Effect Of Antidiabetic Drug on Thyroid Function and Vascular Dysfunction in Diabetic Rat with Methimazole Induced Hypothyroidism

Ankita Sarode*, Dr. Aaishwarya Deshmukh, Dr. S. R. Chaudhari
Trinity College of Pharmacy, Pune
Smt. Kashibai Navale College of Pharmacy, Pune

Abstract: Cardiovascular dysfunctiong in type 2 diabetic patients has interlinked with presence of thyroid hormone dysfunctioning and is one of the leading diabetic complication. SGLT2 inhibiters are seen to have its effect in controlling cardiovascular dysfunctioning as well as imbalance in thyroid hormone levels respectively. Our work aims to elucidate the effect of Dapagliflozin in cardiovascular dysfunctioning and its activity on thyroid hormone levels on diabetic rats induced with hypothyroidism by determining LDH levels and T3, T4, and TSH levels.

Methods: T2DM was induced with a single intraperitoneal (i.p.) injection of freshly prepared streptozotocin (35 mg/kg), 15 min after intraperitoneal injection of nicotinamide (230 mg/kg). Commercially available kits were used to measure thyroid hormone levels and cardiac marker level.

Results: Significant decrease in blood glucose levels were observed 14 days after initial administration in diabetic and hypothyroid treated with dapagliflozin and diabetic treated with dapagliflozin groups compared with diabetic group. After 4 weeks of dapagliflozin treatment, lactate dehydrogenase (LDH) levels and thyroid hormone stimulating (TSH) levels were significantly decreased as compared to diabetic group. Moreover, T_3 and T_4 levels were increased significantly dapagliflozin treated groups compared with a diabetic group. Dapagliflozin treatment also normalised the general symptoms of diabetes.

Conclusion: The study has revealed that, SGLT2 inhibitors demonstrated to impart major cardiovascular safety on T2DM induced rats mainly by decreasing LDH levels in body.

Keywords: Diabetes mellitus, cardiovascular dysfunctioning, thyroid hormones, hypothyroidism, SGLT2 inhibitors.

INTRODUCTION

Hypothyroidism or hyperthyroidism are both associated with diabetes mellitus [(2)]. Hyperglycemia has some harmful effects on vascular between hyperglycemia and poor vascular outcomes [(3)]. Thyroid disorders and diabetes are one of the most prevalent illnesses of everyday life. Thyroid hormone helps in the control of pancreatic activity and carbohydrate metabolism and diabetes have been found to influence thyroid function at variable rates. Therefore, many times thyroid conditions go undiagnosed owing to specific signs and symptoms [(4)]. T2DM is caused by insufficient insulin secretion as a result of defective islet cell function or beta cell mass. Continuous intake of calorie-dense foods, fast food, and a sedentary lifestyle have resulted in a diabetes epidemic that is expected to affect 300 million people worldwide by 2020 [(20)]. Type 2 diabetes mellitus is major cause for diseases such as heart failure and increased risk for coronary and other artery disease and eventually leads to death. Although glucose is consider as a key source of energy for cells, the lipid cell membranes are impermeable to hydrophilic glucose molecules, which thereby makes facilitative glucose transporters (GLUTs) or Na^+ glucose transporters (SGLTs) to make entry for the glucose molecules. In 1960, Crane [(5)] proposed that absorption of glucose molecules was coupled with Na+ transport. SGLT serves as drive to Na+ coupled glucose entry across the sodium gradient. Increase in the levels of sodium chloride consequents S GLT2 inhibitors (SGLT2is) may trigger cascade that reduces the GFR [(6,7,8,9,10)].Thyroid disorders and diabetes are one of the most prevalent illnesses of everyday life. There is a strong connection between diabetes mellitus and thyroid dysfunction. Thyroid hormone helps in the control of pancreatic activity and carbohydrate metabolism. Many times thyroid conditions go undiagnosed owing to specific signs and symptoms. By means of mechanisms that are still partially unknown, the non-genomic actions of thyroid

hormones rapidly activate transduction pathways involving cytoplasmic kinases such as Mitogen-Activated Protein Kinase (MAPK) or Phosphatidylinositol 3-Kinase (PI3 K); these early factors can modulate various downstream reactions and may actually end up triggering nuclear effects such as gene transcription and cell proliferation [16].

MATERIALS AND METHOD

Experimental Animals:

Thirty male Wistar rats were taken for this study by considering mortality rate for diabetes having weight 180-200 g and age 7-8 week from Agharkar Research Institute, Pune, India. The animals were housed in animal house facility given by Smt. Kashibai Navale College of Pharmacy, Kondhwa, Pune. Animals were housed in polypropylene cages with paddy husk as bedding. All animals were kept at a constant temperature of 24 ± 2^0C with relative humidity 30-70% with 12-hour light/dark cycles. High fat diet and water were supplied ad libitum throughout the experimental period. All experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Smt. Kashibai Navale College of Pharmacy, Kondhwa, Pune (Protocol approval no. IAEC-124-21/2019.

Induction of Diabetes mellitus:

Animal model was made by high-fat diet throughout the experiment and was fasted for 12 hours prior STZ administration and FBG was determined. A low dose intraperitoneal injection prepared solution of STZ (35mg/kg Sigma, USA) in citrate buffer (0.1 mol/L, pH 4.5) was administered after 15 mins of NAD (230 mg/kg Arati Dye Chem, Surat). After 72 hours of STZ injection, those animals showed FBG more than 200 mg/dl, were considered as hyperglycaemic and all rats were divided into 4 groups as follows:

Group 1 (Control): Control rats treated with vehicle alone.

Group 2 (Diabetic and hypothyroid group): Diabetic and hypothyroid rats without treatment.

Group 3 (Diabetic and hypothyroid group with Dapagliflozin): Diabetic and hypothyroid rats received dapagliflozin (10 mg/kg, i.p., Alembic Pharmaceuticals, Mumbai) once a day for 4 weeks.

Group 4 (Diabetic group with Dapagliflozin): Diabetic rats received dapagliflozin once a day for 4 weeks.

Diabetic rats in group 2 and 3 received 2% w/v of methimazole (Abbot India Ltd, Nashik) through drinking water.

Dapagliflozine dose:

Dapagliflozine was dissolved in DMSO. The dose was calculated according to formula, HED (mg/kg) = Animal dose (mg/kg) x (Animal Km: Human Km). Where Km is the ratio of body weight in kg to surface area in m2. The control rats were administered with vehicle through-out the study.

Biochemical analysis:

Blood glucose was assessed by Accu-check glucometer on a weekly basis. Under light anaesthesia with ether, blood sample was collected using the retro-orbital method for bioassay. Thyroid hormone levels was estimated by "ELISA method" and "The Modified IFCC method" was followed to measure LDH activity in serum using LDH kit.

Statistical Analysis:

Graph pad prism 8.4.3 software was used to perform statistical analysis using one way ANOVA for T3, T4, TSH and LDH level and two-way ANOVA for blood glucose level, body weight, food and water intake. After ANOVA, Tukey's multiple comparison test was performed. Results were expressed as mean ± SD. Statistical significance was achieved if the $P<0.05$.

RESULTS

Effect of dapagliflozin treatment on blood glucose level and body weight

On day 7^{th} of study, blood glucose level was increased significantly and body weight was found to be significantly decreased in group 2, group 3 and group 4 as compared to group 1 ($^{***}P<0.001$). On day14^{th} of study blood glucose levels was decreased and body weight was found to increased in group 3 and group 4 as compared to the diabetic group 2 ($^{\#\#\#}P<0.001$).

Table 1 Effect of four weeks repeated dose treatment of dapagliflozin on blood glucose level and body weight

Blood glucose levels				
Groups/ Days	Group 1	Group 2	Group 3	Group 4
Day 0	90.6 ± 6.43	77.6 ± 5.71	81.4 ± 7.69	86.80 ± 3.54
Day 7	85.6 ± 5.86	252.8 ± 2.43***	212.2 ± 6.28***	203.0 ± 6.79***
Day 14	86.4 ± 5.86	259.2 ± 2.83	189.2 ± 2.22$^{###}$	183.8 ± 5.43$^{###}$
Body weight				
Groups/ Days	Group 1	Group 2	Group 3	Group 4
Day 0	180 ± 14.14	184 ± 16.73	196 ±16.73	200 ± 20.0
Day 7	204 ± 8.94	152 ± 10.95***	168 ±10.95***	164 ± 8.94***
Day 14	216 ± 16.7	148 ± 10.95	188 ±10.95$^{###}$	184 ± 8.94$^{###}$

Data expressed as mean ± SD, n=6. ***P<0.001 compare to control group, $^{###}$P<0.001 compare to diseased group

Effect of dapagliflozin treatment on food and water intake

In group 2, group 3 and group 4 food and water intake was seen to be significantly increased as compared to group 1 thus, shows hyperphagia condition due to type 2 diabetes mellitus (***P<0.001). Significant decrease in food and water intake was seen upon 14 days of treatment period in group 3 and group 4 as compare to group 2 ($^{###}$P<0.001). Significant difference was seen in group 4 when compared to group 3 ($^{\$}$P<0.05).

Table 2 Effect of four weeks repeated dose treatment of dapagliflozin on Food and water intake

Food intake				
Groups/ Days	Group 1	Group 2	Group 3	Group 4
Day 0	19.56 ± 0.643	24.16 ± 1.519	21.80 ± 0.52	22.34 ± 0.91
Day 7	19.66 ± 0.445	24.54 ± 0.471***	21.56 ± 0.59***	19.82 ± 0.84***
Day 14	19.70 ± 0.283	23.40 ± 0.869	19.98 ± 0.71$^{###}$	19.24 ± 0.37$^{###}$
Water intake				
Groups/ Days	Group 1	Group 2	Group 3	Group 4
Day 0	9.58 ± 0.67	15.46 ± 0.50	12.16 ± 0.27	11.80 ± 0.55
Day 7	10.98 ± 0.28	15.56 ± 0.42***	12.04 ± 0.68***	11.66 ± 0.63***
Day 14	10.79 ± 0.89	14.92 ± 1.16	11.34 ± 0.78$^{###}$	10.12 ± 0.50$^{\$###}$

Data expressed as mean ± SD, n=6. ***P<0.001 compare to control group, $^{###}$P<0.001 compare to diseased group, *P<0.05 compared to group 3

Effect of dapagliflozin treatment on T3, T4 and TSH

The levels of TSH was seen to significantly increased in diseased group as compared to control group (***P<0.001) and simultaneously levels of T_3 was significantly decreased as compared to control group (***P<0.001). Upon treatment in group 3 and group 4 significant increase in the levels of T_3 was observed as compared to diseased group ($^{###}$P < 0.0001) and decrease in TSH levels was seen simultaneously. The T_4 levels in diseased group was found to be markedly increase as compare to the control group (**P<0.01). The significant difference was seen in group 3 when compared to group 1 (***P<0.001). Upon treatment the significant decrease in T_4 levels in group 3 and group 4 was seen as compared to group 2 ($^{###}$P<0.001), significant decrease in T_4 levels was seen in group 4 animals compared to group 3 ($^{\&\&}$P<0.01).

Table 2 Effect of four weeks repeated dose treatment of dapagliflozin on T3, T4 and TSH

Groups/ Parameters	T3	T4	TSH
Group 1	98.00 ± 1.93	6.61 ± 0.139	0.065 ± 0.0029
Group 2	87.48 ± 1.84***	7.52 ± 0.281**	0.076 ± 0.0020***
Group 3	90.44 ± 0.85$^{***###}$	5.88 ± 0.292$^{***###}$	0.070 ± 0.0005$^{*###}$

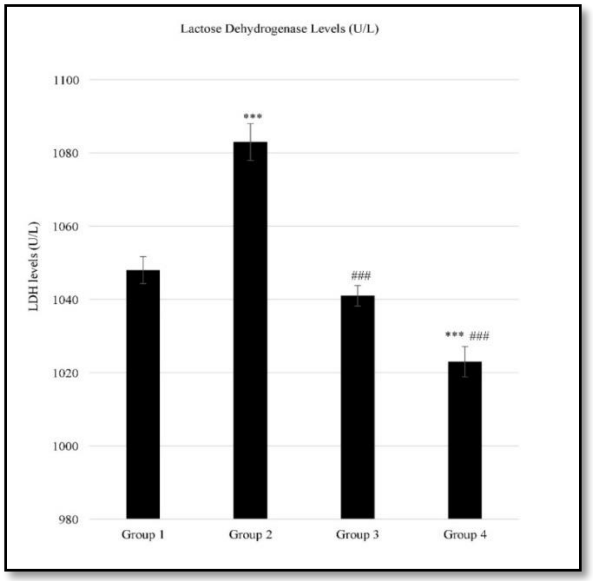
Group 4:	$91.32 \pm 0.63^{\#\#\#}$	$4.86 \pm 0.574^{\&\&\#\#\#}$	$0.062 \pm 0.0031^{ns**\#\#\#}$

Data expressed as mean ± SD, n=6. $^{**}P<0.01$ compare to control group, $^{***}P<0.001$ compared to control group, $^{\#\#\#}P<0.001$ compare to diseased group, $^{\&\&}P<0.01$ compared with group 3

Effect of dapagliflozin treatment on LDH Levels

The LDH levels increased significantly in group 2 as compared to group 1 ($^{***}P<0.001$) The significant decrease in LDH levels was seen in group 4 as compared to group 1 ($^{***}P<0.001$). The LDH levels recovered upon treatment in group 3 and group 4 with significant decrease; as compared to group 2 ($^{\#\#\#}P<0.001$). Upon two weeks of treatment significant decrease was observed in group 4 when compared to group 3 ($^{\&\&\&}P<0.001$).

Figure: Effect of four weeks repeated dose treatment of dapagliflozin on LDH levels



Data expressed as mean ± SD, n=6. $^{***}P<0.001$ compare to control group, $^{\#\#\#}P<0.001$ compare to diseased group. $^{\&\&\&}P<0.001$ compared to group 3

DISCUSSION

Persistent hyperglycemia in diabetes is associated with long-term damage, dysfunction, and failure of various vital organs, contributing to microvascular and macrovascular complications. Selected drug Dapagliflozin is potent $SGLT_2$ inhibitor. $SGLT_2$ inhibitors are operated by a novel mechanism of reduction of renal tubular glucose reabsorption, blood glucose reduction without stimulation release of insulin. Because their action is independent of the function of the β-cell insulin secretion, $SGLT_2$ inhibitors may be used in patients with long-term diabetes have provided renal function; this function is acceptable. When compared to other oral antihyperglycemic agents, $SGLT_2$ inhibitors have been shown non-inferiority with additional metabolic benefits of weight loss and lowering of blood pressure (BP). In the present research, streptozotocin injection induced injury pancreatic cells, which culminated in hyperglycemia. Prolonged hyperglycaemic condition due to impaired insulin-mediated glucose disposal (insulin resistance) and defective insulin secretion due to pancreatic β-cells is a hallmark of diabetes mellitus. (Grundy, 1999) Therefore, a significant increase in blood glucose levels was observed in diabetic rats compared to control rats. Diabetic rats treated with Dapagliflozin at a dose of 10 mg / kg daily for 2 weeks significantly lowered fasting blood glucose levels compared to control rats. Insufficient insulin stops the body from bringing glucose from the blood into the body's cells to use it as energy. And thus, the body begins consuming fat and muscle for energy, which reduces the body's total weight. And hence the body weights of rats induced with type 2 diabetes mellitus in group 2, group 3 and group 4 was seen to be decreased. Upon treatment the body weights of animals in group 3 and group 4 was seen increased as compared to diabetic group. Hyperphagia symptom was seen in group 2, group 3 and group 4 after induction of diabetes, upon treatment reduction in food intake was seen in group 3 and group 4. Polydipsia was prominently seen in diabetic group, including group 3 and group 4. Upon treatment decrease in water intake was observed in group 3 as well as in group 4. We found a significantly higher TSH and lower T_4 in diabetic rats in diseased group, compared with normal rats in control group ($P < 0.001$ for both). Our results are also consistent with other clinical studies, suggesting that serum T_4 was negatively associated and that TSH was positively associated with insulin resistance [17]. Synthesis of thyroid hormones is associated with a variety of factors and sodium iodine symporter (NIS) -mediated uptake of iodine; this is the first stage [16]. The iodine uptake and NIS expression were inhibited and this effect could be reversed by Dapagliflozin, which is the

AMPK inhibitor [11]. The studies were consistent with our results, suggesting that $SGLT_2$ inhibitors may directly affect the synthesis of thyroid hormone and then decrease the level of TSH. Hence significant ($P<0.001$) decrease in TSH levels was observed in group 3 and group 4 after two weeks of treatment with Dapagliflozin. Chronic persistent hyperglycemia contributes to higher rates LDH (lactate dehydrogenase) of myocardial enzymes. Increased amount of LDH suggests injury to the heart muscle. In the current investigation, the levels of LDH were significantly higher in diabetic rats compared to non-diabetic rats. Administration of Dapagliflozin demonstrated a substantial decrease in LDH rates relative to the diseased group.

CONCLUSION

Our study revealed that, SGLT2 inhibitors are a type of anti-hyperglycemic agents demonstrated to even impart major cardiovascular safety on T2DM patients mainly by decreasing LDH levels in body and thus exerting its therapeutic effects on heart attack, anemia, damage and injury decreased amounts of myocardial enzymes. The study has revealed that SGLT2 inhibitors acts as an APMK agonist, thereby imparting their effects on thyroid hormone levels.

Conflict of Interest: There is no conflict of interest.

REFERENCE

[1] Kumar S, Singh R, Vasudeva N, Sharma S. Acute and chronic animal models for the evaluation of anti-diabetic agents. Cardiovascular Diabetology 2012; 11(9):1-13.

[2] Forbes J. M, and Cooper M. E. Mechanisms of Diabetic Complications. Physiological reviews 2013; 93(1):137-188.

[3] Raval R., Mehta R., Sharma P.K.Correlation between Hypothyroidism and Diabetes – A Hospital based Study. International Journal of Contemporary Medical Research 2017; 4(1): 124-125.

[4] Xiaowen Hu, Yang Liu, Changmei Wang, Lulu Hou, XiaoyanZheng, YeqiuXu, Lin Ding and Shuguang Pang. Metformin affects thyroid function in male rats. Oncotarget 2017, 8(64):107589-10759

[5] Crane R.K. Intestinal absorption of sugars. Physiol Rev 1960; 40: 789–825.

[6] Zelniker T.A, Braunwald E. Cardiac and Renal Effects of Sodium-Glucose Co-Transporter 2 Inhibitors in Diabetes. Journal of the American college of Cardiology 2018, 72(15):1847-1855.

[7] Sato T, Aizawa Y, Yuasa S, Yuasa S, Kishi S, Fuse K, et al. The effect of dapagliflozin treatment on epicardial adipose tissue volume. Cardiovascular Diabetology 2018; 17(1):6.

[8] Yoshimoto T, Furuki T, Kobori H, et al. Effects of sodium-glucose cotransporter 2 inhibitors on urinary excretion of intact and total angiotensinogen in patients with type 2 diabetes. J Investig Med 2017; 65:1057–61.

[9] Shin S.J, Chung S, Kim S.J, Lee E.M, Yoo Y.H, Kim J.W, et al. Effect of sodium-glucose co-transporter 2 inhibitor, dapagliflozin, on renal renin-angiotensin system in an animal model of type 2 diabetes. PLoS One 2016; 11(11):e165703

[10] Peti-Peterdi J, Harris R.C. Macula densa sensing and signaling mechanisms of renin release. Journal of the American Society of Nephrol 2010; 21(7):1093–1096.

[11] Vito P.D, Candelotti E, Ahmed R.G, Luly P, Davis P. J, Incerpi S, Pedersen J.Z. Role of Thyroid Hormones in Insulin Resistance and Diabetes. Immunology, Endocrine & Metabolic Agents in Medicinal Chemistry 2015; 15(1):86-93.

[12] Raval R, Mehta R, Sharma P.K. Correlation between Hypothyroidism and Diabetes – A Hospital based Study. International Journal of Contemporary Medical Research 2015; 4 (1):77-83.

[13] Kamble H.V, Bodhankar S.L. Cardioprotective effect of concomitant administration of trigonelline and sitagliptin on cardiac biomarkers, lipid levels, electrocardiographic and heamodynamic modulation on cardiomyopathy in diabetic Wistar rats. Biomedicine & Aging Pathology 2014; 4:335–342.

[14] Are P.C, Raghu, Adidal R, R, Puchchakayala G. Hypoglycemic and antidiabeticactivity of GlochidionVelutinum on streptozotocin-nicotinamide induced type2 diabetic rats. European Journal of Biological Sciences 2011; 3(4):126–30.

[15] Pimple B.P, Kadam P.V, Patil M.J. Ulcer healing properties of different extracts of Origanummajorana in streptozotocin-nicotinamide induced diabetic rats. Asian Pacific Journal of Tropical Disease 2012; 2(4):312-318.
[16] Tabatabai N.M, Sharma M, Petering D.H. Enhanced Expressions of Sodium-glucose Cotransporters in the Kidneys of Diabetic Zucker Rats. Diabetes research and clinical practice 2009; 83(1):1-3.
[17] Mehran L, Amouzegar A, Tohidi M, Moayedi M, Azizi F. Serum free thyroxine concentration is associated with metabolic syndrome in euthyroid subjects. Thyroid. 2014; 24:1566–74.
[18] Jia X, Mehta P.B, Ye Y, Alam M, Birnbaum Y, bajaj M. SGLT2 Inhibitors and Cardiovascular Outcomes: Current Perpectives and Future Potentials. Current Diabetes Reports 2018; 18(9): 11892-018.
[19] Goodman M, Liu Z, Zhu P, Li J. AMPK Activators as a Drug for Diabetes, Cancer and Cardiovascular Disease. Pharmaceutical Regulatory Affairs 2014; 3(2):1-6.
[20] Baxter JD, Dillmann WH, West BL, et al. Selective modulation of thyroid hormone receptor action. Journal of Steroid Biochemistry and Molecular Biology. 2001;76(1–5):31–42.