THE EFFECT OF TOMATO SKIN EXTRACT (*Solanum lycopersicum*) ON INSULIN SENSITIVITY IN RAT MODEL OF DIABETES MELLITUS TYPE 2

Novi Khila Firani*, Prasetyo Adi*, Revianti Anriz**

*Biochemistry-Biomolecular Medical Laboratory of Brawijaya University
**Medical Studies Program of Brawijaya University

ABSTRACT

Diabetes mellitus type 2 (DM type 2) is a metabolic disorder caused by a decrease in tissue sensitivity to insulin. This leads to hyperglycemia and increases free radicals. The increase of free radicals in tissues lead the decrease of insulin sensitivity. It takes an antioxidant to neutralize free radicals in DM type 2. Tomato (Solanum lycopersicum) contains many antioxidants in which the highest antioxidant lies in the tomato skin, such as β-carotene, lycopene, cryptoxanthin β, vitamin A, vitamin C, and vitamin E. This research aimed at determine the effect of tomato skin extracts towards the insulin sensitivity in rat (Rattus norvegicus), Wistar, strain male model of DM type 2. This research used a Randomized Post Test Controlled Group Design. The samples were divided into five groups, consisting of 4 animals, namely the negative control, the positive control (model DM type 2), and 3 treatment groups of rat with DM types 2, were given of 50mg / kg of tomato skin extracts, 100 mg / kg, and 150 mg / kg for 4 weeks. The insulin sensitivity was measured by using the formula QUICKIE = 1 / [log (I (0)) + log (G (0))]. The statistical test results, One Way ANOVA and Post-Hoc showed that there were significant differences (P = 0.037) between the negative and positive control group, but there was no significant difference between the treatment group and negative and positive control group. It can be inferred that there is a significant decrease of insulin sensitivity in rat models of DM types 2 compared with normal rat, and the treatment groups with tomato skin extracts have higher insulin sensitivity than the positive control. However, statistically, it is not significant.

Keywords: diabetes mellitus, tomato skin extract, insulin sensitivity

INTRODUCTION

Diabetes mellitus (DM) is a disease that is in fourth rank based on the priority of public health problems and regularly leads to fatal complications, such as coronary, heart disease, kidney disease, and amputation due to gangrene. Diabetes mellitus type 2 (DM type 2) is a form of diabetes in which it is the most common disease with approximately 20 million people prevalence [1]. DM type 2 is a syndrome disruption of the metabolism of carbohydrates, proteins, and fats, caused by the secretion decrease or a decrease in tissue sensitivity to insulin [2].

Hyperglycemia in DM type 2 causes compensation pancreatic β cell by secreting more insulin, thus insulin levels increase (hyperinsulinemia). These conditions lead to insulin receptor to strengthen themselves (self regulation) by reducing the decrease impact in receptor response,and lead to insulin resistance or the decrease of insulin sensitivity. Hyperinsulinemic conditions also

lead to desensitization of insulin receptors that cause the decrease of insulin sensitivity [3].

Hyperglycemia leads to an increase of free radical production in large quantities, such as reactive oxygen species (ROS) [4]. The free radicals trigger oxidative stress, a condition when the oxidant or ROS production exceeds the antioxidants capacity in the body [5] or leads to imbalance between the amount of free radicals and antioxidants [6].

Antioxidants are compounds that neutralize free radicals [7] and as inhibitors that inhibit oxidation. It aims at protect cells from damage and inflammation [8].

The use of a number of plants and extracts have been commonly used for diabetes. Tomato is one of the plants that is easily found and cultivated [9]. Tomato contains some antioxidants and essential elements for the body, such as potassium, phosphorus, magnesium, iron, vitamin A, vitamin B, vitamin C, vitamin E, β-carotene or pro-vitamin A, folic acid and lycopene. Tomato's skin contributes a lot to the concentration of carotenoids, such as β-carotene, lycopene, lutein, and β cryptoxanthin [10]. Tomato's skin contains very high lycopene and the lycopene configuration is able to deactivate free radicals [11.9].

Some results of the research above encourage the researchers to investigate the effect of tomato skin extracts (Solanum lycopersicum) on insulin sensitivity in rat models of DM Type 2.

RESEARCH METHOD

Research design

Research design that he researcher used is true experimental design in vivo. The research design used was Randomized Post Test Controlled Group. The sampling technique used is Simple Randomized study Sampling [12].

Research subject

The subjects of the research were white rat *Rattus norvergicus* male Wistar aged 6-8 weeks with 150-200 grams weight [13]. The subjects were divided into 5 groups after adaptation in the 1st week, KN group (negative control: normal diet and not given STZ); KP (positive control: normal diet, the induction of STZ, untreated); KP1 (treatment group 1: a high-fat diet, induced STZ, and 50 mg/kgBW of tomato skin extracts); KP2 (2 treatment groups: high fat diet, induced STZ, and 100 mg/kgBW of tomato skin extracts); KP3 (3 treatment groups: high fat diet, induced STZ, and 150 mg/kgBW of tomato skin extracts) (the treatment of animals experiment is corresponding to "*Ethical Clearance*" No. 142 / EC / KEPK / 05/2016).

Making Normal Feed

Normal feed is made from a mixture of BR1, corn flour, mung bean flour, wheat flour, palm olein, and water that is printed and then dried [14]. The normal feed is given as much as 25 grams each day. The normal feed is given to all groups at the 1st week of adaptation period, until the end of the research only in the negative control group.

Animal Experiment of DM Type 2

Making High-Fat Feed

High-fat feed is made from a mixture of BR1, corn flour, mung bean flour, wheat flour, palm olein, water, and pork oil that is printed and then dried [14]. Hifh-fat diet is given as much as 25 grams were replenished each day. High-fat feed is given to the KP, KP1, KP2, and KP3 from 2nd week until the end of the research.

Injection of STZ solution

STZ (Streptozotocin) is injected in intraperitoneal as much as 30mg/kgBW in rat at the 7th week [15].

Making Tomato Skin Extracts

Fresh tomato is weighed and washed first, then put it in a saucepan contains of water, and steamed up until the skin and tomato's flesh are separated. Then, tomato's skin is peeled, and laid on a baking, then dried. Next, the skin is mashed in a blender. Then, mix the skin with acetone, and stored in glass bottles coated by aluminum foil. After that, do the filtration and evaporation to separate antioxidants and acetone by using a rotatory evaporator [16]. Then, mixed tomato skin extract with cortina to make it more soluble with fat, then put it in a capsules. Each mouse in each group got a dose of two capsules every day starting from the 8th week until the end of the research.

Fasting-Blood Glucose Measurement

The blood-glucose level is measured by using a stick from Easy Touch brand. Rats are held use a cloth to prevent too much movement. Rat's tail dipped into warm water to ease the vein more visible. Then, disinfected the tail is by using alcohol, then pinned with a needle. After that, attached the blood to digital-blood-glucose stick and the results will be displayed on the screen in mg/dL unit [17].

Serum Insulin Level Measurement

Serum level of insulin is measured by using the Enzyme-Linked Immunosorbent Assay (ELISA). Rat Insulin ELISA Kit (RayBio), No. Catalog ELR-Insulin. Reagent and sample are placed at room temperature (18-25ºC). Mix it with a 100 mL standard reagent, and incubated for 2.5 hours at room temperature. Then, the solution is washed and filled with 300 mL of a buffer solution by using a multi-channel pipette. Next, combine 100 mL of biotinylated antibody, shaken, and incubated for 1 hour at room temperature. Get rid of the solution and washed. Then, add 100 mL of streptavidin solution, shaken, and incubated for 45 minutes at room temperature. Get rid of the solution and washed. Add 100 mL of TMB substrate reagent, shaken, and incubated for 30 minutes at room temperature. Add 50 mL of stop solution and read at $\lambda = 450$ nm [18].

The Measurement of Insulin Sensitivity

Insulin sensitivity measurement is conducted at the end of the research by using the formula of QUICKIE = 1 / [log (I (0)) + log (G (0))]. This formula need insulin serum level that symbolized with (I(0)), and blood-glucose level that symbolized with (G (0)) [19].

Data analysis

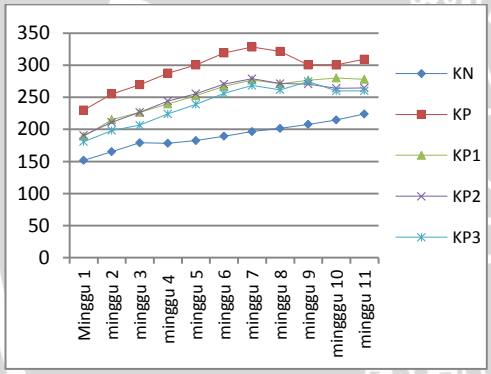
All of the data in this research were analyzed by using SPSS prgram for windows version 16.0. The normality test is tested by using the Shapiro-Wilk test. Homogenity test is tested by using Levene's test. If the data

are distributed normally, and homogeneous (p>0.05), the comparative analysis is measured by using One Way ANOVA and Post-hoc test to determine the significant differences in each group. The statistical test is significant when p<0.05.

RESEARCH FINDING

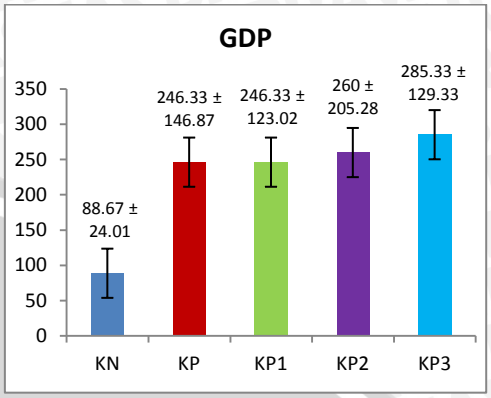
Rat's Weight

Weighing the rat is started from the 1st week until 11th week. Then, the rat's weight in each group is averaged each week to determine the rat's weight growth. The mean results of rat's weight growth per week are as follow.



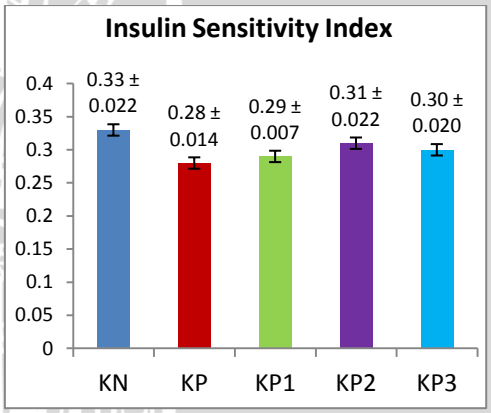
Fasting-Blood Glucose of Rats

Fasting-blood glucose level is measured at the 8th week that taken from the rat's tail to determine whether the rats have DM type 2 or not. The rats are categorized to suspect DM type 2 when their fasting-blood glucose levels are >140mg / dL with typical complaints of DM type 2 such as polyuria , polyphagia, polydipsia, and weight loss. The mean results of rat's fasting blood glucose are as follow.



Insulin Sensitivity of Rats

Insulin sensitivity of rats can be measured by using the formula of QUICKIE = 1 / [log (I (0)) + log (G(0))]. The mean results of rat's insulin sensitivity are as follow.



Then, the data are analyzed. The normality test is tested by using Shapiro-Wilk showed significance results P > 0.05 (P = 0792) which means that the data are distributed normally. Homogeneity test is tested by using Lavene test, showed significance results P > 0.05 (P = 0428) which means that the data are from a homogenous population. If the data are homogeneous and distributed normally, then analysis is performed by using One Way Anova which results P < 0.05 (P = 0.037) which means that there was a difference treatment effect to the rat's insulin sensitivity.

Then, Post-hoc test is performed by using Tukey test,. The result showed that there was a significant difference between KN and KP groups. While, there is no significant difference between KP1, KP2, KP3 with KN and KP groups.

DISCUSSION

Rat's Weight

Based on the results of measurements of rats weight, it can be seen that there is an increase of rats weight starting from the 1st until 7th week. This is because the consumption of a high carbohydrate and fat diet leads to an increasing the amount of fat deposited in adipose tissue. The excessive fat and carbohydrates will be stored in adipose tissue in form of triglycerides. The fat excess in form of triglycerides in adipose tissue is the cause of weight gain [20]. At the 8th week, rats in KP, KP1, KP2, and KP3 groups lost the weight after getting the STZ injection. This is caused by hyperglycemia in DM type 2 which leads to the fat mobilities and lipolysis increase which leads to the weight loss [21].

After the 8th week, rats on unstable weight. Rats undergo an enhancement and weight loss phase which not similar with each groups. This is due to the increase of appetite (polyphagia) and lipolysis activity as the result of DM type 2.

Fasting-Blood Glucose of Rats

Blood glucose level is measured after rats injected with STZ 30mg/kgBW to determine whether the rats already suffered from DM type 2 or not. It is characterized by fasting-blood glucose level ≥140mg/dL. High-fat diet that given to the rats may cause insulin resistance and low-dose of STZ (30mg/kgBW) may cause a bit impairment of insulin secretion. The combination of that will effect on the pancreatic β cells fatigue in secreting insulin and cause DM type 2 [15].

Insulin Sensitivity of Rats

Based on the results, the average of highest insulin sensitivity index is KN groups with 0:33. This result indicates that the group with a normal diet, which not induced by STZ and untreated (KN) has higher insulin sensitivity than the KP group. While lowest insulin sensitivity is the group with a high-fat diet, and induced by STZ, untreated (KP). It showes that insulin sensitivity in a group with a high-fat diet and STZ (DM type 2 model) is decrease. This statement also correlates with Shridar's experiment, 2007 that high-fat diet is proven to reduce insulin sensitivity [22].

Insulin sensitivity of treatment group with 50mg/kgBW, 100 mg/kgBW, and 150mg/kgBW of tomato skin extracts has no significantly difference with KN. However, the treatment group 50mg/kgBW, 100 mg/kgBW, and 150mg/kgBW of tomato skin extracts has a higher insulin sensitivity than KP, but, statistically, it is not significantly different. This indicates that there are improvements or increase of insulin sensitivity in rats after treatment of tomato skin extracts. However, the increasing of insulin sensitivity is not equivalent with the average of insulin sensitivity of KN group.

The increasing of insulin sensitivity after treatment of tomato skin extracts is caused by antioxidants in tomatoes' skin. Antioxidants work by donating an electron to the free radical molecules, thus they can discontinue a chain reaction of free radicals [23]. Antioxidants that can be found in tomatoes' skin, such as lycopene, β-

carotene, flavonoids, vitamin C, vitamin E, and β-cryptoxanthin [10]. Tomatoes' skin contain very high lycopene [11]. Lycopene has ability to neutralize free radicals, especially free radicals that generated by the cellular metabolic reactions (a highly-reactive free radicals in the body). As antioxidants, lycopene has ability to prevent the oxidation by free radicals twice till ten times than β-carotene (vitamin A) and alpha-tocopherol (vitamin E) [24].

Fibrous part in tomatoes, hemicellulose, which located on the skin and seeds of the tomatoes is insoluble fiber. This insoluble fiber can reduce the gluconeogenesis process. It can affect the increase of insulin secretion, thus it will reduce the increasing of blood-glucose levels and increase insulin sensitivity [25].

However, it takes an antioxidant compound which is classified as a powerful antioxidant in reducing the blood-glucose levels of rats model of DM type 2, improving disorders of the β cells of the pancreas, and increasing insulin sensitivity significantly [26].

CONCLUSION

1. There is a significant decrease of insulin sensitivity in rats model of DM type 2 than normal rats (KN).
2. Tomato skin extracts with 50mg/kgBW, 100mg/kgBW, 150mg/kgBW dosage have insulin sensitivity which no significantly difference with normal rats (KN).
3. Tomato skin extracts with 50mg/kgBW, 100mg/kgBW, 150mg/kgBW dosage have higher insulin sensitivity than the rats model of DM type 2, however statistically, it is not significant.

SUGGESTION

1. In the beginning of the research, insulin serum level should measured to compare the level of insulin sensitivity before and after treatment in rats.
2. The measurement of the antioxidants activity that contains in tomatoes' skin

BIBLIOGRAPHY

1. Wahdah, Nurul. 2011. *Menaklukan Hipertensi dan Diabetes (Mendeteksi, Mencegah, dan Mengobati dengan Cara Medis dan Herbal)*. Yogyakarta : MultiPress. Hal: 81-89.
2. Guyton, A. C., and Hall, J. E. 2008. *Buku Ajar Fisiologi Kedokteran*. Edisi 11. Jakarta: EGC. Hal: 3-10.
3. Nugroho, Agung Endro. Hewan Percobaan Diabetes Melitus : Patologi dan Mekanisme Aksi Diabetogenik. 2006, 7 (4): 378-382.
4. Umami Z., Nurdiana, Nugroho F. A. Efek Pemberian Susu Sapi Bubuk Terhadap Kadar Serum HDL (*High Density Lipoprotein*) pada Tikus Putih (*Rattus novergicus*) Galur Wistar Model Diabetes Melitus Tipe 2. *Jurnal Gizi Pangan*. 2015, 10 (1): 1-8.
5. Rahmawati G., Rachmawati F. N., dan Winarsi H. Aktivitas Superoksida Dismutase Tikus Diabetes yang Diberi Ekstrak Batang Kapulaga dan Glibenklamid. *Scripta Biologica*. 2014, 1 (3): 19-23.
6. Rini, Sandra. Sindrom Metabolik. *J. Majority*. 2015, 4 (4): 88-93.
7. Purboyo, Agus. 2009. *Efek Antioksidan Ekstrak Etanol Daun Jambu Bii (Psidium guajava L) pada Kelinci yang Dibebani Glukosa*. Surakarta. Hal: 7-18.
8. Gunawan I. W. G., dan Karda I. M. Identifikasi Senyawa minyak Atsiri dan Uji Aktivitas Antioksidan Ekstrak Eanol

Kulit Batang Kepuh (*Sterculia foetida* L.). *Jurnal Kimia*. 2015, 8 (1): 14-19.
9. Syamsudin. 2013. *Nutrasetikal*. Yogyakarta : Graha Ilmu. Hal: 12-13 dan 33-37.
10. Bhowmik, D., Kumar, K. P. S., Paswan, S., Srivastava, S., 2012. *Tomato-A Natural Medicine and Its Health Benefits. Journal of Pharmacognosy and Phytochemistry*. 1 (1): 33-43.
11. Ranveer, R. C., Patil, S. N., and Sahoo, A. K. Effect of Different Parameters on Enzyme-assisted Extraction of Lycopene from Tomato Processing Waste. 2013, 91 (4) : 370-375.
12. Sastroasmoro dan S. Ismael. 2011. *Dasar-Dasar Metodologi Penelitian Klinis Edisi 4*. Jakarta: Sagung Seto. Hal: 104-116.
13. Zulkifli, Maruni W. D., Yunan Jiwintarum, dan Laksmi Saraswati. Jumlah Eritrosit Darah Tepi Hewan Coba Tikus Putih (Rattus norvegicus) Strain Wistar yang Diberikan Air Seduhan Kelopak Bunga Rosela Merah (Hibiscuss sabdariffa). *Jurnal Media Bina Ilmiah*. 2014,8(4): 58.
14. Handayani W, Rudijanto A, Indra M. R. Susu Kedelai Menurunkan Resistensi Insulin pada Model Diabetes. *Jurnal Kedokteran Brawijaya*. 2009, 25 (2): 60-66.
15. Zhang, Ming, Xiao-Yan Lv, Zhi-Gang Xu, and Li Chen. 2008. *The Characterization of High-Fat Diet and Multiple Low-Dose Streptozotocin Induced Type-2 Diabetes Rat Model*. Experimental Diabetes Research 1-9: 32-39.
16. Andayani, Regina, Maimunah, dan Lisawati, Yovita. Penentuan Aktivitas Antioksidan, Kadar Fenolat Total, dan Likopen pada Buah Tomat (*Solanum lycopersicum). Jurnal Sains dan Teknologi Farmasi*. 2008, 13 (1): 31-37.
17. Suarsana, I Nyoman, Priosoeryanto, B. P., Bintang, M., dan Wresdiyati, T. Profil Glukosa dan Ultrastruktur Sel Beta Pankreas Tikus yang Diinduksi Senyawa Aloksan. *Jurnal Ilmu Ternak dan Veteriner*. 2010, 15 (2): 118-123.
18. Mawarti, Herin, Ratnawati, Retty, Lyrawati, Diana. *Epigallocatechin Gallate* Menghambat Resistensi Insulin pada Tikus dengan Diet Tinggi Lemak. *Jurnal Kedokteran Brawijaya*. 2012, 27 (1): 43-50.
19. Katz A., Nambi S.S., Mather K., Baron A.D., Follmann D.A., Sullivan G., *et al*., 2011. Quantitative Insulin Sensitivity Chech Index: a Simple, Accurate Method for Assessing Insulin Sensitivity in Humans. (Abstract). 85 (7) : 2402-10.
20. Baraas, Faisal. 2003. *Mencegah Serangan Penyakit Jantung dengan Menekan Kolesterol*. Jakarta: Kardia Iqratama. Hal: 2-3.
21. Hernawan U.E., Sutarno, dan Setyawan A.D., Aktifitas Hipoglikemik dan Hipolipidemik Ekstrak Air Daun Bungur (Lagerstroemia speciosa [L.] Pers.) terhadap Tikus Diabetik. *Jurnal Biofarmasi*, 2004, 2 (1): 20.
22. Shridar, M. G. *et al*. 2008. *Bitter Gourd (Momordica charatia) Improves Insulin Sensitivity by Increasing Skeletal Muscle Insulin Stimulated IRS-1 Tyrosine Phosphorylation in High-Fat-Fed Rats*. British Journal of Nutrition vol. 99: 806-812.
23. Chairunnisa, R. Pengaruh Jumlah Pasta Tomat Terhadap Penurunan Kadar Gula Darah pada Mencit Diabetes. *Jurnal Industri* Pertanian. 2012, 1 (1) : 1-12
24. Siagian, Albiner. 2010. *Lycopene: Senyawa Fitokimia pada Tomat dan Semangka*. Halaman: 121-124.
25. Rejeki, M.S.W dan Wirawanni Y. 2015. *Pengaruh Pemberian Jus Mentimun dan*

Tomat Terhadap Kadar Glukosa Darah Postprandial pada Perempuan Overweight dan Obesitas. Tugas Akhir. Tidak diterbitkan, Fakultas Kedokteran Universitas Diponegoro, Semarang.Hal:5-14.

26. Dewanti, T., Wijayanti, N., Handayani, D., dan Rachmawati, N. Efek Hipoglikemik Ekstrak Cincau Hitam (*Mesona palustris* BL) pada Tikus Wistar Diabetes yang Diinduksi Alloxan. *Jurnal Kedokteran Brawijaya*. 2015, 28 (3): 202-207.

.