Med J Indones, Vol. 23, No. 4,
November 2014

Basic Medical Research

Prevention of insulin resistance with *Hibiscus sabdariffa* Linn. extract in high-fructose fed rat

Trinovita Andraini, Sophie Yolanda

Department of Physiology, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia

Abstrak

Latar belakang: *Dislipidemia dan stres oksidatif memiliki peran penting sebagai penyebab resistensi insulin. Salah satu herba yang memiliki efek antioksidan kuat dan dapat memperbaiki dislipidemia adalah Hibiscus sabdariffa* Linn. *Penelitian ini bertujuan mengetahui efek ekstrak Hibiscus sabdariffa* Linn. *terhadap kadar glukosa darah puasa, kadar insulin darah puasa dan indeks resistensi insulin (HOMA-IR) pada tikus yang diberi diet tinggi fruktosa.*

Metode: *Penelitian eksperimental in vivo ini dilakukan pada 25 tikus* Sprague-Dowley *yang diberi diet tinggi fruktosa (10% ad libitum) bersamaan dengan pemberian ekstrak Hibiscus sabdariffa* Linn. *pada dosis 100, 200 dan 400 mg/kgBB/hari selama 5 minggu. Pada akhir penelitian dilakukan pengukuran kadar glukosa darah puasa, kadar insulin puasa, dan indeks resistensi insulin (HOMA-IR).*

Hasil: *Kadar glukosa darah puasa, kadar insulin darah puasa, dan HOMA-IR pada kelompok tikus yang diberi diet tinggi fruktosa bersamaan dengan Hibiscus sabdariffa* Linn. *pada dosis 100 mg/kgBB/hari tidak berbeda bermakna dibandingkan kelompok tikus yang hanya diberikan diet tinggi fruktosa sedangkan pada dosis 400 mg/kgBB/hari lebih rendah secara bermakna dibandingkan pada kelompok tikus yang hanya diberi diet tinggi fruktosa (masing-masing 4,84 mmol/L vs 6,11 mmol/L, 0,07 µU/L vs 0,3 µU/L, dan 0,02 vs 0,08).*

Kesimpulan: *Hibiscus sabdariffa* Linn. *dapat mencegah terjadinya resistensi insulin yang diinduksi diet tinggi fruktosa pada tikus.*

Abstract

Background: Dyslipidemia and stress oxidative play an important role as the cause of insulin resistance. One herb that has potent antioxidant effect and may improve dyslipidemia is *Hibiscus sabdariffa* Linn. The aim of this study was to evaluate the effect of *Hibiscus sabdariffa* Linn. extract on fasting blood glucose level, fasting blood insulin level, and insulin resistance index (HOMA-IR) in high-fructose fed rat.

Methods: This was an experimental study in 25 Sprague-Dawley rats which were administered with a high-fructose diet (10% *ad libitum*) and *Hibiscus sabdariffa* Linn. extract at a dose of 100, 200, and 400 mg/kgBW/d simultaneously for 5 weeks. At the end of study, fasting blood glucose level, fasting blood insulin level and insulin resistance index (HOMA-IR) were measured.

Results: Fasting blood glucose, blood insulin, and HOMA-IR level of rats given high-fructose diet with *Hibiscus sabdariffa* Linn. at dose 100 mg/kgBW/d were not significantly different than the group of rats given only high-fructose fed. While at the dose of 400 mg/kgBW/d, they were significantly lower than the group given only high-fructose fed (4.84 mmol/L vs 6.11 mmol/L, 0.07 µU/L vs 0.3 µU/L, and 0.02 vs 0.08 respectively).

Conclusion: Oral administration of *Hibiscus sabdariffa* Linn. could prevent the development of insulin resistance induced by high-fructose diet in the rat.

Keywords: *Hibiscus sabdariffa* Linn., high-fructose diet, HOMA-IR, insulin resistance

pISSN: 0853-1773 • eISSN: 2252-8083 • http://dx.doi.org/10.13181/mji.v23i4.848 • Med J Indones. 2014;23:192-6
Correspondence author: Trinovita Andraini, trinovita.andraini@gmail.com

Metabolic syndrome is a disorder consisting of several pathologic conditions, which includes obesity, insulin resistance, dyslipidemia, and hypertension. This disease is highly prevalent in both developed and developing countries and is predicted to increase continuously.[1] It increases the risk of type 2 diabetes mellitus and coronary heart disease.[1-4] It has been proven that the progression and development of metabolic syndrome is mainly determined by insulin resistance. Thus, prevention of insulin resistance is an important key to halt the progression of the disease. Insulin resistance causes blood glucose cannot enter the target cells, resulting in increase of blood glucose. To compensate this, pancreatic beta cells will increase insulin secretion to maintain euglycemia, resulting in hyperinsulinemia state.[1,2]

The exact pathophysiology of insulin resistance is still unknown. Recent studies suggest that obesity, dyslipidemia, and oxidative stress play important roles in causing insulin resistance.[3,5-7] Approach to prevent insulin resistance by using natural ingredients containing potent antioxidant and anti-dyslipidemia effect is a safe alternative because it rarely cause unwanted side effects.

In Indonesia, one of the herbs that have the wanted effect is rosella or *Hibiscus sabdariffa* Linn., also known as "red tea". This plant is often used as traditional medicine and is claimed to have some positive effects, such as anti-hypertensive, hepatoprotective effect, anti-hyperlipidemic, anti-cancer, and antioxidant.[4,8-12] *Hibiscus sabdariffa* Linn. contains flavonoids, anthocyanins, alkaloids, β-sitosterol, and citric acid.[10] Anthocyanins have much higher antioxidant effect compared to other antioxidants, such as vitamin E, ascorbic acid, and β-carotene.[10,11,13]

Bunbupha, et al[14] reported that *Hibiscus sabdariffa* Linn. has the effects of lowering fasting blood sugar level and fasting blood insulin level of rats with insulin resistance. However, it is not yet clear whether *Hibiscus sabdariffa* Linn. could prevent insulin resistance. In this study, we want to know the potency of *Hibiscus sabdariffa* Linn. in preventing insulin resistance in a high-fructose diet fed rats. This is a good laboratory animal model for studying insulin resistance and the factors that influence the progression of metabolic syndrome because high-fructose diet contribute in the development of metabolic syndrome.[1,7,14,15]

METHODS

Experimental animals and research protocols

This is an *in vivo* experimental study using Sprague-Dawley male rats aged 10-12 weeks with body weight ranging from 150-180 grams (purchased from Faculty of Animal Science, Institut Pertanian Bogor). Total sample size was 25 rats, calculated based on Federer formula. Rats were treated in accordance with the Helsinki convention. Ethical approval was obtained from the Ethics Committee of the Medical Research - Faculty of Medicine Universitas Indonesia/Cipto Mangunkusumo Hospital (FMUI/RSCM).

Rats were placed in individual cages in a room with proper ventilation, room temperature between 18-26°C and humidity of 30-70%. Lighting of the room was regulated light and dark for 12 hours. Rat cages were cleaned every day and the rat health was well maintained. After one week of acclimatization, rats were randomly divided into 5 groups: group 1, control group, were fed with standard diet (5% fiber, 21-23% protein, 5% fat) and given tap water for drink; group 2, rats were given high-fructose diet (standard food and 10% fructose solution *ad libitum*); group 3, 4, and 5, rats fed with high-fructose diet (standard food and 10% fructose solution *ad libitum*) and were given aqueous ethanol extract of *Hibiscus sabdariffa* Linn. with doses of 100, 200, 400 mg/kgBW/d orally {calyx ethanol extract of *Hibiscus sabdariffa* Linn. was obtained from Laboratory of Research Institute for Spices and Medicinal plants (Balitro) Bogor}. The treatment duration was five weeks for all groups.

Measurement of fasting blood glucose, blood insulin and HOMA (IR)

On the last day of treatment, rats were fasted for one night (approximately 12 hours). Then the blood samples were taken from the tail vein of rats for blood glucose measurement and from the heart for insulin measurement. Blood glucose examination was performed using a portable glucometer (Accu-Check Advantage Performance, Roche Diagnostics, Germany), while insulin levels examination was performed using a standard ELISA kit (Rat insulin ELISA, Mercodia, Sweden). Insulin assays were performed at the immunoendocrinology laboratory, Faculty of Medicine, Universitas Indonesia. Calculation of insulin resistance indicator (HOMA-IR/ homeostasis model assessment values for insulin resistance) was conducted using

the HOMA-IR formula = fasting glucose (mmol/L) x fasting insulin (μIU/L) / 22.5 (Conversion of insulin units: 1 μIU/mL = 6,945 pmol/L).[2]

Statistical analysis

Data processing was performed using SPSS 12 (Statistical Social Sciences 12). Statistical analysis was performed using one-way ANOVA test. Then, post-hoc analysis was performed. Data were previously analyzed for normality with Shapiro-Wilk test.

RESULTS

Fasting blood glucose levels

Fasting blood glucose levels in the group fed with high-fructose diet were significantly higher than in control group (6.11 ± 0.73 mmol/L vs 5.01 ± 0.28 mmol/L, p = 0.002). The fasting blood sugar levels in rats fed with high-fructose diet along with extracts of *Hibiscus sabdariffa* Linn. at a dose of 400 mg/kgBW/d were significantly lower than the group given a high-fructose diet only (4.84 ± 0.51 mmol/L vs 6.11 ± 0.73 mmol/L, p = 0.001). Fasting blood glucose levels on rats fed with high-fructose diet and extracts of *Hibiscus sabdariffa* Linn. at doses of 100 and 200 mg/kgBW/d were not significantly different from the group given high-fructose diet only (p = 0.488 and 0.057 respectively) (Figure 1).

Fasting blood insulin levels

The fasting blood insulin level in rats fed with high-fructose diet was significantly higher than in the control group (0.3 ± 0.12 μU/L vs 0.06 ± 0.04 μU/L, p = 0.002). The fasting blood insulin level in rats fed with high-fructose diet and extracts of *Hibiscus sabdariffa* Linn. at the dose of 400 mg/kgBW/d was significantly lower than in the group given high-fructose diet only (0.07 ± 0.05 μU/L vs 0.3 ± 0.12 μU/L, p = 0.003). The fasting blood insulin level in rats fed with high-fructose diet and extracts of *Hibiscus sabdariffa* Linn. at doses of 100 and 200 mg/kgBW/d were not significantly different from the group of rats that were given high-fructose diet only (p = 0.199 and 0.089 respectively). (Figure 2).

Insulin resistance index (HOMA-IR)

Insulin resistance index (HOMA-IR) in the group of rats fed with high-fructose diet was significantly higher than in the control group (0.08 ± 0.03 vs 0.01 ± 0.01, p < 0.0001). The HOMA-IR in the group of rats fed with high-fructose diet along with extracts of *Hibiscus sabdariffa* Linn. at doses of 200 and 400 mg/kgBW/d was significantly lower than in the group given a high-fructose diet only (0.04 ± 0.03 and 0.02 ± 0.01 vs 0.08 ± 0.03, p = 0.021 and 0.001, respectively). The HOMA-IR in rats fed with

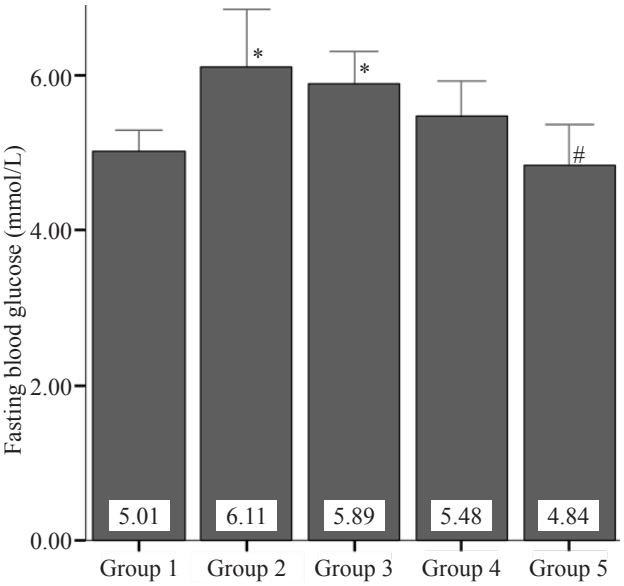

Figure 1. Comparison of fasting blood glucose levels among the five groups of rats. (*significantly different from group 1 (p = 0.002, p = 0.012, respectively), #significantly different from group 2 (p = 0.001). Group 1: control group, group 2: group that were given high-fructose diet only, group 3, 4,5: group that were given high-fructose along with *Hibiscus sabdariffa* Linn. with dose 100, 200 and 400 mg/kgBW/d respectively)

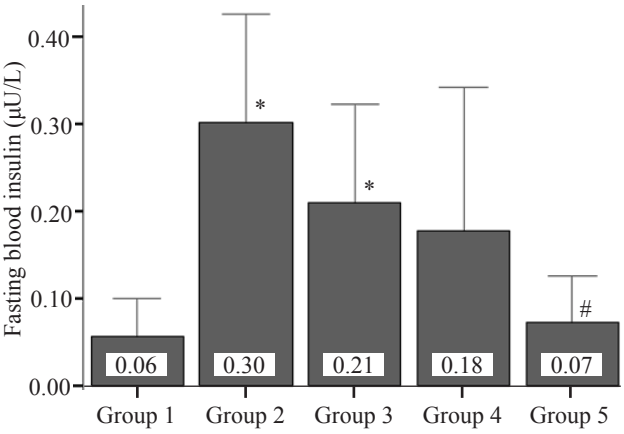

Figure 2. Comparison of fasting blood insulin concentration among the five groups of rats. (μU/L) (*significantly different from the group 1 (p = 0.002, p = 0.038 respectively), #significantly different from the group 2 (p = 0.003). Group 1: control group, group 2: group that was given high-fructose diet only, group 3, 4, 5: group that was given high-fructose along with *Hibiscus sabdariffa* Linn. with dose 100, 200 and 400 mg/kgBW/d respectively

high-fructose diet along with extracts of *Hibiscus sabdariffa* Linn. at dose of 100 mg/kgBW/d were not different significantly from the group of rats that were given high-fructose diet only (p = 0.101). (Figure 3).

DISCUSSION

The significantly higher fasting blood glucose levels, fasting blood insulin levels, and HOMA-IR in rats fed with high-fructose diet compared to the control group indicated that the administration of 10% high-fructose diet *ad libitum* for 5 weeks had led to the development of insulin resistance. The results were consistent with other studies using high-fructose diet models, in which the induction of insulin resistance using fructose can be achieved within 4 to 14 weeks.[14-17]

Rats fed with high-fructose diet along with extracts of *Hibiscus sabdariffa* Linn. at a dose of 400 mg/kgBW/d had lower fasting blood glucose levels, fasting blood insulin levels, and HOMA-IR than the group of rats given high-fructose diet only. These results clearly indicated that *Hibiscus sabdariffa* Linn. was able to prevent the occurrence of insulin resistance. Eventhough the administration of *Hibiscus sabdariffa* Linn. extract at doses of 100 and 200 mg/kgBW/d did not give significant results compared to the group given a high-fructose diet only, there was declining trend in the glucose blood levels, fasting blood insulin levels, and HOMA-IR.

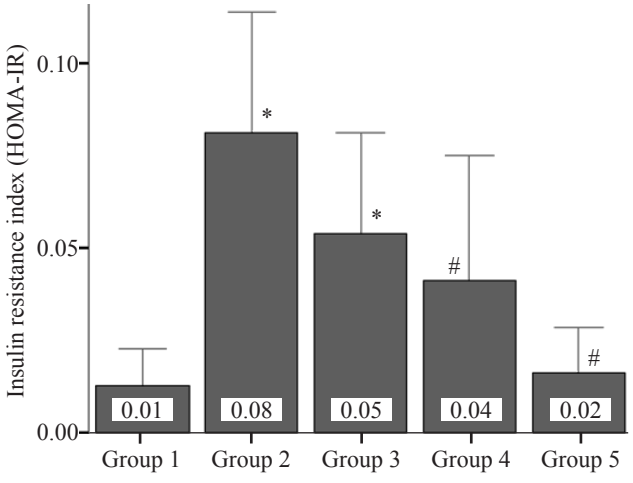

Figure 3. Comparison of HOMA-IR (*significantly different to group 1 (p = 0.00 and p = 0.018, respectively), #significantly different from group 2 (p = 0.021, p = 0.001, respectively). Group 1: control group, group 2: group that was given high-fructose diet only, group 3, 4, 5: group that was given high-fructose along with *Hibiscus sabdariffa* Linn. with dose 100, 200 and 400 mg/kgBW/d respectively)

These results suggested that *Hibiscus sabdariffa* Linn. prevention on insulin resistance works in a dose-dependent manner.

The active substance in *Hibiscus sabdariffa* Linn. that has preventive effect insulin resistance as well as its mechanism of action are not yet fully known. However, the possibility that this effect is mediated through its antioxidant and hypolipidemia effects can not be ruled out.[12,18,19] The most possible active ingredient that has been identified is anthocyanin. This possibility is drawn from analogy of a study conducted by Guo, et al[16] showing that anthocyanin in *Oryza sativa* L could prevent an increase in the production of fatty acids and free radicals in rats fed with high-fructose diet. Previous study in human suggested that extracts of *Hibiscus sabdariffa* are promising for the treatment of hyperlipidemia. In a randomized clinical trial, the daily consumption of tea or extract produced from *Hibiscus sabdariffa* calyxes had favorable influence on lipid profiles including reduced total cholesterol, LDL-C, triglycerides, as well as increased HDL-C.[12] The exact molecular mechanisms of *Hibiscus sabdariffa* Linn. in preventing the occurrence of insulin resistance and the extent to its benefits in human still needs to be further investigated.

In conclusion, the present study suggests that the administration of *Hibiscus sabdariffa* Linn. extract can prevent insulin resistance associated with high-fructose diet, the effect being dose dependent. Further studies are needed to determine the molecular mechanisms and the optimum dose of *Hibiscus sabdariffa* Linn. to provide recommendations for it use in human for the prevention of insulin resistance.

Acknowledgment

This research was funded by Hibah Awal Univesitas Indonesia 2012.

Conflict of interest

The authors affirm no conflict of interest in this study.

REFERENCES

1. Basciano H, Federico L, Adeli K. Fructose, insulin resistance, and metabolic dyslipidemia. Nutr Metab (Lond). 2005;2(1):5.
2. Antuna-Puente B, Disse E, Rabasa-Lhoret R, Laville M, Capeau J, Bastard JP. How can we measure insulin sensitivity/resistance?. Diabetes Metab. 2011;37(3):179-88.

3. Altaş M, Var A, Köse C, Özbilgin K, Arı Z. Endothelial dysfunction in high fructose containing diet fed rats: increased nitric oxide and decreased endothelin-1 levels in liver tissue. Dicle Med J Cilt. 2010;37(3):193-8.
4. Gurrola-Díaz CM, García-López PM, Sánchez-Enríquez S, Troyo-Sanromán R, Andrade-González I, Gómez-Leyva JF. Effects of *Hibiscus sabdariffa* extract powder and preventive treatment (diet) on the lipid profiles of patients with metabolic syndrome (MeSy). Phytomedicine. 2010;17(7):500-5.
5. Henriksen EJ, Diamond-Stanic MK, Marchionne EM. Oxidative stress and the etiology of insulin resistance and type 2 diabetes. Free Radic Biol Med. 2011;51(5):993-9.
6. Tiganis T. Reactive oxygen species and insulin resistance: the good, the bad and the ugly. Trends Pharmacol Sci. 2011;32(2):82-9.
7. Samuel VT. Fructose induced lipogenesis: from sugar to fat to insulin resistance. Trends Endocrinol Metab. 2011;22(2):60-5.
8. Tee PL, Yusof S, Mohamed S, Umar NA, Mustapha NM. Effect of roselle (*Hibiscus sabdariffa* L.) on serum lipids of Sprague Dawley rats. Nutr Food Sci. 2002;32(5):190-6.
9. Hou DX, Fujii M, Terahara N, Yoshimoto M. Molecular mechanisms behind the chemopreventive effects of anthocyanidins. J Biomed Biotechnol. 2004;2004(5):321-5.
10. Mahadevan N, Shivali, Kamboj P. *Hibiscus sabdariffa* Linn – An overview. Nat Prod Rad. 2008;8(1):77-83.
11. Tsai PJ, McIntosh J, Pearce P, Camden B, Jordan BR. Anthocyanin and antioxidant capacity in roselle (*Hibiscus sabdariffa* L.) extract. Food Research Int. 2002;35(4):351-6.
12. Hopkins AL, Lamm MG, Funk JL, Ritenbaugh C. *Hibiscus sabdariffa* L. in the treatment of hypertension and hyperlipidemia: A comprehensive review of animal and human studies. Fitoterapia. 2013;85:84-94.
13. Lila MA. Anthocyanins and human health: an *in vitro* investigative approach. J Biomed Biotechnol. 2004;2004(5):306-13.
14. Bunbupha S, Pakdeechote P, Kukongviriyapan U, Pannangpetch P. Antihyperinsulinemia effect of *Hibiscus sabdariffa* extract in high fructose diet induced insulin resistance rats. Srinagarind Med J. 2012;27:140-3.
15. Abdulla MH, Sattar MA, Johns EJ. The relation between fructose-induced metabolic syndrome and altered renal haemodynamic and excretory function in the rat. Int J Nephrology. 2011;2011:934659.
16. Guo H, Ling W, Wang Q, Liu C, Hu Y, Xia M, et al. Effect of anthocyanin-rich extract from black rice (*Oryza sativa* L. *indica*) on hyperlipidemia and insulin resistance in fructose-fed rats. Plant food Hum Nutr. 2007;62(1):1-6.
17. Suwannaphet W, Meeprom A, Yibchok-Anun S, Adisakwattana S. Preventive effect of grape seed extract against high-fructose diet-induced insulin resistance and oxidative stress in rats. Food Chem Toxicol. 2010;48(7):1853-7.
18. Mardiah, Zakaria FR, Prangdimurti E, Damanik R. The effect of roselle extract (*Hibiscus sabdariffa* Linn.) on blood glucose level and total antioxidant level on diabetic rat induced by streptozotocin. IOSR J Pharmacy. 2014;4(10):8-16.
19. Peng CH, Chyau CC, Chan KC, Chan TC,Wang CJ, Huang CN. *Hibiscus sabdariffa* polyphenolic extract inhibits hyperglycemia, hyperlipidemia, and glycation-oxidative-stress while improving insulin resistance. J Agric Food Chem. 2011;59(18):9901-9.