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Original Article

Effects of SGLT2 inhibitor administration on blood glucose level and body weight in type 1 diabetes rat model

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Abstract

The prevalence of diabetes worldwide is increasing and 629 million people are projected to have diabetes by 2045, and the most significant burden of the disease being concentrated in low- and middle-income countries (LMICs). Type 2 diabetes is mainly treated with insulin adjunctive therapies such as metformin to improve insulin sensitivity and sodium-glucose co-transporter 2 (SGLT2) inhibitors to lower blood glucose levels. However, there was limited study on the application of SGLT2 inhibitors on type 1 diabetes, particularly empagliflozin. Therefore, this study aimed to determine the effect of SGLT2 inhibitors on blood glucose levels and body weights in a rat model of type 1 diabetes. To mimic type 1 diabetes, the rats were injected with streptozotocin 60 mg intraperitoneally. Twenty-four rat models were randomly divided into four groups: normal rat group (negative control), untreated diabetic rat group (positive control), type 1 diabetic rats treated with metformin, and type 1 diabetic rats treated with empagliflozin. Blood glucose levels and body weight were recorded before and after induced with streptozotocin and on weeks 4, 6, 8 and 10 of the treatment with anti-diabetic drugs. This study found that the blood glucose levels before and after treatment significantly decreased in all groups ($p<0.05$), except in the negative control group. Similar results were observed in body weight of the rats, which all groups experienced weight loss, except the negative control. These results suggested that apart from being used in type 2 diabetes, SGLT2 inhibitors may also be used as a treatment for type 1 diabetes.

Keywords: SGLT2 inhibitor, empagliflozin, metformin, type 2 diabetes, type 1 diabetes

Introduction

The global prevalence of diabetes is increasing, with an estimated 629 million people projected to have diabetes by 2045, and the most significant burden of the disease being concentrated in low- and middle-income countries (LMICs) [1]. The treatment strategies involve effective exogenous insulin delivery to sustain glucose levels, effective management of cardiovascular (CVD) risk factors and promoting psychosocial well-being to minimize the burden of living with diabetes [2]. Aside from insulin medication, adjunctive therapies have been considered to improve insulin sensitivity, glycemia and control CVD risk factors, such as metformin, alpha-glucosidase inhibitors, amylin analogs, glucagon-like peptide-1 (GLP-1) agonists, and dipeptidyl

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peptidase-4 (DDP-4) inhibitors [3]. The current popular therapy is the administration of sodium-glucose co-transporter two inhibitors (SGLT2 inhibitors), such as dapagliflozin, canagliflozin and empagliflozin which inhibits glucose reabsorption in the kidney.

SGLT2 inhibitors, recently popularly used drugs in managing type 2 DM, are said to have many pleiotropic effects. First, inhibition of the SGLT2 receptor causes acute osmotic diuresis, resulting in weight loss. SGLT2 inhibitors also trigger natriuresis to balance sodium and body fluid volume to improve endothelial function and reduce vascular stiffness [4]. These three mechanisms provide an antihypertensive effect and prevent left ventricular hypertrophy as a cardiovascular complication due to chronic hyperglycemia. SGLT2 inhibitors can also reduce plasma triglyceride levels and increase HDL cholesterol, a risk factor for cardiovascular complications [5]. The antihypertensive effect of SGLT2 inhibitors is also affected by decreased activity of the sympathetic nervous system and blood uric acid levels [6]. The decrease in blood uric acid levels is caused by increased uric acid secretion through the urine as a substitute for glucose reabsorption through the GLUT9 transporter [5]. Some experts are considering whether SGLT2 inhibitors have a role in managing type 1 diabetes as primary or additional therapy [7-9]. Therefore, this study aimed to determine the effect of SGLT2 inhibitors on blood glucose levels and body weights in a rat model of type 1 diabetes.

Methods

Study design and setting

This study was an in vitro experimental study using the post-test only method with a control group design, using Wistar rats that were introduced with diabetes by injecting streptozotocin (STZ), commonly used to induce type 1 diabetes in rat models. The study was conducted from June 2020 to January 2021 at the Physiology and the Central Laboratories, Universitas Padjadjaran, Bandung, Indonesia.

Sample size and randomization

The minimum sample size was calculated using the Federer formula for experimental research using experimental animals which resulted in six rats each for four treatment groups. Randomization was carried out using the Complete Randomized Design method by drawing.

Animal model

Twenty-four male Wistar rats aged 8 to 10 weeks and weighed 250–350 grams were used as the model organism in this study. The rats were obtained from the Bio Farma Laboratory, Bandung, Indonesia.

Inclusion criteria were healthy male Wistar rats, aged 8–10 weeks, weighing 250–350 grams and engaged in normal activities without external interventions, such as treadmill exercise. Exclusion criteria were rats that were sick or died during the study and rats which blood glucose levels did not increase after administration of STZ.

Intervention

Rats were habituated in sawdust-bedding cages in a room with temperature ranged of 20–28°C and 50–97% humidity, and fed a standard diet for adult rats. After passing through the habituation/adaptation period, the rats were allocated randomly to four groups with six rats each. The allocated four groups were NC: negative control (non-diabetic rat); PC (STZ): positive control (diabetic rats without treatment); STZ+metformin: metformin-treated diabetic rats; and STZ+empagliflozin: empagliflozin-treated diabetic rats (**Figure 1**). Rats were induced to type 1 diabetes with a single dose of 60 mg/kg BW of intraperitoneal STZ injection. Rats were included in this study if their blood glucose levels were more than 250 mg/dL in three blood examinations. Drug administration was carried out for eight weeks, with the drug being delivered via a probe daily in the morning. The dosages were 100 mg/kgBW metformin and 30 mg/kgBW empagliflozin.

Endpoints

Body weight and blood glucose levels were measured six times each: before STZ injection (week 0), two weeks after STZ injection (week 2), week 4, week 6, week 8 and week 10. Glucocard (Arkray, Maharashtra, India) was used to examine blood glucose levels with blood samples taken from the rat tail.

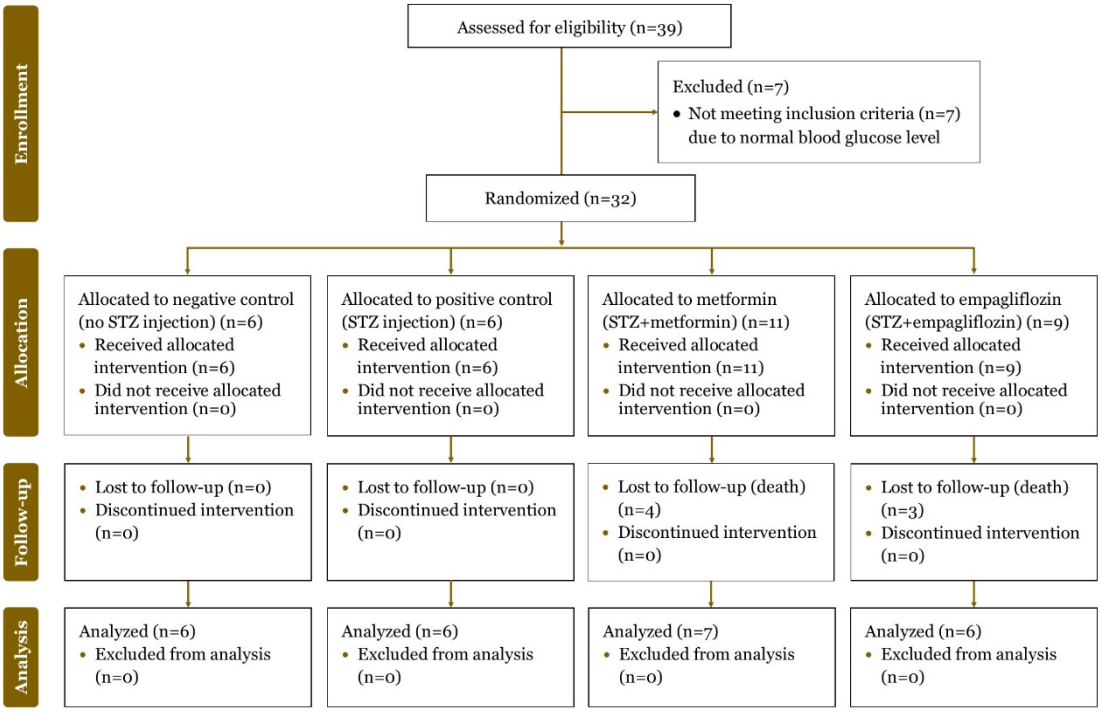

Figure 1. Diagram of the study groups and treatments.

Statistical analysis

The study used one-way ANOVA to assessing the observed decrease and through repeated analyses, a conclusion was made to determine the significance of the observed difference. Data was expressed with a 95% confidence interval (CI) with a significance limit that is accepted when $p<0.05$.

Results

Habituated rats were measured for initial blood glucose levels and body weights (**Table 1**). There was no statistically significant difference of blood glucose levels and body weight before STZ injection across all four groups ($p>0.05$). In post-STZ, there was a significant difference in blood glucose levels ($p<0.05$), which was likely due to the stable value of blood glucose levels in normal rat group (negative control).

Table 1. Baseline data of blood glucose levels and body weight

Variable	Group (mean±SD)				*p*-value
	NC	PC (STZ)	STZ+metformin	STZ+empagliflozin	
Blood glucose levels (mg/dL)					
Pre-STZ	107.50±17.08	110.00±18.08	109.42±17.97	113.83±22.55	0.958
Post-STZ	116.00±8.04	354.67±24.11	333.76±31.52	336.83±52.52	0.000*
Body weight (g)					
Pre-STZ	284.25±12.99	302.83±14.50	271.28±30.99	282.83±13.96	0.116
Post-STZ	297.75±15.19	291.50±16.36	264.14±33.77	269.83±23.48	0.101

NC: negative control (non-diabetic rat); PC (STZ): positive control (diabetic rats without treatment); STZ+metformin: metformin-treated diabetic rats; and STZ+empagliflozin: empagliflozin-treated diabetic rats.

The average weights of the rats initially were similar in all groups, aligning with the inclusion criteria of 250–350 g body weight. ANOVA analysis showed that the difference in body weight between each group was insignificant before STZ injection ($p>0.05$) which could be assumed that rats across four groups had similar baseline characteristics. Two weeks after STZ administration, rats in all groups experienced weight loss except in the negative control group, but there was not significantly different.

Blood glucose levels

There were decreases of blood glucose levels between before and after treatment in all groups (**Figure 2**). However, only three groups (PC, STZ+empagliflozin and STZ+metformin) experienced significant decrease ($p<0.05$). Moreover, the decline occurred more in the rats treated with the empagliflozin, reaching 63.24% decline compared to 35.84% in the metformin group, starting from the second week after drug administration. In addition, throughout the treatment, it could be seen that all the groups were in declining trends (**Figure 3**). The negative control group had relatively stable decrease, while the STZ+empagliflozin group experienced the most drastic decrease. The other two treatment groups showed similar patterns with small difference of blood glucose levels at the last measurement time (STZ+metformin 214.14 mg/dl; positive control 259.67 mg/dl).

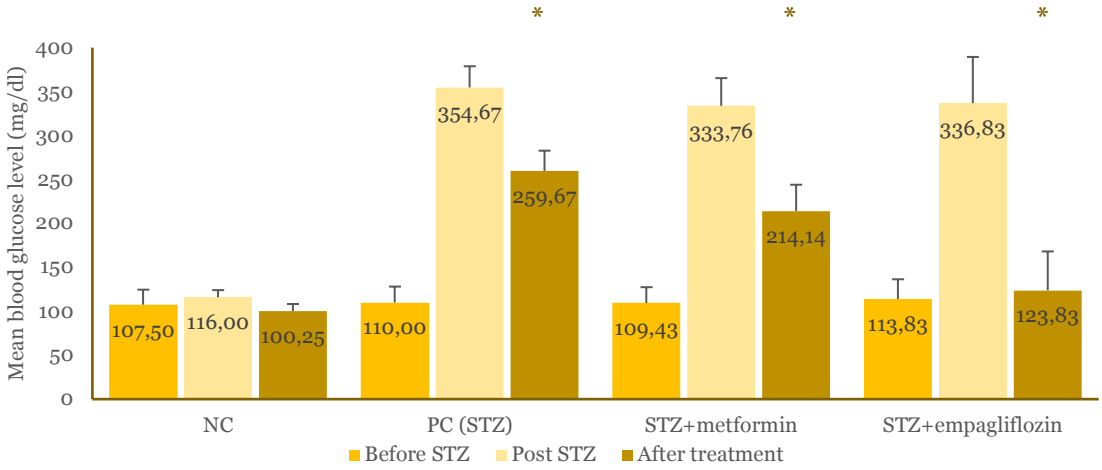

Figure 2. Mean blood glucose levels of rats in each group before STZ injection, after two weeks of STZ injection (baseline/before therapy) and after eight weeks of anti-diabetic administration ($p>0.05$). NC: negative control (non-diabetic rat); PC (STZ): positive control (diabetic rats without treatment); STZ+metformin: metformin-treated diabetic rats; and STZ+empagliflozin: empagliflozin-treated diabetic rats. *Statistically significant at $p<0.05$ compared to before STZ of each group.

Body weight

Body weight of rats were measured before STZ injection, after injection of STZ (baseline/before therapy), and on week 4, 6, 8 and 10 after anti-diabetics administration (**Figure 4** and **Figure 5**). The initial average weights of the rats in all groups were insignificantly different ($p>0.05$), aligning with the body weight inclusion criteria of 250–350 g which could be assumed that all of the rats had similar baseline characteristics. Two weeks after STZ administration, all rats experienced weight loss except in the negative control group. After eight weeks of therapy, rats in the three groups experienced weight gain although there were no significant difference (PC group with $p=0.053$, STZ+metformin with $p=0.181$, and STZ+empagliflozin with $p=0.416$). A significant weight gain only occurred in the negative control group ($p=0.029$). The weight loss experienced by rats after STZ injection coincided with the increase of blood glucose levels. After anti-diabetic medications were given from week two onwards, there were gradual slight weight gains only in the groups treated with metformin and empagliflozin.

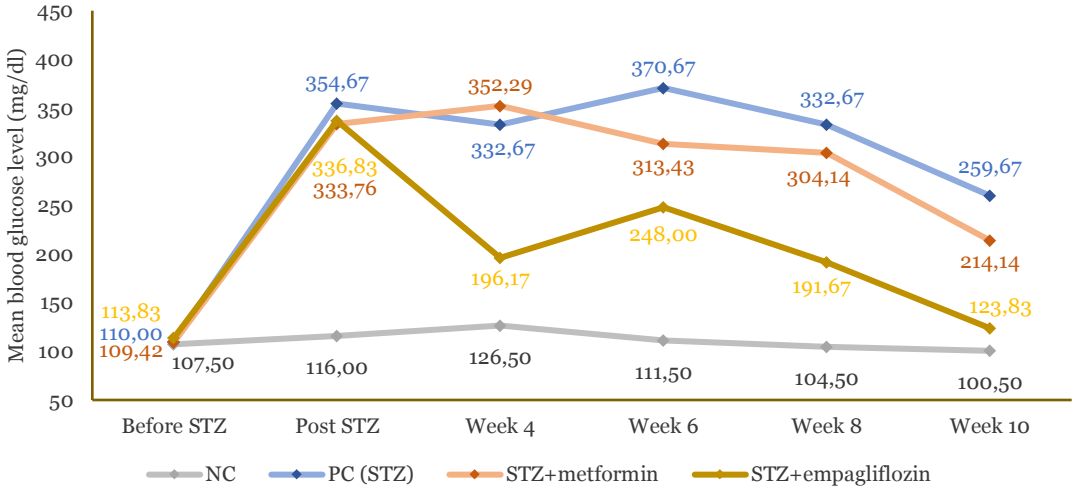

Figure 3. Mean of blood glucose levels of rats in each group before STZ, after injection of STZ (baseline/before therapy), and weeks 4, 6, 8 and 10 after administration of anti-diabetics. NC: negative control (non-diabetic rat); PC (STZ): positive control (diabetic rats without treatment); STZ+metformin: metformin-treated diabetic rats; and STZ+empagliflozin: empagliflozin-treated diabetic rats.

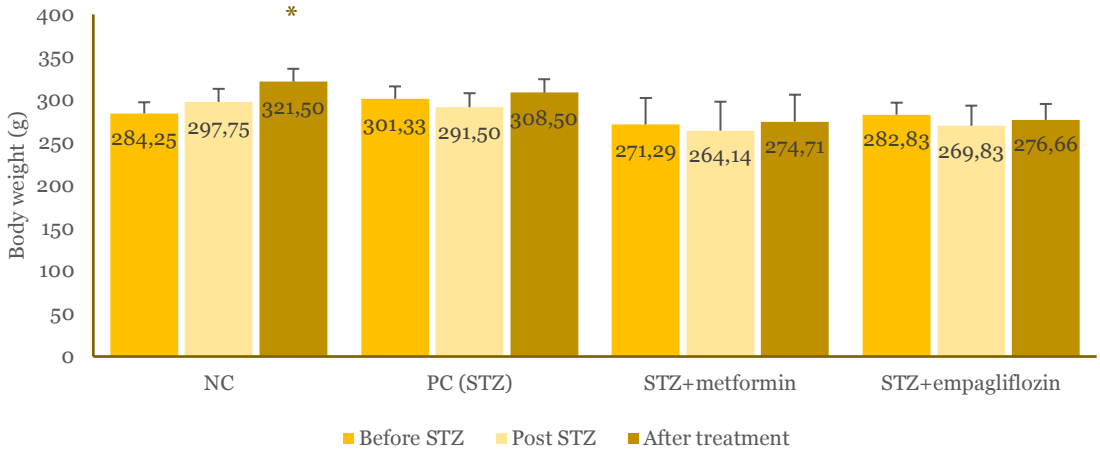

Figure 4. Average body weight of rats in each group before STZ, after injection of STZ (baseline/before therapy), and weeks 4, 6, 8 and 10 after administration of anti-diabetics ($p>0.05$). NC: negative control (non-diabetic rat); PC (STZ): positive control (diabetic rats without treatment); STZ+metformin: metformin-treated diabetic rats; and STZ+empagliflozin: empagliflozin-treated diabetic rats. *Statistically significant at $p<0.05$ compared to post STZ.

Discussion

Blood glucose levels

Unlike for type 2 diabetes, metformin is not commonly recommended as part of a treatment plan for type 1 diabetes. However, previous studies showed that adding metformin as an adjunctive therapy is beneficial, safe, and well-tolerated for type 1 diabetes, even in the adolescent population [10]. Metformin decreases blood glucose concentrations and metabolic syndrome prevalence, as well as lowers insulin dose requirement [11]. In contrast, empagliflozin and other SGLT2 inhibitors have been proven as effective glucose-lowering agents for type 2 diabetes. A recent trial called Empagliflozin as Adjunct to inSulin thErapy (EASE) showed a beneficial effect on glycated hemoglobin (HbA1C), body weight and glucose variability in type 1 diabetes patients [12]. It may be due to its role in improving blood glucose homeostasis through the protective effects on pancreatic beta cells from oxidative stress [13].

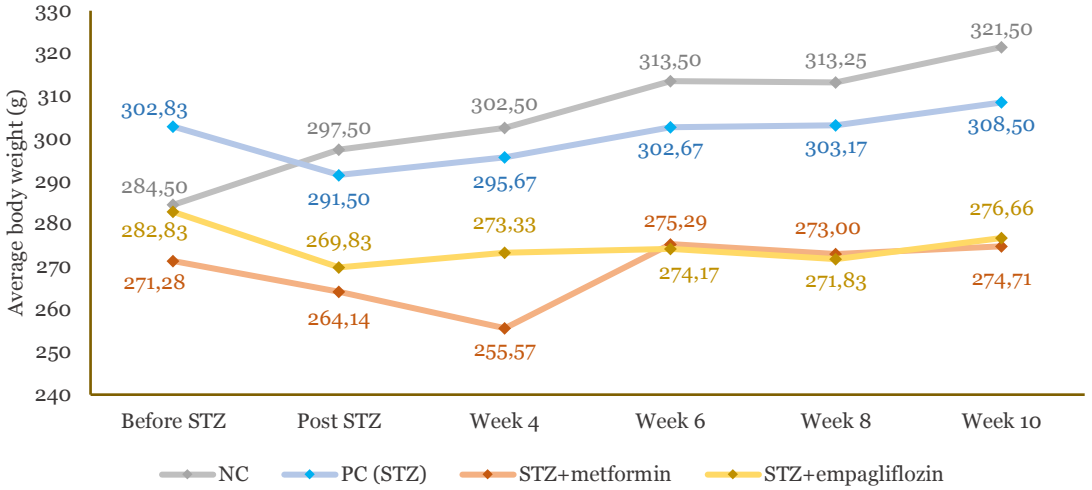

Figure 5. The average weight of rats in each group before STZ, after injection of STZ (baseline/before therapy), and weeks 4, 6, 8 and 10 after administration of anti-diabetics. NC: negative control (non-diabetic rat); PC (STZ): positive control (diabetic rats without treatment); STZ+metformin: metformin-treated diabetic rats; and STZ+empagliflozin: empagliflozin-treated diabetic rats.

This study found that the effect of reducing blood glucose occurred in all three diabetic rat groups, i.e., the positive control, metformin-treated and empagliflozin-treated groups. The blood glucose levels in the positive control group were stable until the sixth week but then decreased. This indicated that a single dose of 60 mg/kg BW of intraperitoneal STZ was able to maintain satisfactory hyperglycemia until the sixth week. However, after the sixth week, it decreased even though it was still in the high range (259.67 mg/dl).

Many researchers have studied the effects of hyperglycemia in the rat model of STZ-induced diabetes [14,15]. Giving a single dose or divided small doses of STZ can induce type 1 diabetes in experimental animals because STZ can cause total destruction or damage to only a portion of the pancreatic beta cells and giving hyperglycemic effect [14,15]. This causes the effects of hyperglycemia to be reversible even without treatment. Usually, a single dose of 60 mg/kg BW of STZ can induce a reversible increase in blood glucose level, but STZ of more than 75 mg/kg BW often causes the death of rats. This could also explain the decrease in blood glucose levels in diabetic rat models not treated with anti-hyperglycemic drugs [14,15].

The group of diabetic rats treated with metformin and empagliflozin, both also experienced decreases, which was clear that there was a higher decrease in the empagliflozin group. Repeated measures analysis proved that the difference in reducing blood glucose levels in rats treated with empagliflozin and metformin was quite significant. Empagliflozin was superior in reducing blood glucose levels compared to metformin (p=0.017). That is, although these two drugs are significant in reducing blood glucose, empagliflozin was superior when compared to metformin.

There were no other studies have compared the effects of reducing blood glucose levels by metformin and SGLT2 inhibitors in experimental animals. A previous study demonstrated the effectiveness of metformin in lowering blood glucose in diabetic mice [16] while another studied rodent models with empagliflozin [17].

Body weight

In normal rats (negative control), the average weight gain increased continuously. In contrast, the average body weight of rats in the positive control decreased two weeks after the STZ injection then it increased slightly (1.43%) compared to normal rats. This finding was similar to a previous study that diabetic rats injected with STZ had an average body weight that decreased within 21 days of injection, whereas normal rats had a body weight that continued to increase [18]. The trend differences of the body weight is caused by the toxic effects of STZ, which resulted in a significant increase in blood glucose level, a decrease in body weight and the chance of experiencing diarrhea [14].

Moreover, the diabetic rats treated with metformin had a lower average body weight compared to diabetic rats who were not given any therapy. This aligned with a study conducted by Yanardag et al., which found that diabetic rats treated with metformin had lower body weight than rats not treated with any drug [20]. Metformin increases fatty acid uptake and adipose tissue utilization and as well as stimulates fatty acid oxidation that inhibits fat accumulation [21,22], therefore, the causes of weight loss in diabetic rats treated with metformin could be explained.

It resonated to the rats treated with empagliflozin, which the average body weight of the rats decreased significantly and the decrease was equivalent to the weight loss that occurred in metformin group ($p>0.05$). Vallon *et al.* found that treating diabetic rats with empagliflozin had no effect on body weight until the 10th week of treatment, but there was a slight increase in body weight at the 15th week of treatment [23]. The inhibition of SGLT2 by empagliflozin increased food intake in Wild-Type rats, possibly to balance ongoing urinary and caloric losses [23]. In addition, empagliflozin treatment found to have an impact on weight loss in diabetic rats with high-fat diet, but not in STZ-induced diabetic rats [24]. The treatment of rats with empagliflozin can reduce body weight by increasing energy expenditure, adipose tissue browning and reducing obesity associated with inflammation and insulin resistance by macrophage activation in white adipose tissue and liver from obese rats [25].

This study confirmed that SGLT2 inhibitors, besides reducing blood glucose, are also able to decrease body weight. The limitation of this study was the absence of direct assessment of pancreatic damage in rat models. Additionally, using oral preparations for both metformin and empagliflozin, rather than pure extracts, may influence the observed outcomes.

Conclusion

Empagliflozin, a SGLT2 inhibitor, was superior in reducing blood glucose levels in rats with STZ-induced type 1 diabetes rat models compared to metformin ($p<0.05$). It was also able to decrease body weight. These results suggest that apart from being used in type 2 diabetes, SGLT2 inhibitors may also be used as a treatment for type 1 diabetes.

Ethics approval

This research was approved by the Research Ethics Committee of Universitas Padjadjaran before conducting the study (No 49/UN11.2.1/PT.01.03/PNBP/2020).

Competing interests

All the authors declare that there are no conflicts of interest.

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This study received no external funding.

Underlying data

All data underlying the results is available as part of the article and no additional source data is required.

How to cite

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References

1. Cheloni R, Gandolfi SA, Signorelli C, *et al.* Global prevalence of diabetic retinopathy: Protocol for a systemic review and meta-analysis. BMJ Open 2019;9(3): e022188.
2. Holt RIG, Devries JH, Hess-Fischl A, *et al.* The management of type 1 diabetes in adults: A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetes Care. 2021;44(11):2589–2625.

3. Bacha F, Klinepeter BS. Insulin resistance, role of metformin and other non-insulin therapies in pediatric type 1 diabetes. Pediatric Diabetes. Pediatr Diabetes. 2016;17(8):545-558.
4. Van Baar MJB, Van Ruiten CC, Muskiet MHA, *et al*. SGLT2 inhibitors in combination therapy: From mechanisms to clinical considerations in type 2 diabetes management. Diabetes Care 2018;41(8):1543-1556.
5. Perkins BA, Cherney DZ, Partridge H, *et al*. Sodium-glucose cotransporter 2 inhibition and glycemic control in type 1 diabetes: Results of an 8-week open-label proof-of-concept trial. Diabet Care 2014;37(5):1480-1483.
6. Wan N, Rahman A, Hitomi H, *et al*. The effects of sodium-glucose cotransporter 2 Inhibitors on sympathetic nervous activity. Front Endocrinol 2018;9:421. doi: 10.3389/fendo.2018.00421.
7. Huang Y, Jiang Z, Wei Y. Efficacy and safety of the SGLT2 inhibitor dapagliflozin in type 1 diabetes: A meta-analysis of randomized controlled trials. Exp Ther Med 2021;21(4):1-11.
8. Snaith JR, Greenfield JR. Sodium-glucose cotransporter 2 inhibitors in type 1 diabetes: A missed opportunity for cardiovascular protection? Med J Aust 2022; 217(3):126-128.
9. Wolfsdorf JI, Ratner RE. SGLT inhibitors for type 1 diabetes: Proceed with extreme caution. Diabet Care 2019;42(6):991-993.
10. Sikorskaya K, Zarzecka I, Ejikeme U, *et al*. The use of metformin as an add-on therapy to insulin in the treatment of poorly controlled type 1 diabetes mellitus in adolescents. Metabol Open 2021;9:100080.
11. Beysel S, Unsal IO, Kizilgul M, *et al*.. The effects of metformin in type 1 diabetes mellitus. BMC Endocr Disord 2018;18(1):1
12. Mathieu C, Van Den Mooter L, Eeckhout B. Empagliflozin in type 1 diabetes. Diabetes Metab Syndr Obes 2019;12:1555-1561.
13. Cheng STW, Chen L, Li SYT, *et al*. The effects of empagliflozin, an SGLT2 inhibitor, on pancreatic β-cell mass and glucose homeostasis in type 1 diabetes. PLoS One 2016;11(1):e0147391.
14. Deeds MC, Anderson JM, Armstrong AS, *et al*. Single dose streptozotocin-induced diabetes: Considerations for study design in islet transplantation models. Lab Anim 2011;45(3):131-140.
15. Goyal SN, Reddy NM, Patil KR, *et al*. Challenges and issues with streptozotocin-induced diabetes – A clinically relevant animal model to understand the diabetes pathogenesis and evaluate therapeutics. Chem Biol Interact 2016;244:49-63.
16. Horakova O, Kroupova P, Bardova K, *et al*. Metformin acutely lowers blood glucose levels by inhibition of intestinal glucose transport. Sci Rep 2019;9(1):6156.
17. Kräker K, Herse F, Golic M, *et al*. Effects of empagliflozin and target-organ damage in a novel rodent model of heart failure induced by combined hypertension and diabetes. Sci Rep 2020;10(1):14061.
18. Pauzi NAS, AA Muhammad, Fakurazi S, *et al*. Preliminary study of the optimization of protocol for developmant of type 2 diabetic model in rats. Indian J Sci Technol 2013;6(7):4960-4965.
19. Pournaghi P, Sadrkhanlou RA, Hasanzadeh S, *et al*. An investigation on body weights, blood glucose levels, and pituitary-gonadal axis hormones in diabetic and metformin-treated diabetic female rats. Vet Res Forum 2012;3(2):79-84.
20. Yanardag R, Ozsoy-Sacan O, Bolkent S, *et al*. Protective effects of metformin treatment on the liver injury of the streptozotocin-diabetic rats. Hum Exp Toxicol 2005;24(3):129-135.
21. He L. Metformin and systemic metabolism. Trends in Pharmacol Sci 2020;41(11):868–881.
22. Kaneto H, Kimura T, Obata A, *et al*. Multifaceted mechanisms of action of metformin which have been unraveled one after another in the long history. Int J Mol Sci 2021;22(5):2596.
23. Vallon V, Gerasimova M, Rose MA, *et al*. SGLT2 inhibitor empagliflozin reduces renal growth and albuminuria inproportion to hyperglycemia and prevents glomerular hyperfiltration in diabetic Akita rats. Am J Physiol Renal Physiol 2014;306(2):F194-F204.
24. Pieber TR, Famulla S, Eilbracht J, *et al*. Empagliflozin as adjunct to insulin in patients with type 1 diabetes: A 4-week, randomized, placebo-controlled trial (EASE-1). Diabetes Obes Metab 2015;17(10):928–935.
25. Xu L, Nagata N, Chen G, *et al*. Empagliflozin reverses obesity and insulin resistance through fat browning and alternative macrophage activation in rats fed a high-fat diet. BMJ Open Diabetes Res Care 2019;7(1):e000783.