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Assessment of SGLT2 Inhibitor, Canagliflozin Effects on Metabolic Syndrome-Induced Cardiovascular Disorders in Rats

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Abstract

Introduction: The metabolic syndrome (MS) is a series of risk factors (glucose sensitivity, dyslipidemia, obesity, hypertension and inflammation) that can lead to type 2 diabetes (T2DM), atherosclerotic cardiovascular disease and stroke. Lipid metabolism disorders are linked to a large degree to the development of MS disorders. The protein proprotein convertase subtilisin/kexin type 9 (PCSK9) is responsible for elevating LDL levels, so PCSK9 has been suggested to induce endothelial cell apoptosis and atherosclerosis progression. Canagliflozin is a relatively recent antihyperglycemic agent that has been shown to improve both blood pressure and hemoglobin A1c in patients with T2DM.

Aim of the study: To demonstrate the protective effect of canagliflozin on cardiovascular affection in fructose-induced MS mainly through amelioration of blood pressure, glycemic indices, lipid parameters, and cardiac PCSK9 concentration.

Materials and Methods: Twenty-four male albino rats were categorized into four groups: Group 1: control; Group 2: taking canagliflozin (10 mg/kg/daily orally); Group 3: taking Fructose (25% fructose in drinking water); and Group 4: taking fructose plus canagliflozin. After seven weeks, systolic blood pressure (SBP) and HR were measured. Serum glucose, insulin, triglycerides (TG), LDL levels, and cardiac tissue concentrations of PCSK9 were estimated. Finally, a histopathological cardiac examination was performed.

Results: Canagliflozin caused significant improvement in SBP, HR, serum glucose, TG, and LDL levels, as well as PCSK9 cardiac levels, in rats that consumed fructose as compared to untreated rats. Therefore, there was an improvement in cardiac tissue degenerative changes and fibrosis with canagliflozin in fructose-fed rats.

Conclusions: Canagliflozin has a cardiac-protective effect in fructose-induced MS.

Keywords: Fructose; Canagliflozin; PCSK9; Cardiac fibrosis.

1. Introduction

Metabolic syndrome (MS) is defined as a group of interconnected risk factors for cardiovascular disease and type 2 diabetes that occur simultaneously in a person's body. Central obesity, hypertension, high triglycerides, low HDL cholesterol, and glucose intolerance are the

main components of MS. The presence of at least three of the previous risk factors is approved to diagnose MS, in spite of the presence of many different criteria for MS [1].

Fructose is a monosaccharide molecule that is formed during glucose metabolism. Excessive fructose consumption triggers MS abnormalities [2]. Fructose is known to stimulate metabolic changes that may contribute to risk factors linked with MS, including hyperuricemia, inflammation, and abnormalities in different metabolic hormones. Unlike glucose, fructose is quickly metabolized in the body and directly converted to fatty acids. It has been linked to lactic acidosis, obesity, insulin resistance (IR), diabetes, liver steatosis, and lipid metabolism disorders, as well as hypertension and cardiovascular diseases [3].

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a protein that aids in cholesterol homeostasis by enhancing hepatic low-density lipoprotein receptor (LDLR) degradation. PCSK9 is produced primarily in the liver. Besides the brain, gut, and pancreas, it is expressed in a wide variety of other tissues and cell types (e.g., macrophages), where it controls the expression and function of LDLR and LDL homeostasis [4]. PCSK9 has been linked to many glucose metabolism indices, including fasting plasma glucose (FPG), insulin, and the IR homeostasis model (HOMA-IR) [5].

Canagliflozin targets the sodium-glucose cotransporter (SGLT) 1 and 2 receptors. Ninety percent of the glucose filtered by the kidneys is reabsorbable through SGLT2, which is expressed in the proximal renal tubules. And since canagliflozin causes an increase in urinary glucose excretion, it therefore causes lower blood glucose levels. Evidence also suggests that in patients with T2DM, as compared to those without T2DM, SGLT-2 plays a larger role in glucose reabsorption. This makes SGLT2 an intriguing prospective therapeutic target. Canagliflozin improved glycemic control, body weight, and blood pressure across phase 3 studies, and it was well tolerated by a wide variety of individuals with T2DM [6].

One of the processes that causes diabetic cardiomyopathy in diabetic people is cardiac inflammation. There is some evidence that SGLT2 inhibitors, through a combination of local and systemic actions, can reduce heart inflammation. Canagliflozin offers cardiovascular benefits in addition to lowering blood glucose levels. They include enhancing hemodynamics, decreasing inflammation and oxidative stress, and boosting the heart's ability to generate its own energy [7].

2. Subjects and methods

2.1. Chemicals:

Fructose was provided in the form of powder. Rats were given fructose (10%) in drinking water [8] for 39 days. On the 21st day, 25% was used as a modified concentration for ten days [9]. The FDW was administered every day for seven weeks.

Canagliflozin was purchased in the form of tablets (Invocana; Janssen Co., Beerse, Belgium). Each tablet contains 100 mg of canagliflozin. Tablets were crushed and dissolved in distilled water before being given to the rats once daily at a dose of 10 mg/kg for seven weeks [10].

2.2. Experimental Animals and Design:

There were 24 Sprague-Dawley male albino adult rats used in this study. The average weights of the rats were 150±20 g. The rats were obtained from Cairo University's National Research Institute animal house. One week before the experiment, all rats were housed in a controlled environment with free access to food and water and an alternating cycle of 12 hours of light and 12 hours of darkness. Rats were housed in sanitary conditions with average humidity in wire cages.

In this study, rats were divided into four different groups, each containing six rats. Groups 1 and 2 were the control groups not given fructose, and groups 3, 4, and 5 were given FDW. These groups were as follows: Group 1: The rats received normal food and drink; Group 2: The rats were given oral canagliflozin; Group 3: The rats were given FDW; and Group 4: The rats were given FDW and oral canagliflozin.

2.3. Sample collection, preparation, section staining, and biochemical indexes:

Systolic blood pressure (SBP) and heart rate (HR) were measured after 7 weeks for all groups. Blood was then collected from the retro-orbital veins of fasting rats, where glucose, insulin, and lipid (TG and LDL) levels were measured after the serum was extracted. Immediately after the collection of blood, the Hearts of rats were removed and cleaned in ice-cold saline after the animals were euthanized via cervical dislocation. Later, each heart was sectioned into two parts: one part was preserved in formalin (10%) for histopathological examination, and the other part was preserved in a deep freezer at -20°C until PCSK9 concentrations were measured by using the enzyme-linked immunosorbent assay (ELISA) kits.

SBP and HR were measured by using the noninvasive blood pressure meter (NIBP) Model No. LE5001 by PANLAB Equipment (Panlab/Harvard Apparatus Co., Barcelona, Spain) in the faculty of medicine at Qasr Al Ainy, Cairo University, Egypt. After letting the sensitive blood pressure meter warm up for 10 minutes, the selector switch located at the back of the equipment was switched to the area marked for rats. The rats were put into the transparent glass restrainer. To help calm the animals, we covered them in bits of black clothing. Putting the tail-cuff/transducer at the tail region base, activating the selector switch, and looking at the results on the screen [11]

2.4. Statistical analysis:

- After re-evaluating the compiled information for accuracy and consistency, changes were made. The information was then entered into SPSS 16 (Social Science Statistical Software) for further statistical examination.
- Data were expressed using descriptive statistics (mean ± standard deviation for quantitative variables).
- The analysis of variance was used to compare the means of quantitative variables across groups, followed by a post hoc test for inter-group comparison.
- A *P value* < 0.05 was considered significant.

3. Results

Fructose-fed rats that were treated with canagliflozin (group 4) showed significant improvement in FSG compared to the untreated fructose-fed group (group 3) (*P< 0.05*). The levels of FSG in group 4 were 6.9 ± 0.7 mmol/l, compared to 13 ± 1.23 mmol/L in group 3. FSI for group 4 was 10.9 ± 1.41 µIU/ml compared to18.9 ± 2.08 µIU/ml for group 3. The fructose + canagliflozin group (group 4) had significantly higher FSG and FSI than the canagliflozin group

(group 2) (*P< 0.05*). The values of FSG for groups 4 and 2 were (6.9 ± 0.7 and 4.6 ± 0.3 mmol/L, while FSI levels were 10.9 ± 1.41 and 7.1 ± 0.86 µIU/ ml, respectively (**Table 1**).

The serum levels of TG and LDL in untreated fructose-fed (group 3) rats were 199.2±9.83, and 153.2±10.79 mg/dl, respectively. The treated group 4 showed a TG level of 149.2±17.82 mg/dl and LDL levels of 105.3±15.21 mg/dl. Therefore, as compared to group 3, the treated group 4 showed a significant decrease in TG and LDL levels. Moreover, group 4 had considerably higher levels of TG and LDL than group 2 (**Table 1**).

Table 1: Effect of canagliflozin on Serum Levels of Glucose, Insulin, TG and LDL in Fructose Induced MS in Male Albino Rats.

GROUPS	Serum glucose (mmol/L)	Serum insulin (µIU/ml)	TG (mg/dl)	LDL (mg/dl)
(1) Control	4.7 ± .29	7.3 ± .06	91.8 ± 13.66	60.8 ± 8.36
(2) Canagliflozin	4.6 ± .3#	7.1 ± .86 #	92.5 ± 9.98 #	48 ± 4.96 #
(3) Fructose	13 ± 1.23*	18.9 ± 2.08*	199.2 ± 9.83 *	153.2 ±10.79 *
(4) Fructose + canagliflozin	6.9 ± .7*#∞	10.9 ± 1.41*#∞	149.2 ±17.82 *#∞	105.3 ±15.21 *#∞

Data are represented mean ± SD (6 rat/ group)
* Comparison of groups to control group.
\# Comparison of groups to fructose group.
∞ Comparison of fructose + canagliflozin group to canagliflozin group

By measuring the SBP of rats as shown in **Table 2**, it was noticed that SBP increased significantly in fructose-fed groups 3 and 4 compared to control group 1. The SBP of control rats was 103.8 ± 8.52 mmHg, and that of fructose-fed groups was 256.3 ± 7.63 and 169.8 ± 5.42 mmHg, respectively. It was also noticed that treatment of fructose-fed rats with canagliflozin in group 4 led to a significant improvement in SBP when compared with untreated group 3. In contrast, the treated fructose group 4 showed a significant increase in SBP when compared to the non-fructose group 2. The SBP of group 4 was 169.8 ± 5.42 mmHg compared to group 2, which exhibited an SBP of 106.2 ± 8.04 mmHg. Meanwhile, Groups 1 and 2 that did not receive fructose showed no discernible alterations.

As regards HR, as shown in Table 2, the untreated fructose-fed group 3 showed a significant increase in HR compared to control group 1 (the HR of groups 1 and 3 were 231.7 ±16.11 and 317.5 ± 50.77 b/min, respectively), whereas in treated group 4 (which showed HR of 241.7± 9.83 b/min), HR decreased significantly from untreated group 3. On the other hand, as shown in **Table 2**, there were no significant changes in HR between groups 1 and 2, between groups 3 and 4, and finally between groups 2 and 4.

As regards PCSK9 levels in the cardiac tissues, it was observed that fructose-fed rats in groups 3 and 4 had significantly higher levels of PCSK9 than non-fructose-fed rats (for control rats, the PCSK9 was 233.07 ± 12.76 pg/mg pt). In

the treated group 4, PCSK9 decreased significantly from the untreated group 3 which exhibited 496.6±77.83 pg/mg ptn of PCSK9. Our results showed that PCSK9 levels were significantly higher in group 4 (PCSK9 was 311± 34.9 pg/mg ptn) compared with those of group 2, which exhibited 228.9± 10.51 pg/mg ptn. Finally, there were no discernible variations between Groups 1 and 2 of the four non-fructose groups (**Table 2**).

Table 2: Effect of canagliflozin on SBP, Heart Rate and PCSK 9 levels in Cardiac Tissues of Male Albino Rats with Fructose Induced MS.

GROUPS	Serum glucose (mmol/L)	Serum insulin (µIU/ml)	TG (mg/dl)
(1) Control	103.8 ± 8.52	231.7 ± 16.11	233.07± 12.76
(2) Canagliflozin	106.2 ± 8.04 #	223.3 ±33.27 #	228.9 ±10.51 #
(3) Fructose	256.3 ± 7.63 *	317.5 ± 50.77 *	496.6 ±77.83 *
(4) Fructose + canagliflozin	169.8 ± 5.42 *#∞	241.7 ± 9.83 #	311 ± 34.9 *#∞

Data are represented mean ± SD (6 rat/ group)
* Comparison of groups to control group.
\# Comparison of groups to fructose group.
∞ Comparison of fructose + canagliflozin group to canagliflozin group

In the present study, we examined histopathological changes in cardiac tissues using hematoxylin-eosin and Masson trichrome stains. There were differences between fructose-fed groups (groups 3 and 4) as shown in **Figures 1, 2,** and **3** and control groups (groups 1 and 2) as light microscopic examination revealed abnormal degenerative changes in fructose-fed groups, including congestion, inflammatory cell infiltration, and fibrosis.

The light microscopic examination of cardiac tissues revealed no abnormality in the cardiac muscles of the control group (group 1). Muscle fibers in the heart seemed cylindrical and branched, with oval nuclei and acidophilic cytoplasm in the center and very little connective tissue separating them. Cardiac tissues in groups taking canagliflozin (group 2) were comparable to the control group and showed no abnormal degenerative changes; in contrast, the fructose-fed groups (groups 3 and 4) showed abnormal degenerative changes, congestion, and tissue inflammatory infiltration of group 3, as shown in **Figure 1.** At the same time, there was an improvement in the sections of cardiac tissues in treated group 4 compared to the untreated fructose-fed group, as shown in **Figure 3A**.

Sections stained by Masson's trichrome were examined for fibrosis. The control groups (groups 1 and 2) showed normal cardiac muscle fibers separated by a minimal amount of connective tissue. Fructose-fed groups (**Figures 2A, 2B, and 3B**) showed fibrosis that was more severe in untreated group 3 and improved in treated group 4.

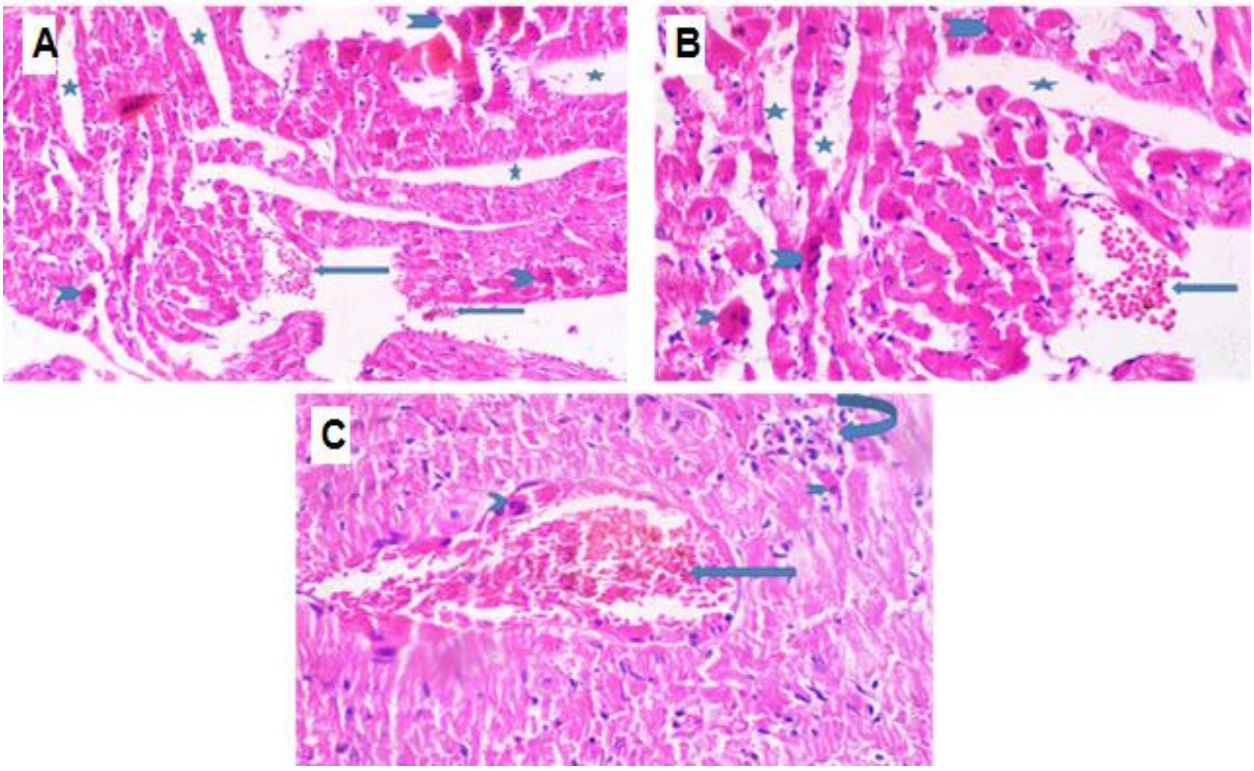

Figure 1: Sections in the rat's heart muscle of group 3 showing hemorrhage (arrows), degeneration (arrowheads), and the cardiac muscle fibers separated by an increased amount of connective tissue (stars) stained by H&E. A) x 200 B) x 400, C) x 200.

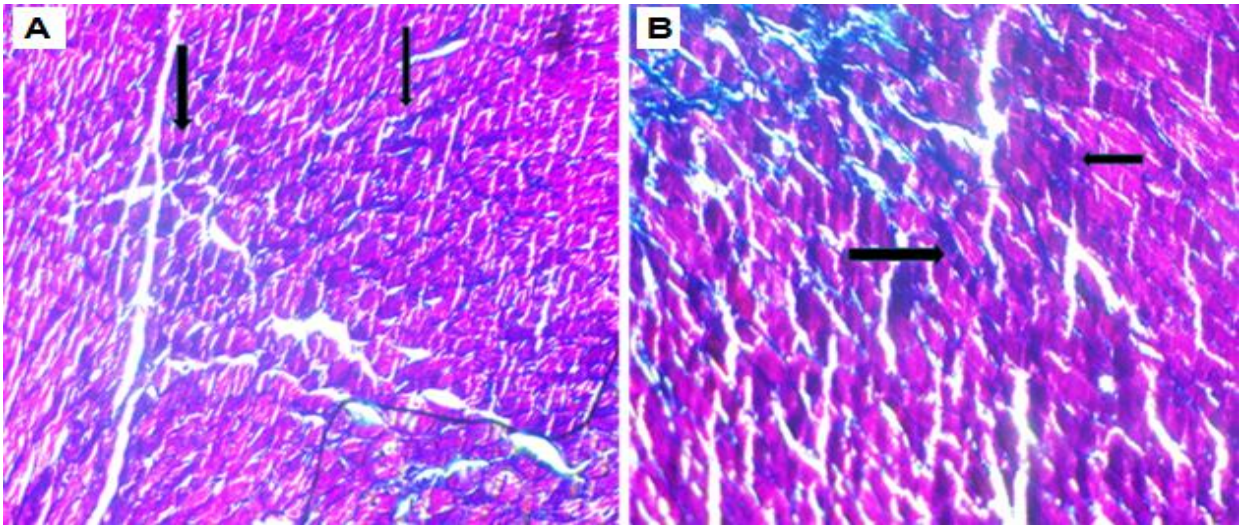

Figure 2: Sections in the rat's heart muscle of the fructose-fed group (group 3) showing marked deposition of collagen fiber between the cardiac muscle fibers (arrows), stained by Masson trichrome A) x200, B) x400.

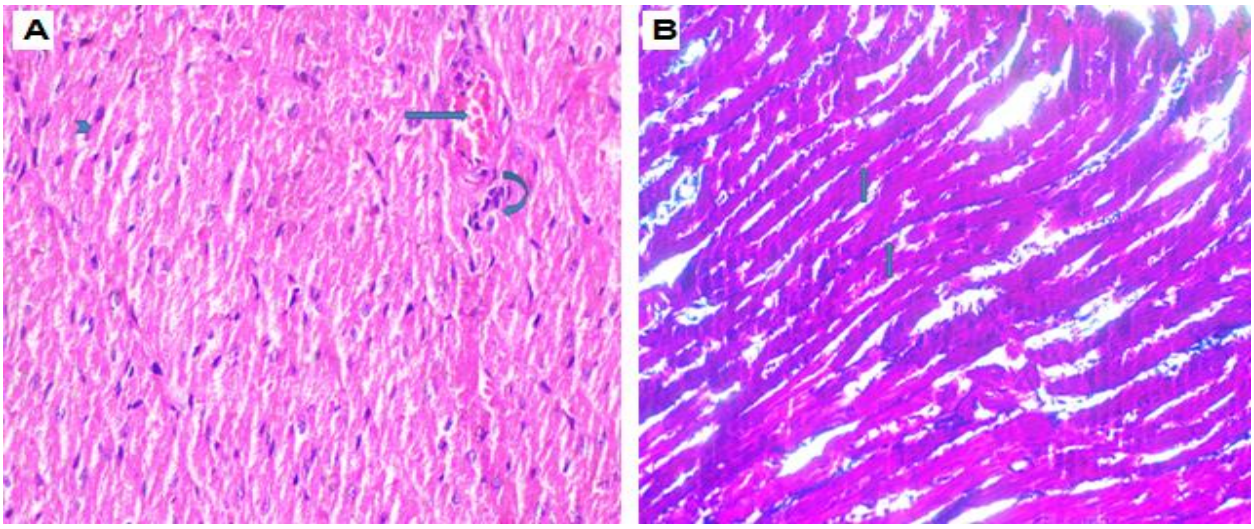

Figure 3: Section in the rat's heart muscle of groups 4 showing mild degenerative changes (arrowhead), infiltration (curved arrow), and mildly dilated and congested blood vessels (arrow), stained by H&E and showing minimal collagen fiber deposition in between the cardiac muscle fibers (arrows) A) x 200, B) x400.

4. Discussion

MS is a group of disturbances such as abdominal obesity, insulin resistance, dyslipidemia, and hypertension that lead to many diseases such as T2DM, atherosclerotic cardiovascular disease, and stroke. Models of fructose drinking are widely used to induce MS, independent of obesity or genetic contributions [8]. Canagliflozin is an SGLT2 inhibitor. It was reported that there was significant improvement in glycemic control, body weight, and BP in patients with T2DM treated with canagliflozin, suggesting that it may be effective in improving the disorders of MS [12].

In the present study, the rats were given fructose in drinking water for 7 weeks to induce MS, and this is compatible with [8, 13], who reported that fructose consumption in diet or drinking water is an important model for MS induction. Fructose-fed rats showed a significant increase in fasting serum glucose and insulin levels. These results coincide with previous studies [8, 14, 15].

The use of fructose has been linked to an increased risk of developing metabolic syndrome, according to both clinical and epidemiological studies. Fructose, unlike other sweets, causes serum uric acid levels to rise rapidly. It was observed that uric acid decreases levels of endothelial nitric oxide (NO), a critical modulator of insulin action. NO improves blood flow to skeletal muscle and boosts glucose absorption. Endothelial nitric oxide deficiency causes insulin resistance and other metabolic syndrome symptoms in animal models. Fructose-induced hyperuricemia has been hypothesized to be a contributing factor to the epidemic of metabolic syndrome by decreasing endothelial NO levels and inducing insulin resistance [16].

The present research was conducted to evaluate the effect of canagliflozin on fructose-induced metabolic syndrome. In the present study, we reported that with oral canagliflozin every day at a dose of 10 mg/kg for 7 weeks, insulin and glucose levels in the serum were significantly reduced in comparison to fructose-

fed rats that did not get treatment. These results coincide with previous studies that revealed significantly lower blood glucose levels in diabetic rats treated with canagliflozin than in untreated diabetic rats [17]. Canagliflozin reduces postprandial glucose even in normal subjects. These results are expected as canagliflozin acts by reducing renal glucose reabsorption and increasing urinary glucose excretion [18].

Regarding the lipid profile, we reported that a high-fructose diet resulted in a significant increase in serum triglyceride and LDL levels, which agrees with previous findings [14, 19]. The dyslipidemia in the present study could be caused by hyperglycemia, as impaired glucose metabolism alters lipid and protein intermediary metabolism [17]. There was a significant improvement in the serum levels of TG and LDL caused by fructose with canagliflozin. These results are consistent with [20]. Canagliflozin's ability to boost AMP-activated protein kinase (AMPK) activity—a metabolic energy sensor crucial for elevating fatty acid oxidation and energy expenditure while tamping down lipogenesis and inflammation—may account for these effects [21].

In the present study, SBP and HR were significantly increased in the fructose-fed group as compared to control rats, and these results coincide with [9, 15]. As BP rose, IR also rose, suggesting a causal relationship between the two. Fructose-diet hypertensive rats were found to have a lower density of insulin receptors in skeletal muscle and liver, providing more evidence for the involvement of IR in the development of fructose-related hypertension. In addition to the sympathetic nervous system, the renin-angiotensin-aldosterone system, the hypothalamus, the autonomic nervous system, and the adrenal glands all appear to contribute to experimental fructose hypertension [22]. Comparing the fructose-fed rats treated with canagliflozin to the fructose-fed rats untreated, there was a substantial reduction in SBP and HR. [23, 24].

Regarding PCSK9, we observed significantly higher levels in the cardiac tissues of fructose-fed rats when compared to the control group. Similarly, previous studies reported a significant increase in circulating PCSK9 levels in patients with MS [25, 26]. It is suggested that High-fructose diets raise plasma PCSK9 concentrations through the transcriptional regulation of the sterol regulatory element-binding protein SREBP1c and decrease LDLR, ultimately lowering PCSK9 clearance from plasma. At the same time, changes in insulin sensitivity have been linked to fructose intake. [27].

Treatment with canagliflozin in fructose-fed rats showed a significant decrease in PCSK9 cardiac levels as compared to untreated fructose-fed rats. In our study, PCSK9 concentrations in cardiac tissues were positively correlated with serum glucose levels, IR, and SBP, and this is compatible with several studies in healthy individuals that reported a positive correlation among fasting plasma PCSK9 and glucose, insulin concentrations, and HOMA-IR [27]. Insulin appears to control PCSK9 expression through the pathway of SREBP1c [28]. Insulin agonists increase SREBP-1c mRNA and mature transcription factors [27]. According to these previous studies, canagliflozin improved the increased concentrations of PCSK9 in cardiac tissues caused by fructose feeding as it ameliorated hyperinsulinemia, hyperglycemia, hyperlipidemia, and increased systolic blood pressure caused by fructose.

Consumption of a high-fructose diet is an important cause of heart disease manifested by hypertrophy, ventricular dilatation, inflammation, and decreased ventricular contractile function [29]. In our study, we examined histopathological changes in cardiac

tissues using hematoxylin and eosin and Masson trichrome stains. This revealed abnormal degenerative changes, congestion, inflammatory cell infiltration, and fibrosis in the cardiac tissues of fructose-fed groups. These results coincide with other studies [29]. With canagliflozin, the histopathological changes in cardiac tissues caused by fructose feeding were improved. These obvious improvements were consequences of the improvement of all features of MS. The present evidence suggests that SGLT-2 inhibitors' direct benefits are mediated by their ability to ameliorate cardiac inflammation, oxidative stress, apoptosis, mitochondrial dysfunction, and ionic dyshomeostasis [30, 31].

Conclusion

In conclusion, canagliflozin improves most of the risk factors of MS (glucose sensitivity, dyslipidemia, obesity, hypertension, and inflammation), which were induced by fructose in drinking water. Our results confirm the cardiac protective role of canagliflozin rather than glycemic control. To verify the experimental data and clarify the potential protective function of canagliflozin on the cardiovascular consequences of MS, more experimental and clinical trials are needed.

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Ethical approval: The study's experimental protocol was confirmed by the Animal Research Ethics Committee of Faculty of Medicine, Fayoum University. Approval No is D 190. The experiment is according to world guidelines of NAH.

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Conflicts of Interest: None declared.

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