The Comparative Effect of Pomegranate Peel Extract and Dapagliflozin on Body Weight of Male Albino Wistar Rats with Type 2 Diabetes Mellitus

Devi Trisna Ramadhani1, Rafi Amanda Rezokia Amradani2, Mila Ulfia2, Suryaningtyas Margi Utami2, Dono Indarto1,2,3*, Brian Wasita1,4

1Postgraduate Program of Nutrition Sciences, Universitas Sebelas Maret, Surakarta
2Biomedical Laboratory, Faculty of Medicine, Universitas Sebelas Maret, Surakarta
3Department of Physiology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta
4Department of Anatomy Pathology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta

*Corresponding author : dono@staff.uns.ac.id

Abstract. Sodium Glucose Co-Transporter 2 (SGLT2) inhibitor is the second line of type 2 diabetes mellitus (T2DM) treatment. From in silico and in vitro studies, ellagic acid in pomegranate fruits has a similar effect to the SGLT2 inhibitor. This study aimed to investigate the effect of pomegranate peel extract (PPE) on body weight (BW) of male rats with T2DM. Male Albino Wistar rats, which aged 8 weeks and weighed 150-200 g were induced with single dose of 230 mg/kgBW nicotinamide and 65 mg/kgBW streptozotocin intraperitoneally. The control group consisted of 14 rats with T2DM and randomly divided into the negative control (NC) and the positive control (PC) treated with 0.18 mg/kgBW/day dapaglifozin. Twenty one rats with T2DM were divided into 3 treatment groups (T1-3) and given with 50, 100, and 200 mg/kgBW/day PPE respectively for 14 days. Collected data were analyzed using ANOVA and LSD post hoc tests with p value <0.05. The mean fasting blood glucose levels of all rat groups were 262.04±4.24 mg/dL and the mean BW was 182.86±4.75 g after induction. After 7 days treatment, the mean BW in T1 (188.14±6.40), T2 (186.85±4.94) and T3 (189.85±5.95) groups were significantly higher than that of in the NC group (179.14±3.02) but only the mean BW in the T3 group significantly different from the PC group (184.00±6.40; p= 0.034). The same results were also observed in 14 days treatment. Administration of PPE is able to resume BW in rats model with T2DM, better than dapaglifozin.

Keywords: Sodium Glucose Co-Transporter-2 inhibitor, Type 2 Diabetes Mellitus, Pomegranate Peel Extract, Body Weight, Albino Wistar Rat

1. Introduction
Type 2 Diabetes Mellitus (T2DM) is commonly found in patients who suffer diabetes but only several among them are able to control their blood glucose level through diet and physical exercise. Therefore, most patients with T2DM need anti-diabetic drugs especially metformin as the first line of T2DM treatment [1]. However, many patients are failed to achieve normal glycaemia just using a mono therapy with metformin [2] and the second line of T2DM treatment is sulfonylurea, dipeptidyl
inhibitor, glucagon like peptide 1 (GLP1) agonist and Sodium Glucose Co-Transporter 2 (SGLT2) inhibitor. Selection of using the second line drugs is based on clinical manifestations of patients with T2DM [3,4].

In physiological condition, SGLT2 protein is expressed in a proximal tubule of the kidney and has a central role in main reabsorption of glucose. Some patients with T2DM have mutation of this protein, which resulting in high glucose reabsorption and worsen hyperglycaemic condition [5]. Therefore, SGLT2 inhibitor, dapaglifozin for instance is preferred to be used as a treatment of those patients. This SGLT2 inhibitor competitively inhibited the kidney SGLT2 protein, which lead to the increase of glucose excretion into the urine [6]. However, administration of this drug for long time period has some side effects, such as genital infections, dehydration, increased risk of fractures and cardiovascular disorders [7-9]. From these side effects, we are therefore looking for natural substances that have a similar effect to SGLT2 inhibitor.

Pomegranate fruit, originally cultivated in Mediterranean countries, has some beneficial effects to treat diabetes [10,11]. From an in silico study, pomegranate fruit contains a secondary metabolite (ellagic acid) that is able to interact with SGLT2 protein, as same dapaglifozin as at Gly79 and His80 residues [12]. In addition, administration of 1.45 ppm pomegranate peel extracted with ethanol, diethyl ether, and water solvents (PPE) has lower toxicity than dapaglifozin [13]. Based on in vivo studies, two studies conducted in India and Pakistan have reported that administration of 200mg/kgBW methanol extract of pomegranate peel for 28 days significantly reduced fasting blood glucose (FBG) levels [14,15] although they used alloxan or streptozotocin to induce T2DM respectively. So far, there has been not reported that BW changes in rats with T2DM are treated with PPE. Therefore, the aim of this study was to investigate the effect of PPE on BW of male Wistar rat with T2DM.

2. Material and Methods

2.1. Preparation on Peel Extract of Pomegranate Fruit
Ripened pomegranate fruits used in this study were obtained from a local market at Karanganyar City, Central Java and were extracted using a maceration method with ethanol, diethyl ether, and water solvents, which was adopted from Singh et al study [16].

2.2. Generation of Animal Model with T2DM
Albino Wistar male rats, which aged 8 weeks and had 150-200 g body weight were used in this experimental study. Each Wistar rat from 35 selected Wistar rats were adapted in a hygienic polypropylene cage for 7 days by controlling temperature (27°C ± 2°C) and light condition (12 hours/day) with ad libitum water access. The following day, Wistar rats were injected intraperitoneally with single dose of 230 mg/kgBW nicotinamide. Fifteen minutes later, Wistar rats were injected intraperitoneally with single dose 65 mg/kgBW streptozotocin. All stages of research experiments followed animal ethics and the research protocol got permission from the Health Research Ethics Committee, Faculty of Medicine, Universitas Sebelas Maret with number 315/UN27.6/KEPK/2018.

2.3. Experimental Design
Once Wistar rats have become T2DM, 35 rats were randomly divided into five groups. Each group consisted of seven diabetic rats and designated to NC, PC, and T1-3. The NC group was given no treatment while the PC group was orally administered 0.18 mg/kgBW/day dapaglifozin. The T1-3 groups were orally administered 50, 100, and 200 mg/kgBW/day pomegranate peel extract (PPE). All diabetic rats were given a standard diet and ad libitum water access for 14 days. BW of diabetic rats were recorded before, during, and after intervention.

2.4. Statistical Analysis
All collected data were presented in mean ± standard deviation. Before running statistical analysis, homogeneity and normality data of BW were analyzed using Levene and Shapiro-wilk tests. The mean
differences of BW before and after intervention in all rats groups were analyzed of variance and followed by LSD post-hoc test. \( P < 0.05 \) was considered as statistically significant.

3. Results and Discussion

After 7 day adaptation, 35 selected rats were injected with NA and STZ intraperitoneally. Figure 1 and 2 indicated that mean fasting blood glucose levels of all rat groups were 262.04±4.24 mg/dL and mean rat BW in all groups reduced by 2.6% after induction with NA and STZ. During adaptation, the highest mean rat BW was found in the T1 group (184.57±3.69 g) while the PC group had the lowest mean rat BW (179.71±6.52 g). The mean differences of rat BW among groups did not reach significantly \( p > 0.102 \). Reduction of rat BW in all groups after induction with STZ and NA had the similar pattern to the mean rat BW before induction but the mean rat BW after induction within group was significantly different from the mean rat BW before induction (\( p \leq 0.002 \)).

In previous studies, administration of 35 - 60 mg/kgBW/day STZ can cause T2DM in animal model [17], which leads to reversible damage of pancreatic \( \beta \) cells [18]. However, STZ also damages other rat tissues such as kidney, liver, heart, and adipocytes. Furthermore, this toxic chemical increases oxidative stress, inflammation, and endothelial dysfunction [19]. Consequently, other chemicals such as NA are needed to protect massive damages of tissues in rats with T2DM model. The addition of NA in Wistar rat induced by STZ has several advantages. STZ-induced hyperglycemia is relatively stable without exogenous insulin treatment to maintain pancreatic \( \beta \) cells, secreting insulin and preventing glucose intolerance [20-23]. Secondly, NA administration before STZ helps regenerate pancreatic \( \beta \) cells and inhibit their apoptosis [24]. Therefore, this rat model is suitable for biochemical and pharmacological studies to evaluate the potential of PPE for anti-diabetic treatment.

![Figure 1. Fasting Blood Glucose Levels of Wistar Rats Induced by NA and STZ. After 7 days adaptation, Wistar Rats were intraperitoneally injected with single dose of 230 mg/kgBW NA and 65 mg/kgBW STZ. Fasting blood glucose levels were measured in the day 3 after induction and presented as mean ± standard deviation.](attachment:Figure_1.png)
**Figure 2.** BW changes in Wistar Rats Before and After Induction with NA and STZ. Wistar Rats were intraperitoneally injected with single dose of 230 mg/kgBW NA and 65 mg/kgBW STZ. BW was measured before and after induction and presented as mean ± standard deviation. The difference of rat BW was analyzed using the paired t-test and *designated significant difference within rat groups with p<0.05.

**Figure 3.** The effect of PPE on the BW of Wistar Rats with T2DM. BW was measured in the day 1, 7, and 14 days treatment and presented as mean ± standard deviation. The difference of rat BW was analyzed using the paired t-test and ANOVA, followed by LSD post hoc test with p<0.05. *designated significant difference within rat groups. **was comparison between T1-3 and NC groups and *** was between T1-3 and PC groups.
We evaluated rat BW in the day 7 and 14 after PPE treatment. In general, increased mean BW occurred in rats treated with Dapaglifozin and PPE (Figure 3). In the day 7, all rats with T2DM had mean BW >180 g that is significantly higher than rats with T2DM in the NC group (179.14±3.02 g). Mean rat BW treated with 200 mg/day/BW PPE was also significantly different from mean rat BW treated with dapaglifozin (p=0.034). The highest rats’ mean BW was found in the T3 group (189.85±5.95 g), followed by T1, T2, and PC groups. The same pattern of BW increase was also observed in the day 14 treatment. Compared to the NC group, significantly higher BW mean was found in all treatment groups. Mean BW of rat in T3 group was significantly higher than mean rat BW in the PC group (p=0.019). From our results, it clearly indicated that administration of PPE was able to improve metabolism in T2DM rat model although we do not evaluate pancreatic β cells, hepatocytes and muscle cells.

The increased BW in rats treated with dapaglifozin and PPE is probably caused by improvement of insulin secretion, which leads to increased uptake of glucose in all tissues. Our findings are in accordance to the finding of Lee et al. study that diabetic mice treated with dapaglifozin have increased BW and reduced blood glucose level [25] although we used a different method to generate diabetic rat model. We used 230 mg/kgBW NA and 65 mg/kgBW STZ to induce T2DM while Lee and co-workers made a point mutation in the leptin receptor gene to generate T2DM. Therefore, increased rat BW in this present study indicates protective effects, which leads to improvement of health outcome. In contrast, diabetic rats underwent significant reduction of BW after 7 and 14 days intervention.

In the present study, increased BW in rats treated with 200 mg PPE is higher than increased BW in rats treated with dapaglifozin. It implied that PPE has a better effect in T2DM treatment because pomegranate fruit containing ellagic acid is able to interact with the SGLT2 protein, it was similar with dapaglifozin analysis based on in silico study [12]. This study suggested that PPE is likely to have the same mechanism as dapaglifozin.

4. Conclusion
Rat BW with T2DM back to normal after administration of 50, 100, or 200 mg/kgBW/day PPE for 7 and 14 days. However, higher weight gain is found in T2DM rats treated with 200 mg/kgBW/day PPE, compared to dapaglifozin. Further studies are required to confirm this result with pure ellagic acid whether or not ellagic acid is responsible for increasing BW.

Acknowledgments
We would like to thank all staff in the Laboratory of Food and Nutrition Study Center, Gadjah Mada University, Yogyakarta for looking after rats during this study, testing blood glucose levels and measuring BW. We also appreciate staff members at the unit 2, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta for making PPE.

References
[1] Marín-Peñalver, JJ., Martín-Timón, J., Sevillano-Collantes, C., and Cañizo-Gómez, FJD. Update on the treatment of type 2 diabetes mellitus. World J Diabetes. 2016, vol. 15, pp.354–395.
[2] Cook, MN., Girman, CJ., Stein, PP, and Alexander, CM. Initial monotherapy with either metformin or sulphonylureas often fails to achieve or maintain current glycaemic goals in patients with Type 2 diabetes in UK primary care. Diabetic Medicine. 2007, vol. 24, pp.350-358.
[3] Hayden, J., Huang, R., McConnell, L.M., Sainsbury, CA and Jones, AC. Evaluation of a combination of SGLT2 inhibitor and GLP-1 receptor agonist treatment in type 2 diabetes. Diabetes & Primary Care. 2016, vol.18, pp.135–138.
[4] Gurgle, HE., White, K., and McAdam-Marx, C. SGLT2 inhibitors or GLP-1 receptor agonists as second-line therapy in type 2 diabetes: patient selection and perspectives. Vasc Health
Bays, H. Sodium Glucose Co-transporter Type 2 (SGLT2) Inhibitors: Targeting the Kidney to Improve Glycemic Control in Diabetes Mellitus. Diabetes Ther. 2013, vol.4, pp.195–220.

Abdul-Ghani, M., Prato, S., Chilton, R., and Defronzo, R. SGLT2 Inhibitors and Cardiovascular Risk: Lessons Learned From The EMPA-REG OUTCOME Study. Diabetes Care. 2016, vol.39, pp.717–725.

Somasundaram, N., dan Wijesinghe, A. Therapy For Type 2 Diabetes Mellitus: Targeting The “Unlucky Thirteen”. Jacobs Journal of Diabetes and Endocrinology. 2016, vol.2, pp.1-12.

Bethel, MA., and McMurray, JJV. Class Effect for Sodium Glucose-Cotransporter-2 Inhibitors in Cardiovascular Outcomes. Circulation. 2018, vol. 137, pp. 1218-1220.

Lytvyn, Y., Bjornstad, P., Udell, JA., Lovshin, JA., and Cherney, DZI, Sodium Glucose Cotransporter-2 Inhibition in Heart Failure Potential Mechanisms, Clinical Applications, and Summary of Clinical Trials. Circulation. 2017, vol. 136, pp.1643-1658

Jurenka, JS. Therapeutic Applications of Pomegranate (Punica Granatum L.): A Review. Alternative Medicine Review. 2008, vol.13, pp. 128–144.

Medjakovic, S., and Jungbauer, A. Pomegranate: A Fruit that Ameliorates Metabolic Syndrome. Food & Function. 2013, vol.4, pp. 19–39.

Amradani. Molecular docking : exploration of sodium glucose co-transporter 2 inhibitors of Indonesian herbal plant compounds as a type 2 diabetes mellitus therapy. 2015. Bachelor thesis, Sebelas Maret University.

Utami, SM., Indarto, D., and Yudhani, RD. Methanol extract of pomegranate fruits containing ellagic acid and cytotoxicity in Vero cell line. AIP Conference Proceedings. 2018. vol. 2021, issue.1.

Parmar, HS., and Kar, A. Medicinal Values of Fruit Peels From Citrus Sinensis, Punica Granatum, And Musa Paradisiaca With Respect to Alterations In Tissue Lipid Peroxidation and Serum Concentration of Glucose, Insulin, And Thyroid Hormones. J Med Food. 2008, vol.11, pp.376-381.

Shujaat, A., and Hussain, MM., Effect of Pomegranate Peel Alone and in Combination With Rosiglutzoin on Hyperglycemia and Dislipidemia in Type 2 Diabetes Mellitus. Pak Armed Forces Med J. 2016, vol.66,pp.460-474.

Singh, S., Usman, K., and Banerjee, M. Pharmacogenetic Studies Update in Type 2 Diabetes Mellitus. World Journal of Diabetes. 2016, vol.7, pp.302–315.

Ghasemi, A., Khalifi, S., and Jedi, S. Streptozotocin-Nicotinamide-Induced Rat Model Of Type 2 Diabetes. Acta Physiologica Hungarica. 2014, vol.101, pp. 408–420.

Akbarzadeh, A., Norouzian, D., Mehrabi, MR., Jamshidi, S., Farhangi, A., Verdi, AA., Mofidian, A., and Lame Rad, B. Induction of Diabetes by Streptozotocin in Rats. India Journal of Clinical Biochemistry. 2007, vol.22, pp.60-64.

Eleazu, CO., Eleazu, KC., Chuckwuma, S., and Essien, UN. Review of the Mechanism of Cell Death Resulting from Streptozotocin Challenge in Experimental Animals, Its Pactical Use and Potential Risk to Humans. Journal of Diabetes & Metabolic Disorders. 2013, vol.12, pp.60.

Chang, KC., Tseng, CD., Chou, TF., Cho, YL., Chi, TC., Su, MJ., and Tseng, YZ. Arterial stiffening and cardiac hypertrophy in a new rat model of type 2 diabetes. Eur. J. Clin. Invest. 2006, vol.36, pp.1–7.

Fierabracci, V., De Tata, V., Pocai, A., Novelli, M., Barbera, A., and Masiello, P. Oral tungstate treatment improves only transiently alteration of glucose metabolism in a new rat model of type 2 diabetes. Endocrine. 2002, vol.19, pp.177–184.

Masiello, P. Animal models of type 2 diabetes with reduced pancreatic beta-cell mass. Int. J. Biochem. Cell. Biol. 2006, vol.38, pp.873–893.

Novelli, M., Pocai, A., Lajoix, AD., Beffy, P., Bezzi, D., Marchetti, P., Gross R., and Masiello P. Alteration of beta-cell constitutive NO synthase activity is involved in the abnormal
insulin response to arginine in a new rat model of type 2 diabetes. *Mol. Cell. Endocrinol.* 2004, vol.219, pp.77–82.

[24] Pandya, KG., Patel, MR., and Lau-Cam, CA. Comparative study of the binding characteristics to and inhibitory potencies towards PARP and in vivo antidiabetogenic potencies of taurine, 3-aminobenzamide and nicotinamide. *J. Biomed. Sci.* 2010, vol.17, suppl.1.

[25] Lee, DM., Battson, ML., Jarrell, DK., Hou, S., Ecton, KE., Weir, TL., and Gentile, CL. SGLT2 inhibition via dapagliflozin improves generalized vascular dysfunction and alters the gut microbiota in type 2 diabetic mice. *Cardiovasc Diabetol.* 2018. Vol. 17, pp. 1-14.