ZINC POTENTIATES THE ANTIOXIDANT EFFECT OF DAPAGLIFLOZIN IN RATS WITH EXPERIMENTAL-INDUCED DIABETES

Irina Claudia Anton [1,2], Liliana Mititelu-Tartau[2*], Radu Iliescu[2], Ionela Lăcrămioara Șerban[2], Monica Hăncianu[3], Cornelia Geanina Mircea[3]
"Grigore T. Popa" University of Medicine and Pharmacy Iasi, Romania
1. Ph.D. Student
Faculty of Medicine
2. Department of Morpho-functional Sciences (II)
Faculty of Pharmacy
3. Department of Pharmaceutical Sciences (II)
[*]Corresponding author. E-mail: lylytartau@yahoo.com

ZINC POTENTIATES THE ANTIOXIDANT EFFECT OF DAPAGLIFLOZIN IN RATS WITH EXPERIMENTAL-INDUCED DIABETES (Abstract). Diabetes mellitus (DM) is a multifaceted disorder that disrupts the body's overall metabolic equilibrium, giving rise to various organ-related complications such as cardiovascular, neuronal, and renal problems. In this research, we **aimed** to investigate the effects of zinc chloride (Zn), dapagliflozin (DAPA) and their combination on some biochemical parameters evaluating the liver function, lipid metabolism and cellular oxidative processes in rats with streptozotocin-induced diabetes. **Material and methods:** The rats were distributed into five groups (of five animals each): Control: citrate buffer; STZ and fat diet; STZ+Zn and fat diet; STZ+DAPA and fat diet; STZ+DAPA+Zn and fat diet. DM was induced in rats by intraperitoneal administration of a single daily dose of streptozotocin (STZ) of 20 mg/ kg body weight (kbw), for three consecutive days, and fat diet (10 g cholesterol/100 g diet) for 4 weeks. Citrate buffer (0.1 mL/100 g body) was intraperitoneal administered to the control rats. DAPA 1 mg/kbw, and Zn 5 mg/kbw were administered orally as a single daily dose for 4 weeks. **Results:** The treatment with Zn, DAPA and their combination resulted in weight loss and a decrease in blood glucose level, the higher effect being evidenced in STZ+Dapa+Zn group. The use of Zn, DAPA and Zn+DAPA in STZ-induced DM in rats with fat diet led to a decrease in blood values of transaminases, total cholesterol, triglycerides and in malondialdehyde activity, most substantially in the group treated with DAPA and Zn association. **Conclusions:** These outcomes suggest that the supplementation with Zn of DAPA treatment, enhanced the DAPA effect on decreasing the glycemia, restored the liver function, improved the lipid metabolism, and reduced the oxidative stress in STZ-induced DM in rats. **Keywords:** ZINC, STREPTOZOTOCIN, DIABETES, RATS, ANTIOXIDANT.

Type 2 DM is a chronic and widespread disease that arises from a combination of genetics and environmental factors, including sedentary lifestyle and obesity. The disease and its complications pose a significant public health challenge globally affecting both developed and developing countries with high rates of morbidity and mortality.

Unfortunately, the prevalence of type 2 DM is rapidly increasing, particularly in populations undergoing modernization. The disease often remains undiagnosed until serious complications emerge, and many risk factors are associated with it. The existing therapies are often ineffective and expensive, highlighting the need for new, efficient prevention and treatment strategies.

The selective glucose-sodium cotransporter 2 (SGLT2) inhibitors (empagliflozin, dapa-gliflozin, canagliflozin) belong to a new class of drugs approved for use as antihyperglycemic therapy (1). They reduce plasma concentrations of glucose through the renal inhibition of its reabsorption, inducing glucosuria (2). Their actions include: the decreasing glycated hemoglobin, the lowering postprandial blood glucose, reducing body weight and blood pressure (3-5).

In healthy individuals, 90% of the filtered glucose is reabsorbed by the glucose-sodium cotransporter 2 (SGLT-2) in the renal proximal convoluted tubule, and the remaining 10% is reabsorbed by the SGLT-1 transporter with a high affinity, in the right segment of the proximal descending tube (6, 7). In nondiabetic subjects, no glucose appears in the urine until the plasma glucose concentration exceeds 180-200 mg/dl, at which point all excess filtered glucose is eliminated. In people with DM, the threshold at which glucose appears in the urine, as well as the maximal renal tubular reabsorption capacity, are significantly increased, thus contributing to the maintenance of hyperglycemia (8, 9).

SGLT2 is expressed in the proximal tubule and mediates the reabsorption of approximately 90% of the filtered glucose. SGLT2 inhibitors promote renal glucose excretion and therefore modestly reduce high blood glucose levels in patients with type 2 diabetes (10). The ability to lower blood glucose and glycated hemoglobin levels is limited by the amount of filtered glucose and the osmotic diuresis caused by this medication. Although the SGLT2 inhibitors currently on the mark*et al*most completely block tubular glucose reabsorption, the measured inhibition is less than 50%, estimated based on urinary glucose excretion (11,12).

Zinc is a trace element with an essential role for growth, immunity, has antioxidant properties, and participates in the storage and processing of insulin in the body (13). Data from the literature show that the administration of zinc supplements in patients with type 2 DM improves the symptoms of the disease, as a result of the decrease in the level of cholesterol and glycated hemoglobin in the blood (14-16). Normally, zinc is concentrated in the pancreatic beta cells, at the level of the dense nucleus of the insulin-secreting granules, being essential for the formation of insulin crystals, its release, transport, storage and processing in the body (17,18).

The **purpose** of our study was the experimental research on the effects of DAPA and its association with Zn in rats with experimental-induced DM.

MATERIAL AND METHOD

Substances

STZ (molecular weight of 265.221 g/mol, catalogue code: S0130), buffer citrate (0.1 M, pH 4.5, catalogue code: P4809), DAPA (≥ 98% HPLC, molecular weight of 408.87 g/mol, catalogue code: SML2804) and Zn (molecular weight of 136.30 g/mol, catalogue code: 429430) were procured from Sigma-Aldrich, Chemical Co (Steinheim, Germany).

Animals

Healthy, non-genetically engineered white Wistar rats, weight between 150 and 200 g weight) were purchased from the National Medical-Military Institute for Research and Development, Băneasa, Bucharest, Romania through the Biobase of "Grigore T. Popa" University and Medicine and Pharmacy from Iasi and CEMEX (Advanced Research and Development Center for Experimental Medicine), Iasi, Romania.

The rats were housed in individually plexiglass cages and acclimatized for 7 days, in constant laboratory environment with a controlled temperature of 21±2°C, a relative humidity of 50-70% and 12-hours light/12-hours darkness alternative cycle. Standard granulated food and tap water were *ad libitum* offered, the daily intake was measured and the animal`s behavior was carefully monitored. All animals were weighted after one and four weeks in the experiment.

The protocol of the experiment

Rats were randomly assigned into five groups (5 animal per group), as follows: Control: citrate buffer; STZ and fat diet; STZ+Zn and fat diet; STZ+DAPA and fat diet; STZ+DAPA+Zn and fat diet. DM was induced in rats, by intraperitoneal administration of a single daily dose of STZ of 20 mg/ kg body weight (kbw), for three consecutive days, and fat diet (10 g cholesterol/100 g diet) for four weeks. After dissolving in citrate buffer (25 µg), STZ was immediately injected. Citrate buffer (0.1 mL/100 g body) was intraperitoneal administered to the control rats. DAPA 1 mg/kbw and Zn 5 mg/kbw were administered orally as a single daily dose, for four weeks.

Few drops of blood collected from caudal vein were used to measure glycemia in twelve hours fasting rats, using an Accu-Chek Go digital glucometer (Roche, Warsaw, Poland). The animals with levels higher than 200 mg/dL were recognized as having diabetes and included in the investigation.

Laboratory investigations

Before inducing diabetes (moment zero - baseline), a week and four weeks after, 0.3 mL blood were collected for biochemical analysis to assess the: ALT (alanine-aminotransferase), AST (aspartate-aminotransferase), cholesterol, HDL (high density lipoprotein), triglycerides using the VITROS 750 XRC Analyzer (Alphasoft, Bochum, Germany) with specific Johnson & Johnson (Johnson, New Haven, USA) reactors.

The influence of substance tested on the oxidative processes was investigated by evaluation of SOD (superoxide dismutase) and MDA (malondialdehyde) activity. The estimation of SOD activity was carried out by spectrophotometric analysis, using specific kits from the RANSOD kit (catalogue code19160-1KT-F) of RANDOX Laboratories Ltd. (Crumlin, UK). The analysis of SOD activity is based on the inhibition of the reduction of nitroblue tetrazolium, with xanthine-xanthine oxidase, used as a superoxide generator (19). The measurement of MDA blood values was performed by chromatography, using the thiobarbituric acid test as a marker of lipid peroxidation and free radical generation (20). For the HPLC (high performance liquid chromatography) method, a particular kit (catalogue code ABIN772058) bought from Redox, Bucharest, Romania was used.

Ethical aspects of the research

The research methodology was approved by our University Committee for Research and Ethical Issues (Certificate No. 30/14.01.2021) and was carried out in accordance with international standards (21)

about the handling of laboratory animals.

Statistical analysis

The mean ± standard deviation (S.D.) of average values was calculated for each group and the statistical differences among the groups were assessed using SPSS software version 17.0 for Windows and EXCEL application (IBM, New York, United States). The probability (*p*) values below 0.05 were estimated to be significant versus control group.

RESULTS

At the beginning of the experiment, no significant variations in the animals body weight between STZ, STX+Zn, STZ+DAPA, STZ+DAPA+Zn groups, and the group treated with buffer citrate were observed (fig. 1). The administration of STZ and cholesterol was associated with a continuous increase in the rats' body weight over time, which became statistically relevant versus control group (*p<0.05), but also compared to the beginning of the experiment (♦p<0.05), after one month (fig. 1). The use of Zn, DAPA and DAPA+Zn in rats with STZ and fat diet, lowered the body weight; but no significant differences were revealed between groups, as well as compared to buffer citrate group, and to baseline, at both moments of the evaluation (fig. 1).

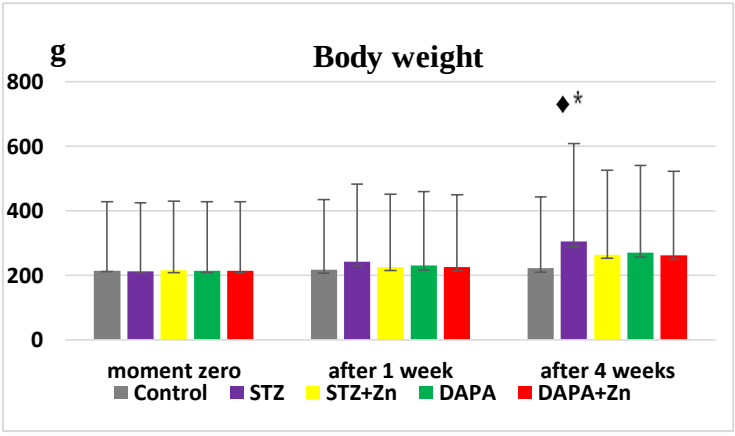

Fig. 1. The influence of DAPA, Zn and their association on the body weight in rats with STZ-induced diabetes. *p<0.05 compared to control; ♦p<0.05 compared to moment zero.

Before the inducing of DM, no modifications in glycemia values between the groups studied were observed (fig. 2). The rats with DM after the administration of STZ injection and fat diet, exhibited a progressive increase in serum sugar levels, with a statistical relevance significant compared to control group, after 7 days (**p<0.01), respectively after 4 weeks (**p<0.01). At both moments of the observation the blood glucose values were considerably higher (♦♦p<0.01) versus baseline (fig. 2).

In the diabetic rats the treatment with Zn, DAPA, or their association, prevent the increase in glycemia, which were still slightly elevated, but without relevance compared to buffer citrate group, after one week. Thereafter, the blood glucose levels were progressive diminished becoming insignificant compared to the control group after one month in the experiment (fig. 2).

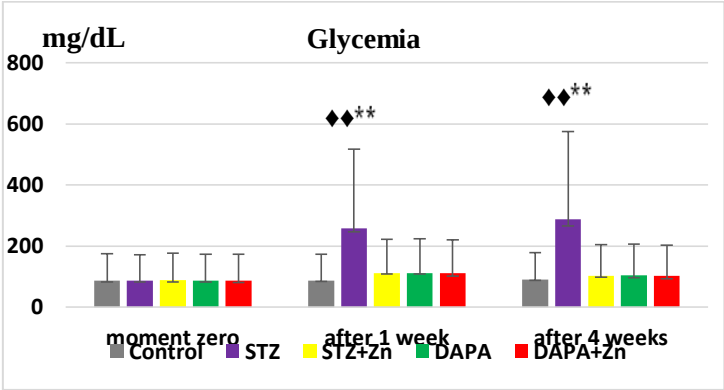

Fig. 2. The effects of DAPA, Zn and their association on the blood glucose levels in rats with STZ-induced diabetes. **p<0.01 compared to control; ♦♦p<0.01 compared to moment zero.

The induced DM with STZ on rats was accompanied by an increase of ALT and AST activity which became relevant after one month, compared to citrate buffer group (*p<0.05), as well as to the moment zero (♦p<0.05) in the experiment (tab. I). The use of Zn, DAPA, respectively DAPA+Zn in animals with STZ-induced diabetes resulted in a considerable decrease in the blood values of these two liver enzymes, their levels being comparable with those of control group at both moments of the investigation (tab. I).

The effects of the substances tested on the decreasing of ALT respectively of AST activity after 7 days, were in the descending comparison as following: STZ+DAPA+Zn > STZ+Zn > STZ+DAPA. Similar ranking was revealed for the blood levels of the transaminases after 4 weeks in the experiment (tab. I).

There were evidenced no important dissimilarities in the blood values of total cholesterol, HDL and triglycerides in the groups studied at moment zero (tab. II). In the STZ group a significant increase in serum total cholesterol levels, after one week, as well after four weeks of monitoring, statistically significant compared to control group (*p<0.05), respectively to the moment zero (♦p<0.05) (tab. II). At the same time an important increasing in triglyceride and a reduction in HDL values were noted after one month in the experiment, statistically relevant versus buffer citrate group, as well as versus the baseline (tab. II).

The treatment with Zn, DAPA and DAPA+Zn was accompanied by a diminution of the cholesterol and triglycerides and increasing of the HDL blood values during the experiment. At both moments of the determination, the intensity of the effects produced by the substances investigated on these parameters of the lipid metabolism, were in descending ordering as following: STZ+ DAPA+Zn > STZ+Zn > STZ+DAPA (tab. II).

No evident variations in the SOD and MDA blood values were shown between STZ, STZ+Zn, STZ+DAPA, STZ+DAPA+Zn and buffer citrate group at the beginning of the experiment (tab. III). In rats with STZ-induced diabetes, was revealed a continuous reduction of SOD, and increase of MD activity, with a statistical significance after four weeks, compared to the control group (*p<0.05), respectively to baseline (♦p<0.05) (tab. III). The treatment with Zn, DAPA and DAPA+Zn alleviated the disturbances of these two enzymes activity

during the interval of monitoring. At both time moments of the evaluation their serum levels were similar to control group, respectively to baseline (tab. III).

TABLE I.

The influence of DAPA, Zn and their association on the ALT and AST activity in rats with STZ-induced diabetes.

***p<0.05 compared to control; ♦p<0.05 compared to moment zero**

	Moment of evaluation	ALT (U/L)	AST (U/L)
Control	moment zero	40.54±4.29	91.64±7.27
	after 1 week	42.72±5.55	90.87±7.43
	after 4 weeks	41.36±4.17	91.25±6.67
STZ	moment zero	40.63±4.83	90.45±6.33
	after 1 week	48.24±4.45	113.39±7.45
	after 4 weeks	**69.33±5.22*♦**	**124.63±7.18*♦**
STZ+Zn	moment zero	41.48±4.67	91.82±6.83
	after 1 week	48.51±5.13	100.36±6.33
	after 4 weeks	45.75±5.05	99.48±6.29
STZ+DAPA	moment zero	41.28±3.83	91.52±5.37
	after 1 week	51.17±4.29	101.36±6.45
	after 4 weeks	48.21±5.67	100.65±6.33
STZ+DAPA+Zn	moment zero	40.92±4.41	91.68±5.29
	after 1 week	46.46±3.83	97.36±5.41
	after 4 weeks	44.21±3.55	95.45±7.17

TABLE II.

The influence of DAPA, Zn and their association on the blood values of cholesterol, HDL-cholesterol and triglycerides in rats with STZ-induced diabetes.

***p<0.05 compared to control; ♦p<0.05 compared to baseline**

	Moment of evaluation	Cholesterol (mg/dL)	HDL (mg/dL)	Triglycerides (mg/dL)
Control	moment zero	65.48±5.67	45.44±2.43	51.82±4.25
	after 1 week	64.33±5.21	46.62±2.17	52.25±4.67
	after 4 weeks	67.26±6.30	45.73±1.55	52.16±5.43
STZ	moment zero	64.82±4.13	45.35±2.21	52.44±5.21
	after 1 week	**78.63±6.45*♦**	35.36±2.67	60.59±5.33
	after 4 weeks	**80.54±6.33*♦**	**34.28±2.05*♦**	**63.78±6.17*♦**
STZ+Zn	moment zero	66.42±4.55	46.14±2.33	51.34±4.83
	after 1 week	74.37±5.05	36.65±1.29	56.26±4.79
	after 4 weeks	75.76±5.83	37.30±1.43	54.26±5.55
STZ+DAPA	moment zero	66.56±5.17	46.52±2.17	51.67±4.37
	after 1 week	76.45±5.33	36.54±2.35	57.48±5.43
	after 4 weeks	75.86±6.21	36.78±2.21	55.42±5.67
STZ+DAPA+Zn	moment zero	65.78±4.55	46.22±1.41	51.24±5.21
	after 1 week	71.43±5.05	41.64±2.55	54.67±5.33
	after 4 weeks	70.36±5.83	43.13±1.67	53.83±4.45

TABLE III.

The influence of DAPA, Zn and their association on the activity of SOD and MDA in rats with STZ-induced diabetes.

***p<0.05 compared to control; ♦p<0.05 compared to moment zero**

	Moment of evaluation	SOD (U/mg protein)	MDA (nmol/mg protein)
Control	moment zero	104.48±6.43	22.54±1.35
	after 1 week	105.63±6.55	23.27±2.11
	after 4 weeks	106.39±7.27	22.65±1.67
STZ	moment zero	105.22±6.43	22.83±1.43
	after 1 week	98.33±4.51	29.48±2.13
	after 4 weeks	**79.85±5.13*♦**	**32.67±1.25*♦**
STZ+Zn	moment zero	105.34±5.83	21.25±1.33
	after 1 week	103.62±6.67	26.71±2.21
	after 4 weeks	104.29±6.21	25.44±1.17
STZ+DAPA	moment zero	104.88±6.41	22.36±1.41
	after 1 week	100.32±6.45	27.34±1.55
	after 4 weeks	101.48±6.37	26.82±1.41
STZ+DAPA+Zn	moment zero	104.64±6.55	21.74±1.52
	after 1 week	104.13±7.21	25.22±1.13
	after 4 weeks	104.29±6.21	23.46±2.17

The effects on the specific markers of oxidative reactions varied in intensity following the exposure to the studied substances, and were observed both at one and four weeks, being in descending classification as following: STZ+DAPA+Zn > STZ+Zn > STZ+DAPA (tab. III).

DISCUSSION

Literature data revealed that SGLT2 inhibitors readily reduce blood pressure and body weight, particularly due to their glycosuric effect. Currently, empagliflozin and canagliflozin have also been shown to improve cardiovascular status in high-risk patients and slow the progression of kidney disease in diabetics (22, 23). Regarding their clinical efficacy, it is important to emphasize the obvious metabolic benefit of SGLT2 inhibitors, when used in place of other antihyperglycemic agents, such as sulfonylureas, or to reduce the daily dose of insulin (24, 25). Very important are their osmotic diuretic and natriuretic effects, which contribute to the reduction of plasma volume and the decrease of systolic and diastolic arterial pressure, which explains the cardiovascular and renal benefits (11, 26). SGLT2 inhibition is also associated with a rapid, dose-dependent decrease in glomerular filtration rate and reduction in albuminuria (27, 28).

It is known that in patients with diabetes, the zinc content of the pancreas is very low, the serum zinc levels are low, as a result of its loss due to excessive urination. Low levels of zinc in the blood plasma prevent the islets of Langerhans from secreting and producing insulin (29). Also, this divalent cation plays an important role in the antioxidant defense in patients with type 2 diabetes, in particular by acting as a cofactor of

superoxide dismutase, by modulating glutathione metabolism and metallothionein expression, by competing with iron and copper, at the cell membrane level and by inhibiting nicotinamide adenine dinucleotide phosphate oxidase. Zinc alleviates oxidative stress in these patients, by reducing chronic hyperglycemia, with the promotion of insulin receptor phosphorylation, as a result of increased glucose transport in cells (30).

Recent outcome trail data indicate that DAPA not only improves hyperglycemia but also slows the progression of DM-associated glomerulosclerosis and liver fibrosis by improving hyperglycemia-induced tissue inflammation and oxidative stress by reducing levels of ROS, MDA and increased antioxidant enzymes, including SOD and glutathione peroxidase (31,32). Other studies highlight that diabetic rat subjected to treatment with Zn experienced weight loss and a reduction in blood sugar levels. The addition of Zn to their diet resulted in a significant decrease in oxidative stress, preserved the pancreatic architecture, and restored both liver and kidney function and structure in rats with STZ-induced diabetes (33, 34).

In our research we evidenced an increase in body weight occurred in all groups in the study throughout the duration of the experiment, both the one measured at seven days and the one measured at four weeks. It was found that in the control group, the weight increase was the lowest, the highest increase being registered in the STZ and fat diet group. Although an increase in body weight versus baseline was noticed in all the other groups, this was the lowest in the STZ+Dapa+Zn group, probably due to the synergistic action of DAPA and Zn. Similar observation could be done about the modification in blood glucose values.

In rats with STZ and fat diet-induced DM, an increase in ALT and AST enzymes activity, in total cholesterol and triglyceride levels and a decrease in HLD-cholesterol blood values were observed in all study groups compared to the control group and baseline. Also, the weight gain caused by the administration of STZ, and high-fat diet was recorded in all study groups, but the most significant decrease was observed in the group treated with DAPA+Zn, probably due to the synergistic action of the two substances.

The treatment with Zn, DAPA and their combination resulted in a significant decrease in blood sugar levels in diabetic rats, in all groups studied. The administration of Zn, DAPA, respectively DAPA+Zn in animals with STZ-induced DM led to a significant improvement in the blood values of liver enzymes ALT and AST, total cholesterol, triglycerides and HDL-cholesterol, most substantially in the DAPA+Zn group.

CONCLUSIONS

In our experimental conditions, we demonstrated that supplementation with Zn of DAPA treatment enhances the DAPA effect, reduced oxidative stress, restored the liver function and improve lipids levels in STZ-induced DM in rats.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

This work is part of Irina Claudia Anton's Ph.D. studies and was co-funded from the European Social Fund - the Human Capital Operational Program, Project / Grant No: POCU/993/6/13/154722.

REFERENCES

1. Batra S, Bamrah PK, Choudhary M. Sodium-glucose transporter (SGLT2) inhibition: a potential target for treatment of type-2 diabetes mellitus with natural and synthetic compounds. Egypt J Basic Appl Sci 2023; 10(1): 69-82.
2. Mascolo A, Di Napoli R, Balzano N *et al*. Safety profile of sodium glucose co-transporter 2 (SGLT2) inhibitors: A brief summary. *Front Cardiovasc Med* 2022; 9: 1010693.
3. Zelniker TA, Wiviott SD, Raz I, *et al*. SGLT2 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes in type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. *Lancet* 2019; 393(10166): 31-39.
4. Minze MG, Will KJ, Terrell BT, Black RL, Irons BK. Benefits of SGLT2 inhibitors beyond glycemic control - a focus on metabolic, cardiovascular and renal outcomes. *Curr Diabetes Rev* 2018; 14(6): 509-517.
5. Shin H, Schneeweiss S, Glynn RJ, Patorno E. Evolving channeling in prescribing SGLT-2 inhibitors as first-line treatment for type 2 diabetes. *Pharmacoepidemiol Drug Saf* 2022; 31(5): 566-576.
6. Srinivas N, Sarnaik MK, Modi S, *et al*. Sodium-glucose cotransporter 2 (SGLT-2) inhibitors: delving into the potential benefits of cardiorenal protection beyond the treatment of type-2 diabetes mellitus. Cureus 2021;13(8):e16868.
7. Suga T, Kikuchi O, Kobayashi M, *et al*. SGLT1 in pancreatic α cells regulates glucagon secretion in mice, possibly explaining the distinct effects of SGLT2 inhibitors on plasma glucagon levels. *Mol Metab* 2019; 19: 1-12.
8. Duan S, Lu F, Song D, Zhang C, Zhang B, Xing C, Yuan Y. Current challenges and future perspectives of renal tubular dysfunction in diabetic kidney disease. *Front Endocrinol (Lausanne*) 2021; 12: 661185.
9. Granata A, Pesce F, Iacoviello M, *et al*. SGLT2 inhibitors: a broad impact therapeutic option for the nephrologist. *Front Nephrol* 2022; 2: 867075.
10. Vallon V, Nakagawa T. Renal tubular handling of glucose and fructose in health and disease. *Compr Physiol* 2021; 12(1): 2995-3044.
11. Zelniker TA, Wiviott SD, Raz I, *et al*. SGLT2 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes in type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. *Lancet* 2019; 393: 31.
12. Tuttle KR. Digging deep into cells to find mechanisms of kidney protection by SGLT2 inhibitors. *J Clin Invest 2023*; 133(5): e167700.
13. Jarosz M, Olbert M, Wyszogrodzka G, Młyniec K, Librowski T. Antioxidant and anti-inflammatory effects of zinc: zinc-dependent NF-κB signaling. *Inflammopharmacology* 2017; 25(1): 11–24.
14. Pompano LM, Boy E. Effects of dose and duration of zinc interventions on risk factors for type 2 diabetes and cardiovascular disease: a systematic review and meta-analysis. *Adv Nutr* 2021; 12(3): 1049.
15. Grădinaru D, Margină D, Ungurianu A, *et al*. Zinc status insulin resistance and glycoxidative stress in elderly subjects with type 2 diabetes mellitus. *Exp Ther Med* 2021; 22(6): 1393.
16. Choi S, Liu X, Pan Z. Zinc deficiency and cellular oxidative stress: prognostic implications in cardiovascular diseases. *Acta Pharmacol Sin* 2018; 39: 1120-1132.
17. Norouzi S, Adulcikas J, Sohal SS, Myers S. Zinc transporters and insulin resistance: therapeutic implications for type 2 diabetes and metabolic disease. J Biomed Sci 2017; 24: 87.
18. Fatehi M. Impact of zinc on insulin resistance and hepatic fat accumulation. *Preprints* 2023; 2023 010012.

19. Carmo de Carvalho e Martins M, da Silva Santos Oliveira AS, da Silva LAA, Primo MGS, de Carvalho Lira VB. Biological indicators of oxidative stress [malondialdehyde, catalase, glutathione peroxidase, and superoxide dismutase] and their application in nutrition. In: Patel VB, Preedy VR. (eds) *Biomarkers in nutrition. Biomarkers in disease: methods, discoveries and applications*. Springer, Cham. 2022.
20. Senthilkumar M, Amaresan N, Sankaranarayanan A. Estimation of malondialdehyde (MDA) by thiobarbituric acid (TBA) assay. In: *Plant-microbe interactions*. Springer Protocols Handbooks. Humana, New York, 2021.
21. Directive 2010/73/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32010L0073.
22. Brawn P. Diabetes Distilled: More evidence that SGLT2 inhibitors slow CKD progression. Are we optimizing care? *Diabetes & Primary Care* 2023; 25: 19-20.
23. Liu AYL, Low S, Yeoh E, *et al*. A real-world study on SGLT2 inhibitors and diabetic kidney disease progression. *Clin Kidney J* 2022; 15(7): 1403-1414.
24. Lam D, Shaikh A. Real-life prescribing of SGLT2 inhibitors: How to handle the other medications, including glucose-lowering drugs and diuretics. *Kidney360* 2021; 2(4): 742-746.
25. George J, Lobkovich A, Nardolillo J, Farhat N, Kolander S, Thomas E. Real-world evaluation of insulin requirements after GLP1 agonist or SGLT2 inhibitor initiation and titration. *Am J Health Syst* Pharm 2022; 79(14): 1151-1157.
26. McGuire DK, Shih WJ, Cosentino F, *et al*. Association of SGLT2 inhibitors with cardio-vascular and kidney outcomes in patients with type 2 diabetes: a meta-analysis. *JAMA Cardiol* 2021; 6(2): 148-158.
27. Xu L, Li Y, Lang J, *et al*. Effects of sodium-glucose co-transporter 2 (SGLT2) inhibition on renal function and albuminuria in patients with type 2 diabetes: a systematic review and meta-analysis. *PeerJ* 2017; 5: e3405.
28. Heerspink HJL, Kosiborod M, Inzucchi SE, *et al*. Reno protective effects of sodium-glucose cotransporter-2 inhibitors. *Kidney Int* 2018, 94(1): 26-39.
29. Khanam S. Role of zinc supplementation on diabetes. *Reports in Endocrine Disorders: Open Access* 2018, 2(1): 2.
30. Olechnowicz J, Tinkov A, Skalny A, *et al*. Zinc status is associated with inflammation, oxidative stress, lipid, and glucose metabolism. *J Physiol Sci* 2018; 68: 19-31.
31. Oraby MA, El-Yamany MF, Safar MM, Assaf N, Ghoneim HA, Dapagliflozin attenuates early markers of diabetic nephropathy in fructose-streptozotocin-induced diabetes in rats. *Biomed Pharmacother* 2019; 109: 910-920
32. Yang H, Mei Z, Chen W, *et al*. Therapeutic efficacy of dapagliflozin on diabetic kidney disease in rats. *Int Immunopharmacol* 2022; 113(Part A): 109272.
33. Anton IC, Mititelu-Tartau L, Popa EG *et al*. Zinc chloride enhances the antioxidant status, improving the functional and structural organic disturbances in streptozotocin-induced diabetes in rats. *Medicina* 2022; 58(11): 1620.
34. Martins MDPSC, Oliveira ASDSS, Martins MDCCE, *et al*. Effects of zinc supplementation on glycemic control and oxidative stress in experimental diabetes: A systematic review. *Clin Nutr ESPEN* 2022; 51: 28-36.