Injection of STZ Solution

STZ was injected with a low dose of 30mg/kgBW to the rat intraperitoneal at the 7th week [14].

Tomato Skin Extract Production

Fresh tomatoes, red and ripe weighed and washed first, then put in a bowl containing water and steameduntil the skin and flesh of tomatoes detached. Tomato skin was peeled, laid out on a baking sheet and dried. The skin was later crushed in a blender. After that, mixed with acetone and stored in glass bottles coated with aluminum foil. Continued with filtration and evaporation processes to separate antioxidant with acetone using rotatory evaporator tools [15]. Tomato skin extract mixed with cortina to be more soluble in fat, then put into capsules. Each rat received two capsules in accordance with each group dose every day starting from the 8th week until the end of the research.

Fasting Blood Glucose Measurement

The measurement of blood glucose level was using a stick Easy Touch. The rat were fasted for 8 hours prior to blood sampling. The rat held in a cloth to refrain its rebel. Later, tail of the rat dipped in warm water to increase vasodilation of blood vessels, so that the veins are more visible. Tail disinfectioned with 70% alcohol then pierced with a needle. The blood that came out attached to the stick of digital blood glucose meter then the results was seen on the screen in mg/dL units [16].

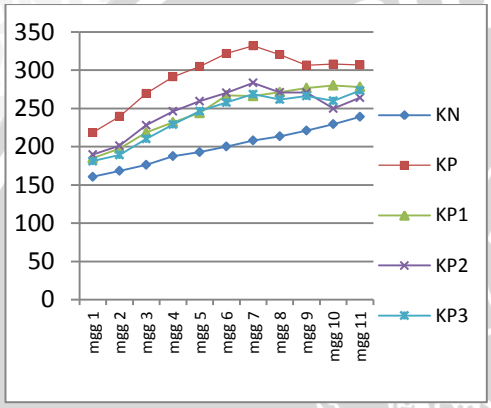
Data Analysis

All data were analyzed using SPSS for Windows version 16.0. Normality test was using the Shapiro-Wilk test, if the data were normally distributed ($p > 0.05$). Paired comparison test usedT test for normally distributed data and Wilcoxon Signed Rank Test for data that is not distributed normally. The correlation was tested to determine the relation between fasting blood glucose levelwith tomato skin extract. The statistical test revealed significant when $P < 0.05$.

RESEARCH FINDING

Rat's Weight

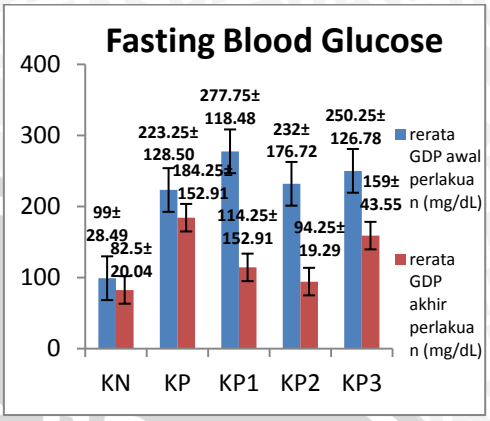
The rat body weight measurement started from the 1st week until the 11th week. The body weight median in each group was calculated weekly to determine the weight development of the rat. Here are the results of the development of the average weight of rat each week.



Fasting Blood Glucose of Rats

.Measurement of fasting blood glucose level in rat held at the 8th week after STZ injection in the tail of the rat to figure out whether the rat have had diabetes mellitus type 2 and after tomato skin extract consumption to determine the effect oftomato skin extract on fasting blood glucose level.

The rat categorized as having diabetes mellitus type 2 if the fasting blood glucose level is more than 140mg/dL with diabetes type 2 typical complaints, which are polyuria, polyphagia, polidipsi, and body weight reduction. Here is the median of early and late fasting blood glucose level in rat.



The datas were analyzed. The Shapiro-wilk thatwas used for normality test showed that inthe beginning of treatment, the KN group. KP, KP1 has a normal distribution of data (p value> 0.05), whereas in the end of treatment showed that the normal distributed data is in KN group, KP1, KP2 and KP3. The next step performed was comparative tests with Paired T Test method and Wilcoxon Signed Rank Test.

Furthermore, the comparative test was held by using Paired T Test for the distribution of normal data and Wilcoxon Signed Rank Test for the distribution of data that is not normal and showed P> 0.05 (P = 0.040) as the result, which means that there are significant differences in fasting blood glucose level in a dose of 50 mg/BW. Then the correlation test to determine the relation between the dose of tomato skin extracts with fasting blood glucose level by using the Pearson test showed that there is no significant relation between the dose of tomato skin extracts with fasting blood glucose level.

DISCUSSION

Rat's Weight

The results of measurements of the rat body weight were shown an increasing body weight in all groups of rat starting from

the first week until the seventh week.This happened because of the high fat feed diet in rat. A diet rich in fat carbohydrate consumption will lead to an increasing amount of fat deposited in adipose network. Excessive fats and carbohydrate indirectly used by the body is stored in adipose network in the form of triglycerides. Too much fat in the form of triglycerides in adipose network may cause an increasing body weight [17]. At 8th week, a decreasing body weight in rat happened after STZ injection. This causes disruption in pancreatic β cells resulting in decreased insulin secretion, that lead to a condition of hyperglycemia, hipoinsulinemia, polyphagia, polyuria, and polidipsi accompanied by body wight lose after STZ injection [18,19].

After the 8th week, there was instability in the rat body wight. Those rat under went different phase of increasing and decreasing body weight in each group. The reason is that rat experienced a high appetite (polyphagia), but in the same time a breakdown of fat (lipolysis) in rat happened, resulting in instability of its body weight [20].

Fasting Blood Glucose Level of Rats

FBG normal level in rat ranges from 71-112 mg/dL, whereas rat that had higher level of FBG ≥140 mg/dL suspected that it suffers from DM [21]. In the non-fasting situation, the normal blood glucose level in rat ranges from 102-142 mg/dL, while the fasting rat suffers DM if the blood glucose level ≥200 mg/dL [21].

Early measurements of FBGon rat conducted after high fat diet and STZ injection to determine whether the rat have had diabetes mellitus type 2. It is characterized by FBG level in rat ≥ 140 mg / dL [19]. The groups given a high fat diet and injection STZ consist of four groups: KP, KP1, KP2, and KP3. It is shown by FBG level ≥ 140 mg / dL (> 200 mg / dL) in the four treatment groups. While the negative control group that did not get high fat diet and STZ injection, had a normal FBG level of 71-112 mg / dL (99 mg / dL).

In normal circumstances, the muscle will use the glucose in the blood to produce energy. However, high fat diet and low dose STZ injection (30 mg / kg) causes mild disruption in insulin secretion resulting in pancreatic β cell exhaustion. These conditions will decrease the sensitivity of body network to insulin. High fat diet also plays a role in increasing free fat acid level in the blood that causes the muscles to perform the oxidation of fat acids and increased acetyl level CoA in mitochondria. This condition will activate the enzyme pyruvate dehydrogenase and induces increasing level of sitratinstraseluler, there by inhibiting the accumulation of fosfofruktokinase and glucose-6-phosphate. Then there will be obstacles glucose uptake by muscles, resulting in hyperglycemia [19,22].

Tomato skin extract dose of 50 mg/BW, 100 mg/BW, 150 mg/BW give the effect of decreasing FBG in each treatment group. Statistical analysis showed that there are significant differences in the tomato skin extract dose of 50 mg/BW. It is proved that the optimal dose to reduce FBG level in rat is equal to 50mg/BW.Meanwhile in tomato skin extract dose of 100 mg/BW and 150 mg/BW showed no significant difference. This has been proven in the research [23] that showed that an increased dose is'nt contributing in improving the effectiveness of the treatment given. This was occurred because the receptor is unable to bind and cause interactions with chemical compounds contained in the treatment given. Therefore, when the receptor has been saturated or not capable of binding, then the escalation of

therapeutic doses can not achieve the maximum effect.

The increasing dose of medication should be improving response as much as the dose increased, but with increasing dose, increased responsiveness will eventually decline because already reached doses that were not able to improve the response again [24]. This often happens in medication from natural materials because they're not consisting of a single component or consist of a wide variety of chemical compounds. If the number of chemical compounds which contained inside get more and more amount, there will be harm, which the reduction of the effects [23]. Increasing the dose of medication should be improving response proportional to the dose increased, but with increasing dose, increased responsiveness will eventually decline because already reached doses that were not able to improve the response again [24]. This often happens in medicine from natural materials because they contain no components of a single compound or consist of a wide variety of chemical compounds. If the number of chemical compounds which contained more and more, there will be adverse interactions, namely the reduction in the effects [23].

Hyperglycemia in diabetes type 2 become the roots of the thediabetes complications caused by the increasing oxidative stress. Oxidative stress is an escalation in free radicals and is a pathological condition resulted from increased production of free radicals or decreased antioxidant level [7].Oxidative stress also plays an important role in the pathophysiology of several diseases, such as diabetes mellitus and its complications. Antioxidants needed by the body to cope with and prevent oxidative stress. Various natural materials from native Indonesia contain antioxidants with different active ingredients [25].Tomato skin extract in this research acts as an antioxidant to reduce FBG levelin rat to prevent oxidative stress from happening, thus preventing ROS escalation in the body and prevent further complications. Some of the antioxidants contained in tomatois a β-carotene (vitamin A), flavonoids (quercetin), vitamin E, and vitamin [9].

The research result showed that the highest amount of lycopene found in tomato skin of 417.97 µg/g, while the amount of lycopene in intact tomato is 83.85 µg/g, and on the tomato flesh 47.6 µg/g [26]. Based on research conducted known that lycopene can serve as a powerful antioxidant. Lycopene works by stopping cell damage by free radicals by reducing the toxic effects of Reactive Oxygen Species (ROS) [27,28].

Secondary antioxidants are antioxidants that have the function to capture free radicals and prevent a chain reaction of complications in DM [30]. Vitamin C plays a role in processing insulin and glucose as aldose reductase enzyme inhibitor, so that the use of equivalent reduced which will prevent the accumulation of sorbitol in network [9.31]. Vitamin E also has effect in the prevention of diabetes, insulin sensitivity. Vitamin E improves the potential of free radical defense system as well as improving glucose transport and insulin sensitivity [31]. Quercetin is a flavonoid that can be found in fruits and vegetables, and has biological traits that can reduce the risk of infection. Quercetin has antioxidant benefits, as well as able to inhibit lipid peroxidation which is a complication of diabetes [31].

CONCLUSION

1. The giving of 50mg/BW tomato skin extract dose resulted a significant decrease of FBG level, whereas the

100mg/BW and 150mg/BW tomato skin extract dose decreased the level of FBG as well but not too significant.
2. There is no correlation between the doses of tomato skin extracts with level of FBG.

SUGGESTION

1. Further research is needed to determine the condition of pancreatic β cells after treatment.
2. Quantitative analysis of antioxidants found in tomato skin extract is necessary so the amount of antioxidants that canaffect a decrement in fasting blood glucose level can be known.
3. Development of further research to determine the effectiveness of the tomato skin extracts to decrease fasting blood glucose level in rat is required with better methods and research design.

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