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Potential Reno-protective Influence of Canagliflozin in Male Albino Rats with Metabolic Syndrome

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Abstract

Introduction: Metabolic syndrome (MetS) is a pathological condition of protein, fat, and carbohydrate metabolism. Persistent inflammation caused by MetS can affect renal structure and function. Renal inflammation due to MetS can be controlled, in part, by activating the nucleotide-like receptor protein 3 (NLRP3) inflammasome. The sodium-glucose cotransporter 2 (SGLT2) inhibitor canagliflozin has been shown to benefit people with and without type 2 diabetes by lowering metabolic risk factors and protecting renal function.

Aim of the study: Canagliflozin was tested for its influence on renal inflammatory changes in rats with fructose-induced metabolic syndrome.

Materials and Methods: Four groups of male albino rats were studied; Control, Canagliflozin (10 mg/kg/daily orally), Fructose (25% fructose in drinking water), and Fructose + Canagliflozin. After seven weeks, measurements of systolic blood pressure (SBP), IL-6 levels, the HOMA test for insulin resistance (IR), renal NLRP3 levels, and histopathological renal examination were estimated.

Results: Fructose-induced MetS caused significant increases in SBP, IR, serum IL6 level, and NLRP3 renal levels associated with marked renal interstitial inflammatory infiltration. Canagliflozin ameliorated IR and serum IL6 levels by 70% and 63%, respectively; SBP by 33.7%; NLRP3 renal levels by 58%; and finally, it attenuated the interstitial inflammatory infiltrations caused by MetS.

Conclusions: Canagliflozin has a protective renal effect in fructose-induced MetS not only by controlling glycemia but also through its potential anti-inflammatory effect. The anti-inflammatory role of canagliflozin is related to some extent to its ability to decrease the activation of the NLRP3 inflammasome.

Keywords: Fructose; MetS; NLRP3; Canagliflozin; kidney inflammation.

1. Introduction

Visceral obesity, high blood sugar, abnormal lipid profiles, and hypertension make up the main components of metabolic syndrome (MetS). According to the World Health Organization (WHO), impaired glucose metabolism, diabetes mellitus, or insulin resistance (IR) are the cornerstones of a diagnosis of MetS. The International Diabetes Federation

considers central adiposity to be the most fundamental condition. The prevalence of MetS varies even among the same group due to variations in diagnostic criteria [1].

MetS has a global prevalence of 20–25% and is considered a major risk factor for chronic renal disease. It has been shown that MetS can influence renal pathophysiology by inducing glomerular hyperfiltration, microalbuminuria, profibrotic factors, and renal podocyte injury [2]. MetS-related kidney injury has a multifactorial etiology that includes IR, obesity, hypertension, dyslipidemia, oxidative stress, inflammation, and endothelial dysfunction [3]. Abnormal secretion and release of adiponectin, leptin, resistin, IL6, tumor necrosis factor (TNF), and other adipokines characterize MetS and contribute to the development of oxidative stress, endothelial dysfunction, inflammatory effects, amplified sympathetic activity, and, ultimately, alterations in renal function and structure [3].

The nucleotide-binding domain like receptor protein 3 (NLRP3) inflammasome is a large polyprotein complex with a molecular weight of roughly 700 kDa that controls the body's chronic inflammatory response. Three components make up the NLRP3 inflammasome: a nucleotide-binding domain-like receptor (NLR), a speck-like protein with a caspase recruitment domain (ASC), and a protease activating domain (PAD) [4]. The middle NACHT domain, downstream adapter protein pyrindomain (PYD) or caspase recruitment domain (CARD), and leucine-rich repeats are the key structural components of NLRs (LRRs). Caspase-1 is the active form of pro-caspase-1, and it is responsible for mediating a unique kind of programmed cell death called pyroptosis as well as cleaving cytokine precursors like interleukin (IL)1b and IL18 to produce mature cytokines [5].

The kidney's inflammatory reaction to either viral or non-infectious activators is known as the renal inflammatory response. Dendritic cells and macrophages are two types of renal mononuclear phagocytes that can contain NLRP3 inflammasome components and, potentially, trigger cell death by activating caspase-1 [4]. Additionally, the NLRP3 inflammasome's activation has been linked to a wide variety of clinical disorders, from metabolic syndrome to kidney illnesses. Interestingly, the NLRP3 inflammasome is a possible target in the therapy of metabolic inflammatory illnesses, including renal injury in diabetes, when activation of the inflammasome is prevented in the glomerulus by a particular decrease in mitochondrial reactive oxygen species [ROS] [6].

Around 90% of the glucose that is filtered in the proximal renal tubules is reabsorb-able through the action of sodium-glucose co-transporter-2 (SGLT2). Canagliflozin lowers blood sugar levels and promotes the excretion of glucose in the urine by inhibiting SGLT2. Patients with T2DM have been shown to have more glucose reabsorption by SGLT2 than those without T2DM, elevating this transporter to the status of a prospective therapeutic target [7]. Canagliflozin has been shown to significantly lower pathological remodeling of the kidneys, arterial stiffness, and systemic blood pressure [8]. Canagliflozin has been shown to prevent cisplatin-induced nephrotoxicity in mice by lowering ROS and RNOS levels and increasing the activity of endogenous antioxidants such as superoxide dismutase and catalase [9].

Therefore, this study was intended to demonstrate the protective effect of canagliflozin on inflammatory changes in the kidneys caused by MetS.

2. Subjects and methods

2.1. Chemicals:

Fructose was purchased from El-Farouk Company, El-Fayoum, Egypt. Canagliflozin was purchased in the form of tablets (Invocana; Janssen Co., Beerse, Belgium).

2.2. Experimental Animals and Design:

Twenty-four adult, age-matched, male albino Sprague Dawley strain rats were procured from the animal house of The National Research Institute, Cairo University, and were maintained under observation for one week before the study with free access to food and water. There were four distinct groups of rats (n = 6), as follows: Control (rats received normal food and tap water), canagliflozin (10mg/kg orally once daily) [10], Fructose (rats were given 25% fructose in drinking water) [11], and Fructose + canagliflozin (rats were given fructose and canagliflozin) for seven weeks.

2.3. Blood pressure measurement:

The LE 5001 non-invasive blood pressure meter was used to monitor SBP after the seventh week (Panlab; Harvard Apparatus, Barcelona, Spain). After 20 minutes of acclimatization, the sensitive blood pressure meter was activated. Dark clothing was used to ease the rats' nerves while they were introduced to the restrainer. After inserting the transducer/tail cuff into the tail's base, activating the selector switch, and waiting for the readings to appear, the device was used. [12].

2.4. Blood sampling and Histological study:

Under light general anesthesia with diethyl ether, blood samples were collected from the retroorbital veins of fasting rats, centrifuged, and serum was separated for the determination of IR and IL6. Soon after rats were sacrificed by cervical dislocation, their two kidneys were removed, cleaned in ice-cold saline, and then sectioned into two sections each: one part was frozen at -60 C to measure NLRP3 concentrations, and the other part was preserved in formalin (10%) for histopathological examination. Serial sections of 4-micron thickness were obtained and stained with Hematoxylin and Eosin (H & E) to assess renal histopathological changes.

2.5. Determination of biochemical parameters:

Serum glucose and serum insulin were measured according to the manufacturer's instructions (Atlas Medical Co., Cambridge, UK). Then IR was calculated by homeostatic model assessment for insulin resistance (HOMA-IR) according to the following equation [13]: IR = glucose (mg/dL) X Insulin (μU/mL)/405.

2.6. Enzyme-linked immunosorbent assay (ELISA) assessment:

By using ELISA kits, the procedures and methods for measurement of serum insulin (Cat. No. MBS045315), IL-6 (Cat. No. MBS175908), and tissue NLRP3 (Cat. No. MBS 7612469) were according to the manufacturer's instructions of MyBiosource, Southern California, San Diego, USA.

2.7. Statistical analysis:

The obtained data were updated for completeness and conformity with reasoning. The data were then imported to version 16 of the Statistical Program for Social Science (SPSS) for statistical analysis, utilizing the following levels of analysis:

- The descriptive statistics (mean standard deviation for quantitative variables) to summaries our findings.
- The ANOVA test, followed by a post hoc test for inter-group comparison, was used to

compare quantitative variables across treatment groups.

- * Pearson's Rank correlation was done to test the association between variables and expressed using (r).

If r value ≥ +0.7, → Very strong positive relationship

If r value ≥ -.7, → Very strong negative relationship

- A *P value* < 0.05 was considered significant.

3. Results

Compared to the control and canagliflozin groups, the fructose group experienced a statistically and clinically significant increase in SBP in the current study (*P< 0.05*), while with canagliflozin, SBP was significantly improved (*P< 0.05*) in fructose-fed rats. Canagliflozin and the placebo groups showed no discernible change (*P > 0.05*) (**Figure 1**).

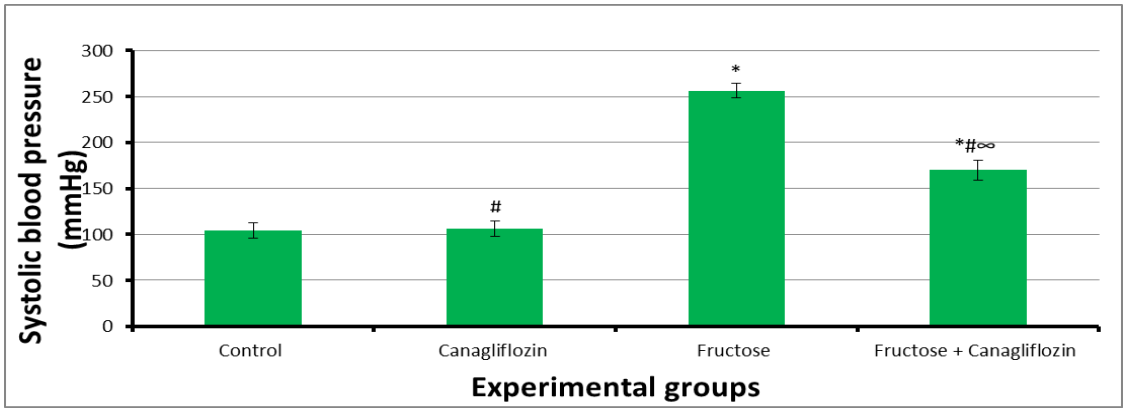

Figure 1: Description of SBP among the study groups.

IR and serum IL6 levels were significantly higher in the fructose and fructose + canagliflozin groups than in the control group. Meanwhile, there were no significant differences between the control and canagliflozin groups. With canagliflozin, IR and IL6 levels improved significantly, as compared to the fructose group (**Figures 2 and 3**).

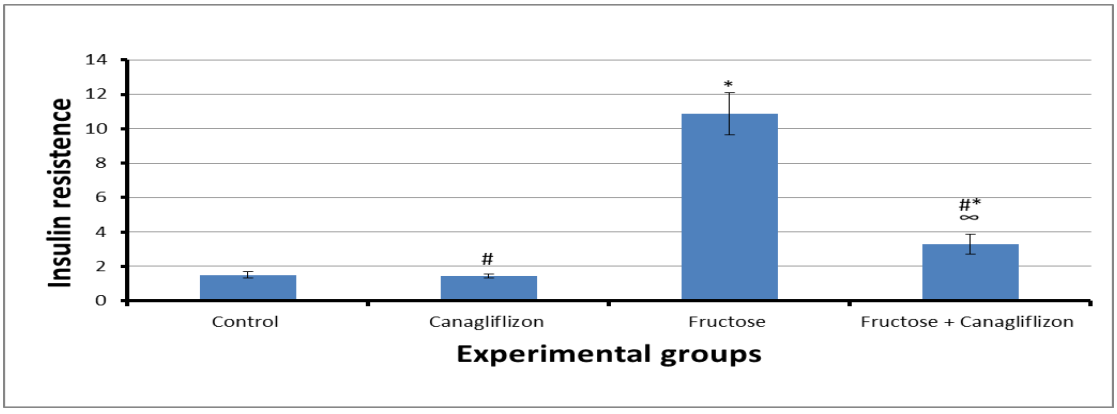

Figure 2: Description of IR among the study groups.

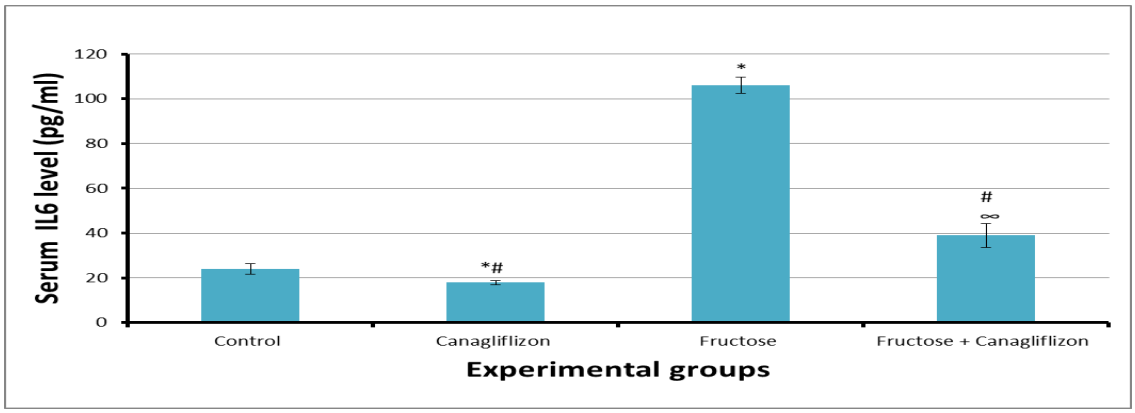

Figure 3: Description of IL6 levels among the study groups.

Rats that received fructose showed significantly higher levels of the NLRP3 inflammasome than those in the control group (*P< 0.05*) while neither group differed in a noteworthy way between the control and canagliflozin groups (*P> 0.05*). Fructose + canagliflozin showed significantly lower NLRP3 levels than the fructose group (*P< 0.05*) (**Figure 4**).

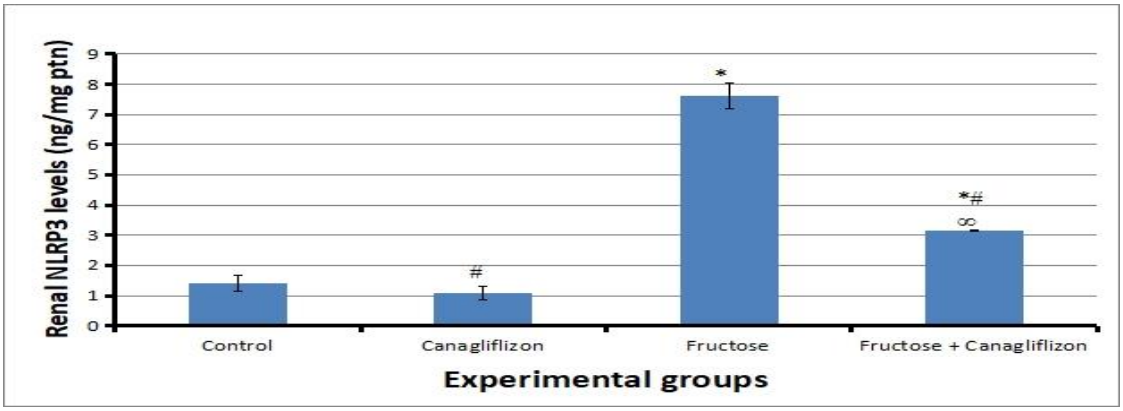

Figure 4: Description of NLRP3 renal levels among the study groups.

We further explored the association between serum IL6 and IR as well as SBP of all groups; the results revealed a significant positive correlation of the parameters (*P< 0.05* and r>0.696) (**Figure 5**).

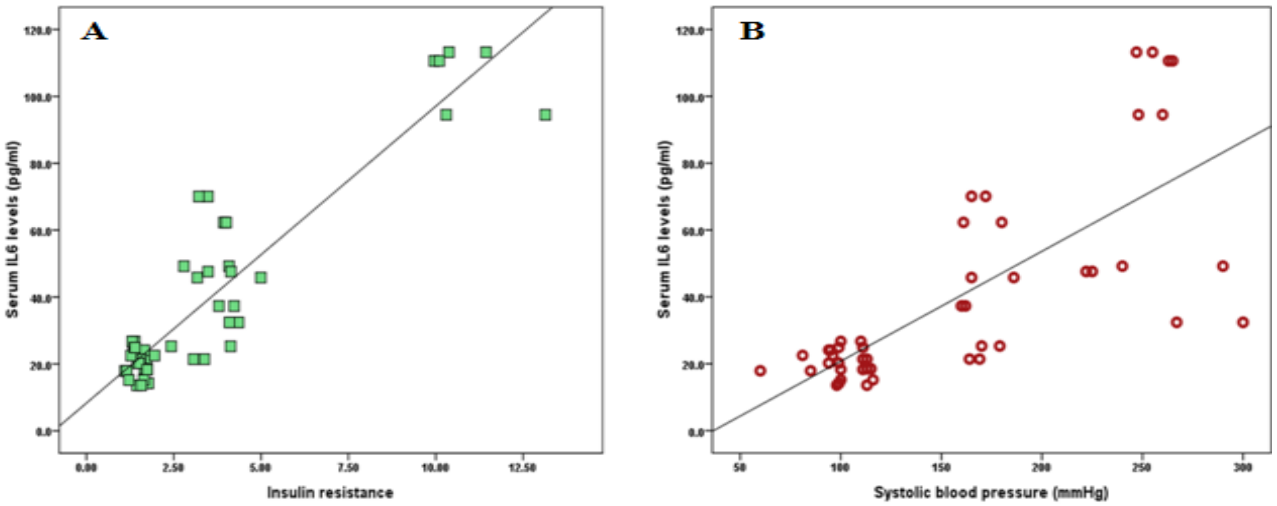

Figure 5: Association between IL6 and A) IR, and **B)** SBP.

Our results showed a significant direct association between renal NLRP3 concentrations and both IR and SBP ($P< 0.05$ and r >0.696) **(Figure 6).**

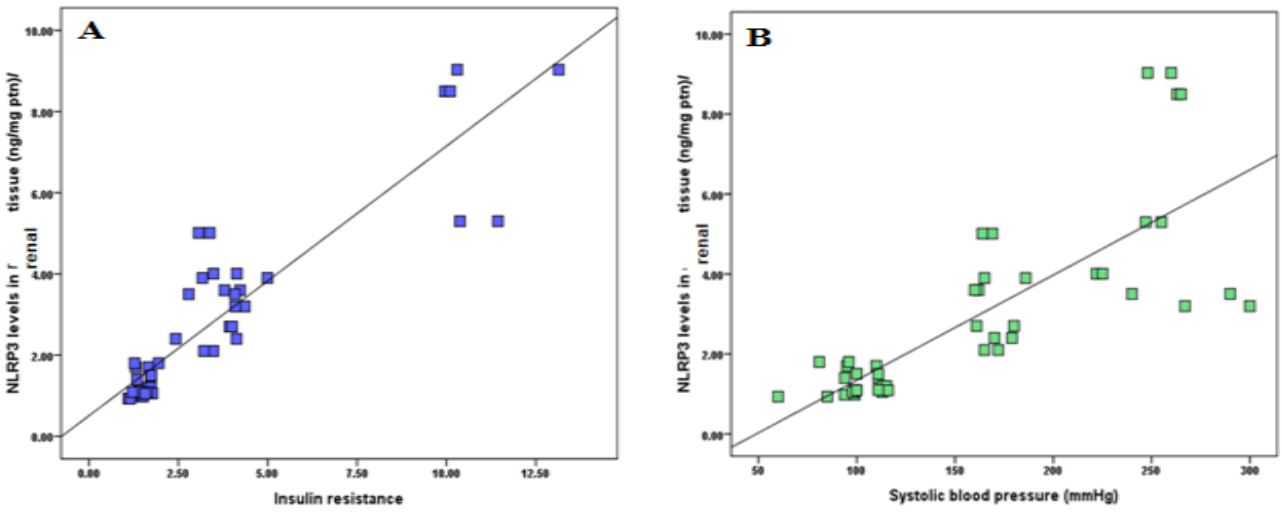

Figure 6: Correlation of Renal NLRP3 Levels and A) IR, and **B)** and SBP.

Histological examination of renal tissues from Control and canagliflozin rats (**Figure 7A**) revealed the normal histological architecture of renal corpuscles, formed of Bowman's capsules lined with simple squamous epithelia. The glomeruli, proximal tubules, and distal tubules had normal epithelial linings. whereas the fructose group (**Figure 7 B, C**) exhibited marked interstitial inflammatory infiltration.

Widening of the capsular space as well as glomerular loss were marked in the fructose group. Renal tubules were exhibiting marked distortion. Extravasation of erythrocytes in between glomerular capillaries as well as obvious

blood vessel congestion were clear findings. There was an improvement in the histological structure of kidney tissues with canagliflozin in fructose-fed rats (**Figure 7D**), with few intraluminal eosinophilic debris in few tubules and less extravasation of erythrocytes in-between glomerular capillaries than fructose group.

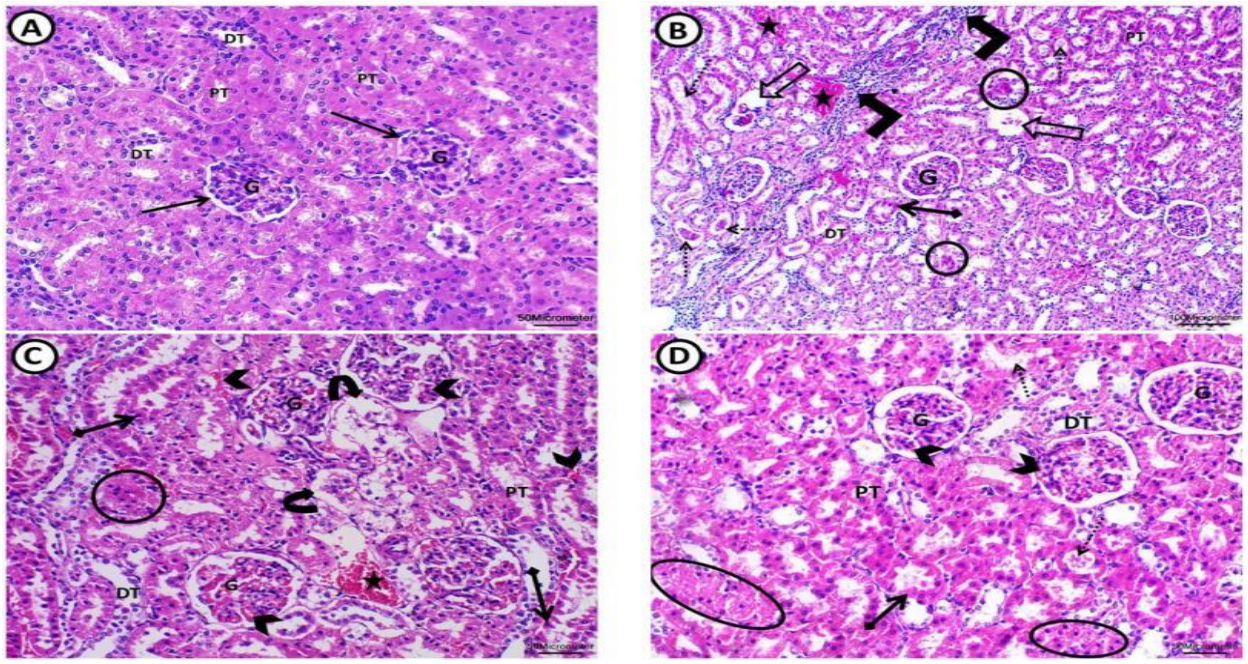

Figure 7: Photomicrographs of H&E-stained sections of the renal cortex from all experimental groups:(arrows); normal lining, (right-angled arrows); interstitial inflammatory infiltration (hollow arrow); Widening of the capsular space; Renal tubules are exhibiting marked distortion (encircled) (arrowheads); extravasation of erythrocytes (*); blood vessel congestion (dotted arrows); Intraluminal eosinophilic debris.

4. Discussion

In the present study, MetS was induced by the consumption of fructose in drinking water for 7 weeks with a significant increase in fasting serum glucose and insulin levels and IR, which is compatible with [14–17]. The development of IR and hyperglycemia after consumption of fructose could be the result of the down-regulation of insulin receptors caused by high fructose levels, which subsequently led to a reduction in insulin-stimulated glucose utilization and a reduction in insulin sensitivity [18].

With oral canagliflozin, there was a significant improvement in IR in fructose-fed rats. These results coincide with previous studies [19, 20]. This improvement is expected as canagliflozin acts specifically by blocking glucose reabsorption in the kidney due to SGLT2 inhibition, leading to increased urinary excretion of glucose, especially when hyperglycemia is present. This mechanism of action isn't dependent on the presence of endogenous insulin [21]. Canagliflozin is also a weak inhibitor of SGLT1 that contributes to glucose homeostasis in other tissues, especially the small intestine. Therefore,

canagliflozin is very important for lowering postprandial glucose and insulin levels [22].

As regards blood pressure, administration of a high fructose diet for seven weeks resulted in a significant elevation in SBP; these results coincide with [11, 17]. Three broad causes may play a role in fructose-induced hypertension: 1. The primary fructose transporter GLUT5, the sodium-hydrogen exchanger 3 (a sodium transporter), and the putative anion transporter 1 are all up-regulated by fructose in the small intestine and proximal renal tubule, resulting in increased salt absorption and decreased salt excretion (a chloride transporter). Second, abnormalities in the endothelium Long-term stimulation of the sympathetic nervous system [23].

Canagliflozin caused a significant decrease in SBP in fructose-fed rats when compared with the untreated fructose-fed group, and these findings are compatible with previous studies [20, 24]. The antihypertensive effect of canagliflozin in T2DM was explained by its role in the prevention of intrarenal angiotensinogen upregulation caused by hyperglycemia [25]. Besides that, canagliflozin acts by inhibiting the reuptake of sodium and glucose in the proximal tubule. The natriuretic and osmotic diuretic effects have been suggested to cause reductions in intravascular volume and SBP [26].

Variable studies reported that MetS is accompanied by low-grade inflammation induced by IL6 and other mediators [27, 28]. In our study, consumption of fructose led to a significant increase in serum IL6. This finding is explained by hyperglycemia which is associated with oxidative stress and inflammation and is considered a risk factor for cardiovascular disease and diabetic nephropathy (DN) [29]. Chronic inflammation participates in the development of microvascular complications of DM. Inflammatory cytokines, such as IL6, were shown to be higher in the serum of DN patients, and this elevation was linked favorably with the severity of nephropathy in earlier research. Podocytes, mesangial cells, interstitial tissue, and tubules can all be triggered by hyperglycemia to produce IL6, which contributes to the local and systemic inflammatory process in DN. [30].

We also revealed amelioration in serum IL6 levels in fructose-fed rats taking canagliflozin, and this is compatible with previous studies that reported the ability of canagliflozin to decrease levels of inflammatory markers (IL6, TNFα and CRP) in plasma and liver of high-fat diet and streptozotocin–nicotinamide- induced type 2 diabetic mice and rats [31].

From a different angle, MetS components aid in the formation of a state of inflammation known as metaflammation, which has been linked to an early innate immune response via the assembly of the multiprotein complex inflammasome. Among inflammasome platforms, the NLRP3 family has received the greatest attention [32]. Hence, we looked into how fructose intake affected NLRP3 concentrations in renal tissues and we revealed a significant increase in NLRP3 levels in fructose-fed groups when compared to control groups. This coincides with previous studies [33].

Researchers have found that elevated NLRP3 expression in adipose tissue is highly linked to IR and obesity. Activation of the NLRP3 inflammasome appears to play a critical role in regulating adipocyte differentiation and pushing adipocytes in an IR-promoting direction. Insulin sensitivity is enhanced in obese people with type 2 diabetes when they lose weight by calorie restriction or exercise-mediated weight loss. [34].

Canagliflozin improved NLRP3 renal levels as compared with the untreated fructose group which is compatible with other studies that reported that the SGLT2 inhibitors have

inflammatory properties in the experimental models and in vitro studies of type 1 and type 2 diabetes mellitus and revealed that SGLT2 inhibitors caused significant decrease in the diet-induced activation of NLRP3 inflammasome in the kidney of rats after chronic exposure to high fat-high sugar diet [35]. A previous study revealed that canagliflozin inhibits the activation of the NLRP3 inflammasome by inhibiting the mRNA and protein levels of NLRP3 by inhibiting the activity of intracellular caspase-1 and reducing the release of caspase-1 in the supernatant [36].

Renal tissues were shown to have undergone inflammatory alterations in response to fructose, providing more evidence that this sugar may have a role in the onset and progression of chronic kidney disease. Many diabetic vascular problems, such as nephropathy, have been linked to oxidative stress, which is thought to result from the creation of glycosylated products, glucose autooxidation, and sorbitol when glucose levels are elevated. [37]. It was revealed that NLRP3 inflammasome activation is involved in the inflammatory changes of the kidneys by induction of IL-1β and IL-18 secretion [38].

The histopathological results also confirmed the ameliorated effect of canagliflozin in fructose-fed rats. These obvious improvements were consequences of the improvement of all features of MetS. It is thought that this improvement is related to the improvements in renal oxygenation due to improved glycemia, IR, SBP and a decrease in inflammatory markers including NLRP3 and IL6 [39].

Conclusion

In conclusion, canagliflozin modulates most of the degenerative changes caused by MetS which was induced by fructose in drinking water. The results of this work confirm the role of IL6 and NLRP3 in the pathogenesis of some features of the MetS model considering them as a strong renal inflammatory sensor. These results show promising renal protective effects of canagliflozin to be used clinically in MetS rather than glycemic control. To corroborate the experimental observations and clarify the potential preventive function of canagliflozin on renal consequences of MetS, more experimental and clinical trials are needed.

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Ethical approval: The Ethics Committee of Research at Fayoum University's School of Medicine gave its stamp of approval to the trial (D190/56-2019). Animals used in these investigations were treated humanely and tested in accordance with European Council Directive for the Care and Use of Laboratory Animals (86/609/EEC), issued on November 24, 1986 and adopted by the National Institutes of Health.

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Conflicts of Interest: None declared.

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