The Efficacy of Canagliflozin on Type 2 Diabetic Nephropathy in Male Rats

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Abstract

Background: Sodium-glucose Cotransporter-2 inhibitors [SGLT2Is] are new antidiabetic group. Canagliflozin is the first member in this drug class approved for management of patient with type 2 diabetes (T2DM), that affects reabsorption of glucose from kidney, but its impact on kidney structure and function has not been clarified.

Objectives: The target of this study is assessment of the canagliflozin effect on body weight, blood glucose, serum creatinine, urea and evaluation of the possibility of urinary tract infection (UTI) development in type 2 diabetic rats.

 Materials and Methods: The search was carried out on 24 male Wistar rats of weighting 160-300 g and ageing 2.5-3 months. They were splitted into 3 equal groups each of eight rats: negative control, positive control (diabetic) and Diabetic-canagliflozin treated groups. The positive control group was given a single dose intraperitoneal (IP) Streptzotocin (STZ) (35mg/kg diluted in 1ml of 0.1 M citrate buffer in 4.5 PH). The diabetic-canagliflozin treated group which received STZ was given canagliflozin (30 mg/kg/day, orally) for 12 weeks. Blood samples were collected and used for estimation of blood glucose, creatinine and urea levels. Urine samples were collected for urine analysis and urinary albumin creatinine ratio assessment. Kidney tissue samples were obtained for histopathological screening.

Results: Diabetic-canagliflozin treated group had highly significantly decrease in body weight (287 ± 34.6) in comparison with positive control group (309.3 ± 21.1) at the end of the study as their weight were (234.2 ± 15.7 and 232.2 ± 11.01, respectively) at the srart. In addition to the highly significant decrease in blood glucose (255 ± 5.7) in Canagliflozin-treated group after treatment compared to (515.7 ± 49.2) before treatment. Also, there were a high significant decrease in urinary albumin (1.8 ± 0.2) and urinary albumine/creatinine ratio (1.1 ± 0.18) after treatment by canagliflozin. Adding to this, canagliflozin increase the level of urinary glucose excretion (875 ± 104) and development of UTIs (13.75 ± 3.5). However, no significant difference (p-value = 0.149) in serum creatinine or urea levels were detected (p-value = 0.112). Treatment with canagliflozin improved histological structure changes in the kidney.

Conclusions: Canagliflozin has a renoprotective effect. It improves renal tissue damage and decreases blood glucose levels.

Keywords: Canagliflozin; Diabetic nephropathy; Renoprotection; SGLT2 inhibitors.

Introduction

In 2021, the International Diabetes Federation was recorded that there were 537 million people with diabetes in the whole world. These analysis are expected to increase the number of diabetic patients to 783 million by 2045, so diabetes mellitus has become a serious public health problem **(Tong et al., 2023).**

Diabetic nephropathy (DN) is one of the most prevalent consequences of diabetes. The three levels of DN are generally referred to as normoalbuminuria, microalbuminuria and macroalbuminuria. Glomerular hyperfiltration is the defining symptom of early illness. The progressive decrease in glomerular filtration rate [GFR] occurs because of albuminuria but, there are other factors such as female gender, obesity, and the presence of hyperlipoproteinemia.The amount of albuminuria is in relation with blood pressure and blood glucose **(Rosaria et al., 2023).**

The pathogenesis of DN involves metabolic, hemodynamic, growth, inflammatory and fibrotic factors. In addition, increased circulating inflammatory mediators and renin angiotensin system activation are observed in patients with DN. The management theories of DN include (a) good control of blood glucose; (b) medications for normalization of blood pressure as angiotensin converting enzyme inhibitors or angiotensin-II receptor blockers; (c) weight management with bodily activity and sports; (d) high free protein diet; (e) smoking discontinuation; (f) therapy of hyperlipoproteinemia; and (g) evasion of nephrotoxic drugs such as non steroidal anti inflammatory drugs, antibiotics, contrast agents, Cyclosporins and Amphotericin B **(Rosaria et al., 2022).**

The use of finerenone has been one of the more recent DN therapy theories. Finerenone, a non-steroidal selective mineralocorticoid receptor antagonist, differs from spironolactone, eplerenone, and other steroidal congeners in terms of its pharmacological and pharmacokinetic properties **(Juan et al., 2020).**Renal protection and decreasing the course of disease are the main goals of DM treatment. SGLT-2 inhibitors increase the rate at which glucose is excreted in the urine, lowering blood glucose levels. Additionally, they have shown favorable protective benefits on cardiac or renal individuals with or without type 2 Diabetes (T2DM). They reduce the advancement of chronic kidney disease (CKD), which lowers the risk of dialysis and mortality from kidney illnesses **(Rajiv et al.,2022).**

Recent studies of cardiovascular and renal protection have clarified that the effects of SGLT2 inhibitors on eGFR are more distinct in patients with T2DM than those without diabetes, and that their effect on albuminuria is more pronounced in patients with T2DM than in patients with CKD without diabetes, keeping in mind that the effect of these agents on kidney replacement endpoints may be modified by the degree of glycemic control **(Jongs et al., 2021).**

Canagliflozin may reduce the kidney's ability to absorb glucose. By controlling glucose metabolism and autophagy, it produces anti-inflammatory and anti-tumor actions in addition to its hypoglycemic impact. Compared to placebo, canagliflozin dramatically reduced the incidence of renal failure by its effect on urinary creatinine ratio and eGFR. This was approved by Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation (CREDENCE) **(Van der et al., 2023).**

Materials and methods

The current experimental procedure was approved by the Animal Ethics Committee, Qena Faculty of Medicine, South Valley University. **Ethical Approval code:** SVU-MED-PHA006-1-21-11-265.

1) **Chemicals and drugs:** STZ, Canagliflozin and Ketamin were purchased from Sigma Chemical Corp., Germany, stored at 2-4 ◦C, and protected from sunlight, citrate buffer and saline.

2) **Induction of Diabetes:** Animals were recieved a high-fat diet for eight weeks then fasted overnight and injected with freshly prepared single dose of streptozotocin (35 mg/kg diluted in a volume of 1 ml/kg, I.P.) for induction of type 2 diabetes **(Zeinab et al., 2020)**, while negative control group rats recieved a normal palatable diet (NPD) all over the experiment. Seven days later, blood glucose level and body weight were determined to ensure the diabetic model **(Enas et al., 2020),** and nephropathy was occured 12 weeks after the onset of diabetes mellitus **(Manal et al., 2020).**

3) **Animals and experimental design:** This study was performed in the pharmacological Department, Qena Faculty of Medicine, South Valley University, Egypt. The experimental animals were gained from the laboratory animal house, Faculty of Veterinary Medicine, South Valley University, Egypt. Twenty-four male Wistar albino rats of 160-300 g body weight and 2.5-3 months of age were used. Rats were housed in cleanly stainless cages (42 x 21 x 20) in a well ventilated room and supplied with food and water ad libitum. Before starting of the experimental study, all rats were adapted one week .

Rats were randomly classified into three equal groups; each group involved eight rats.

- **Group 1:** Negative (-ve) controlled group.

- **Group 2:** Positive (+ve) controlled (diabetic) animal group administered a single inta-peritoneal dose of STZ (35 mg/kg diluted in 1 ml of 1 M citrate buffer in 4.5 PH) **(Zeinab et al., 2020).**

- **Group 3:** Diabetic-Canagliflozin-treated animal group administered a single inta-peritoneal dose of STZ (35 mg/kg diluted in 1 ml of 1 M citrate buffer in 4.5 PH) then given Canagliflozin (30 mg/kg/day, orally) dissolved in saline and given once daily and repeated daily for 12 weeks **(Ali et al.,2016).**

4) **Body weight measurement:** At the start of the experiment, rats were weighted with a top loader weighting balance (Model D0030, A&D Company Limited, USA) and they were weighted also at the end of the study.

5) **Samples collection:** We anesthetized the rats by useing single dose Ketamine (50mg/ml/kg, IP) **(Maya et al.,2016).** Blood samples were collected from the orbital vein removed in a sterile plain tube. Samples were stored at -20°C to determine serum creatinine and urea. For collection of 24-hour urine, we put

each animal into the metabolic cage with food and water. Kidney sample was sliced for histopathological studies.

6) **Blood glucose measurement:** Drops of blood were gained from rat`s tail vein for monitoring blood glucose at the start of the study, before induction by STZ and at the end of the study using a glucometer,Accu-Chek®Active, Roche Diagnostic Corporation, Mannheim, Germany, catalogue no: 06656757.

7) **Biochemical analysis**
The collected blood samples were centerfuged at 3000 rpm/min and stored at -20 ± 2°C. Commerical kits from Roche Diagnostics GmbH, Germany were used for measurement of creatinine and urea:

- Roche Cobas Creatinine Jaffe (CREJ2), catalogue no: 04810716190.
- Cobas Urea C311, catalogue no: 04460715190.

8) **Urine analysis:** The collected urine samples were centerfuged at 1500 rpm/min and the clear supernatant was stored at -20 ± 2°C. For measurement of urinary albumine, urinary albumine / creatinine ratio and detection the possibility of UTIs development using Chemstrip®10 UA, supplied by Roche Diagnostics GmbH, Germany, catalogue no: 11895354160.

9) **Histopathological Examination: Bancroft and Stevens (2013)** described the procedure for histological preparations. Briefly, kidney sample was sliced to 3-4 mm thick, fixed in 10% neutral buffered formalin (10% NBF), dehydrated in graded concentrations of ethanol, cleared in xylene, and embedded in paraffin. The paraffin blocks were sectioned with a microtome at (4-6µm) thickness and dyed with Hematoxylin and Eosin stain to study general tissue structure. H&E-stained sections were examined via using Leica microscope (CH9435 Hee56rbrugg) (Leica Microsystems, Switzerland)

10) **Statistical analysis**
Data were analyzed using SPSS version 25*. Descriptive statistics: Means and standard deviations (SD) were calculated. The following tests were done:

- **The ANOVA test:** was calculated to test the mean differences of the data.
- **Post Hoc analysis:** was calculated using Tukey corrections.

All statistical comparisons were two-tailed with AP-value less 0.05 being considered significant, AP- value less 0.001 being considered significant and AP- value less 0.001 being considered highly significant.
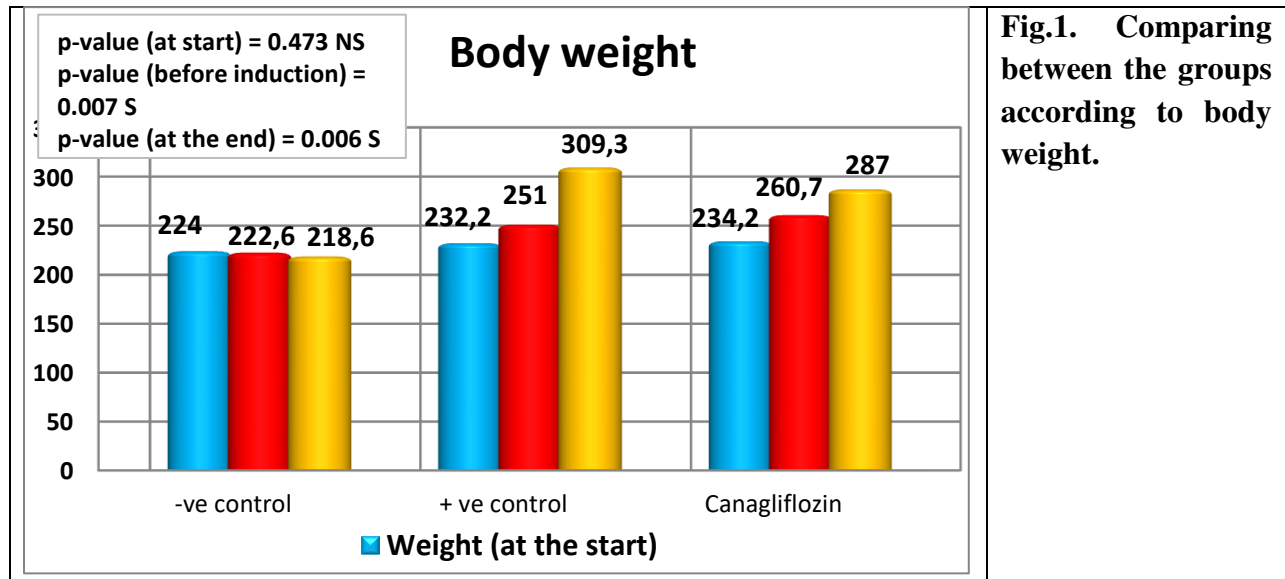
Results

Effect of canagliflozin on body weight

An insignificant rise (AP-value = 0.473) was observed in the diabetic canagliflozin-treated group when compared to the negative and positive groups, according to body weight at the beginning of the trial. According to **Table 1 and Fig. 1**, it was 224 ± 10.9 in the negative group, 232.2 ± 11.01 in the positive group, and 234.2 ± 15.7 in the Canagliflozin-treated group. Body weight prior to STZ treatment showed a substantial increase (AP-value = 0.007). According to **Table 1 and Fig. 1**, it was 222.6 ± 7.9 in the negative group, 251 ± 9.5 in the positive group, and 260.7 ± 21.1 in the Canagliflozin-treated group. A considerable drop in body weight (AP-value = 0.006) at the end of the research. The results were 218.6 ± 32.7 in the negative group, 309.3 ± 21.1 in the positive group, and 287 ± 34.6 in the group that had been given canagliflozin, these findings are displayed in **(Table 1 and Fig. 1).**

Table 1. Effect of Canagliflozin treatment on kidney function, blood glucose, and animal weights in various study groups

Variables	Negative control group	Positive control group	Canagliflozin treated group	P-value
Creatinine (mg/dl)	1.26 ± 0.12	1.77 ± 0.62	1.48 ± 0.17	0.149 NS
Urea (mg/dl)	33.5 ± 2.9	43.9 ± 8.7	49.4 ± 15.8	0.112 NS
Weight at the start (g)	224 ± 10.9	232.2 ± 11.01	234.2 ± 15.7	0.473 NS
Weight before STZ induction (g)	222.6 ± 7.9	251 ± 9.5	260.7 ± 21.1	**0.007 S**
Weight at the end (g)	218.6 ± 32.7	309.3 ± 21.1	287 ± 34.6	**0.006 S**
Glucose before TTT (mg/dl)	111.2 ± 17.5	384 ± 26.8	515.7 ± 49.2	**< 0.001 HS**
Glucose after TTT (mg/dl)	99.8 ± 13.5	309.3 ± 21.1	255 ± 5.7	**< 0.001 HS**

S: AP-value less 0.05 is significant. **T**: Independent sample T-test.
HS: AP-value less 0.001 is highly significant.
NS: AP-value more 0.05 is non-significant.

Fig.1. Comparing between the groups according to body weight.

Effect of canagliflozin on blood glucose level

The results of contrasting the diabetic canagliflozin-treated group with the negative and positive groups were as follows: High statistically significant increase according to blood glucose levels prior to treatment (AP-value 0.001). It was 384 ± 26.8 in the

positive group, 111.2 ±17.5 in the canagliflozin-treated group, and 515.7 ± 49.2 in the negative group **(Table 1 and Fig. 2)**. After treatment, there was high statistically significant reduction in blood glucose (AP-value 0.001). According to **Table 1 and Fig. 2**, it was 99.8 ± 13.5 in the negative group, 309.3 ± 21.1 in the positive group, and 255 ± 5.7 in the Canagliflozin-treated group.
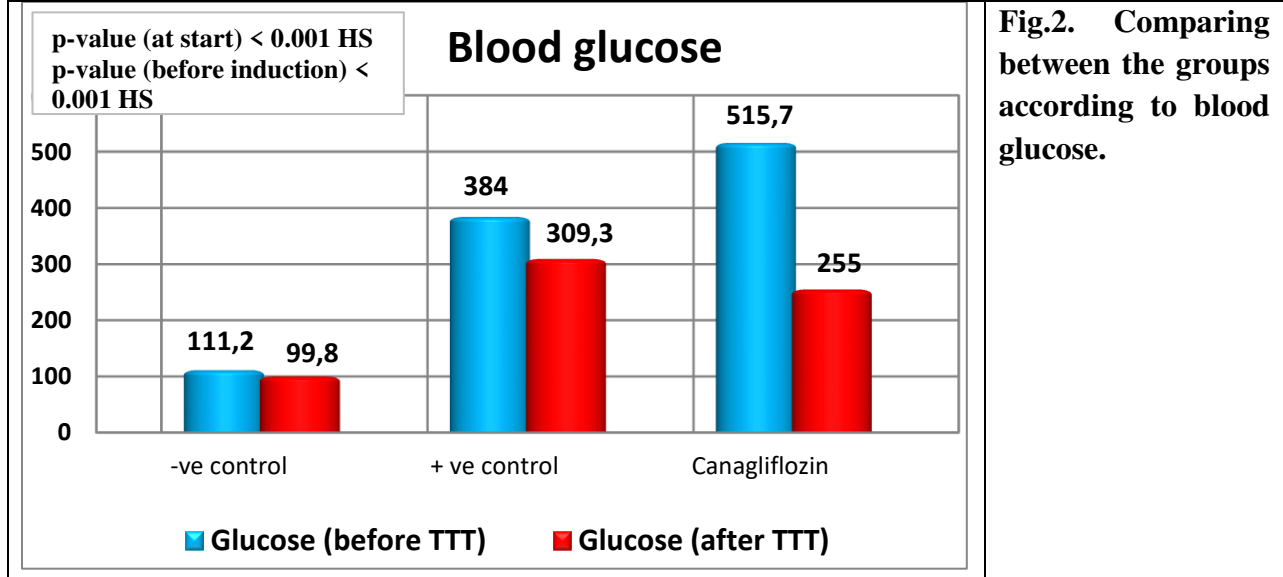
	Fig.2. Comparing between the groups according to blood glucose.

Effect of canagliflozin on serum creatinine level

The serum creatinine ratio improved when the diabetic canagliflozin-treated group was compared to the negative and positive groups, although the difference was statistically insignificant (AP-value = 0.149) according to serum creatinine. It was 1.26 ± 0.12 in the negative group, 1.77 ± 0.62 in the positive group, and 1.48 ± 0.17 in the canagliflozin-treated group **(Table 1 & Fig. 3)**.

	Fig.3. Comparing between the groups according to serum creatinine level.

Effect of canagliflozin on serum urea

Comparing the canagliflozin-treated group to the negative and positive groups revealed a negligible increase (AP-value = 0.112), per urea. As shown in **Table 1 and**

Fig. 4, it was 33.5 ± 2.9 in the negative group, 43.9 ± 8.7 in the positive control group, and 49.4 ± 15.8 in the Canagliflozin-treated group.
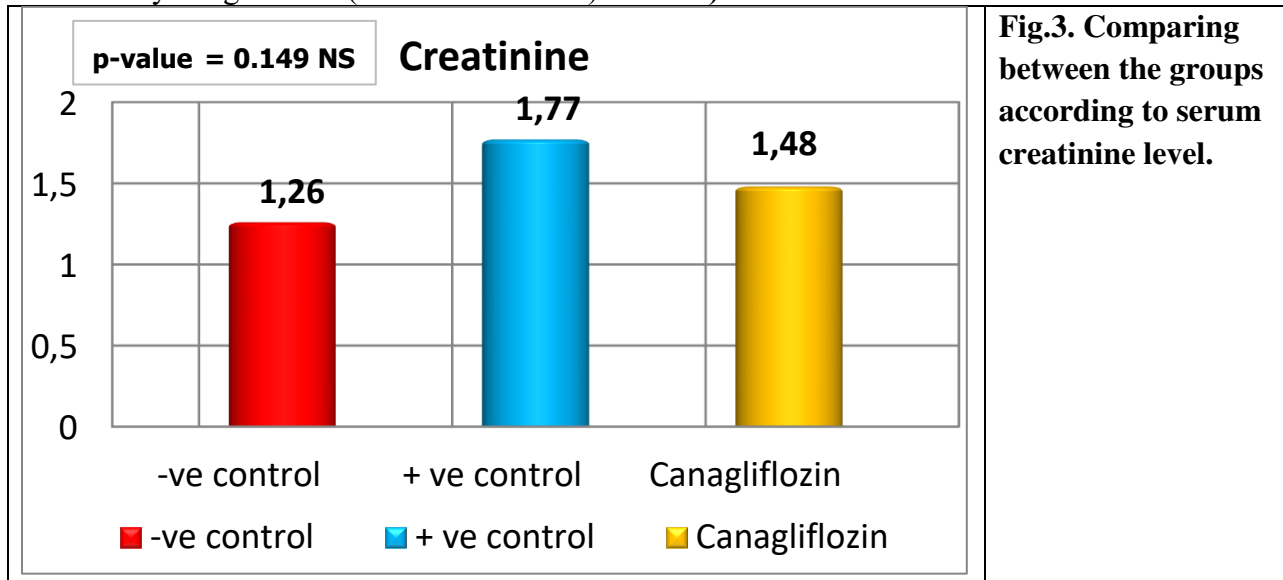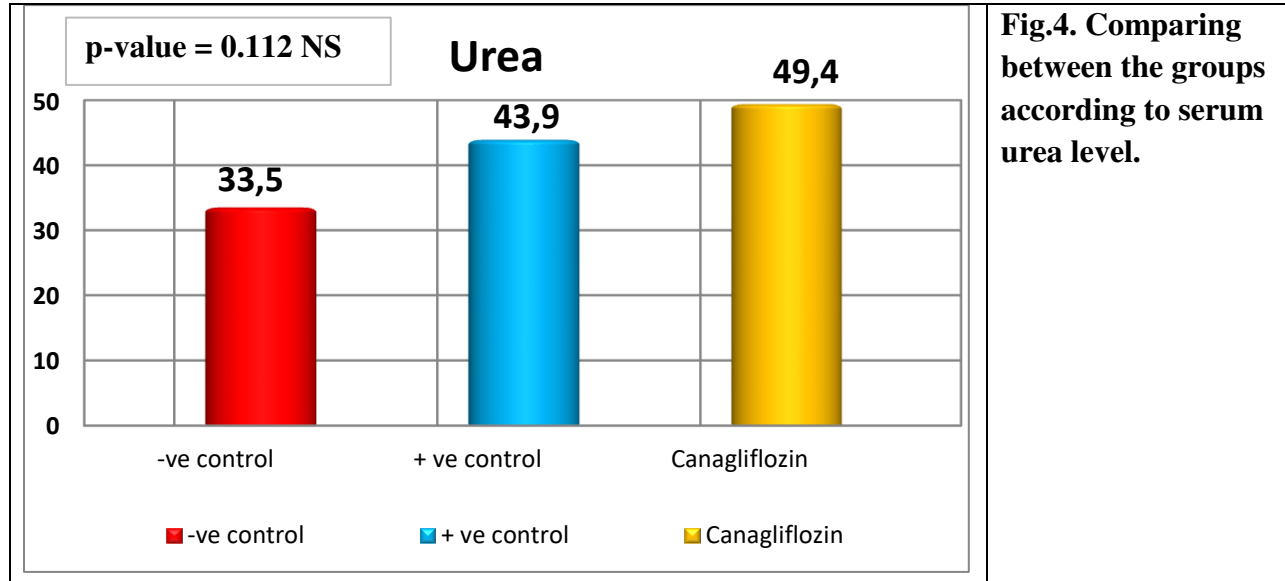

Fig.4. Comparing between the groups according to serum urea level.

Effect of canagliflozin on urinary albumin

When the diabetic canagliflozin-treated group was compared to the negative and positive groups, urinary albumin revealed a high statistically significant decrease (AP-value = 0.000). In the control

group, it was 0.4 ± 0.04; in the positive group, 3.7 ± 0.02; and in the group receiving canagliflozin, 1.8 ± 0.02 **(Table 2 & Fig. 5).**

Table 2. Effect of Canagliflozin treatment on urinary albumin in various study groups

Variables	Groups	Mean	Standard Deviation	P value	P value (1&2)	P value (1&3)	P value (2&3)
Urinary albumin	**negative control(1)**	0.4450	0.04435	**0.000**	**0.000**	**0.000**	**0.000**
	positive control (2)	3.7750	0.22174				
	Canagliflozin(3)	1.8750	0.22174				
Albumin/creatinine ratio	**negative control**	0.8000	0.18257	**0.003**	**0.002**	0.09	0.07
	positive control	1.4250	0.17078				
	canagliflozin	1.1000	0.18257				

Effect of canagliflozin on urinary albumin / creatinine ratio

When the diabetic-canagliflozin-treated group was compared to the negative and positive groups, the urinary albumin/creatinine ratio exhibited a very

statistically significant decrease (AP-value = 0.003). In the control group, it was 0.8 ± 0.18, in the positive group, 1.4 ± 0.17, and in the group receiving canagliflozin, 1.1 ± 0.18 **(Table 2 & Fig. 5).**
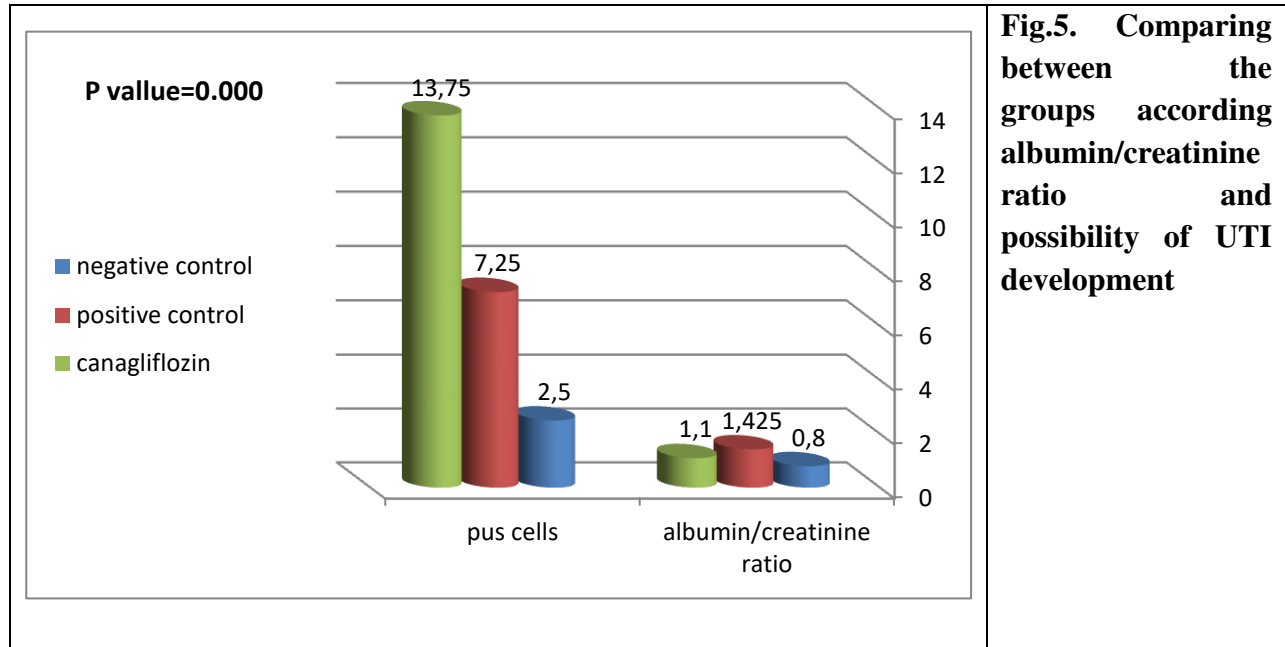
Fig.5. Comparing between the groups according albumin/creatinine ratio and possibility of UTI development

The relationship between canagliflozin and urinary tract infections (UTIs)

Comparing the Diabetic-Canagliflozin-treated group with negative and positive groups, there was high significant statistical increase (AP-value =

0.000) according to presence of pus cells. It was 2.5 ± 1.29 in the negative group, 7.25 ± 1.7 in the positive group and 13.75 ± 3.5 in Canagliflozin-treated group **(Table 3 & Fig.5).**

Table 3. The relationship between Canagliflozin and UTIs possible developments.

Variables	Groups	Mean	Standard Deviation	P value	P value (1&2)	P value (1&3)	P value (2&3)
Pus cells	**negative control**	2.5000	1.29099	**0.000**	**0.04**	**0.000**	**0.009**
	positive control	7.2500	1.70783				
	canagliflozin	13.7500	3.50000				
Urine glucose excretion	**negative control**	18.7500	2.98608	**0.000**	0.9	**0.000**	**0.000**
	positive control	0.9000	0.25820				
	canagliflozin	875.0000	104.08330				

Effect of canagliflozin on urinary glucose excretion

A high significant elevation (AP-value = 0.000) in urine glucose excretion was seen when the diabetic canagliflozin-treated group was compared to the negative and positive groups. According to **Table 3 and Fig. 5**, it was 18.75 ± 2.9 in the negative group, 0.9 ± 0.25 in the positive group, and 875 ± 104 in the group that received canagliflozin.

Post-Hoc test

As regards weight (at the start), there was insignificant differences (AP-value = 0.395) between – ve control and +ve control groups. Insignificant difference (AP-value = 0.262) between – ve control and Canagliflozin groups. Insignificant difference (AP-value = 0.849) between + ve control and Canagliflozin groups. (before STZ induction), there was statistically significant difference (AP-value = 0.022) between – ve control and +ve control groups. A statistically significant difference (AP-value = 0.003) between – ve control and Canagliflozin groups. No statistically significant difference (AP-value = 0.849) between + ve control and Canagliflozin groups. (at the end), there was statistically significant difference (AP-value = 0.003) between – ve control and + ve control groups. A statistically significant difference (AP-value = 0.01) between – ve control and Canagliflozin groups. No statistically significant difference (AP-value = 0.374) between + ve control and Canagliflozin groups **(Table 4).**

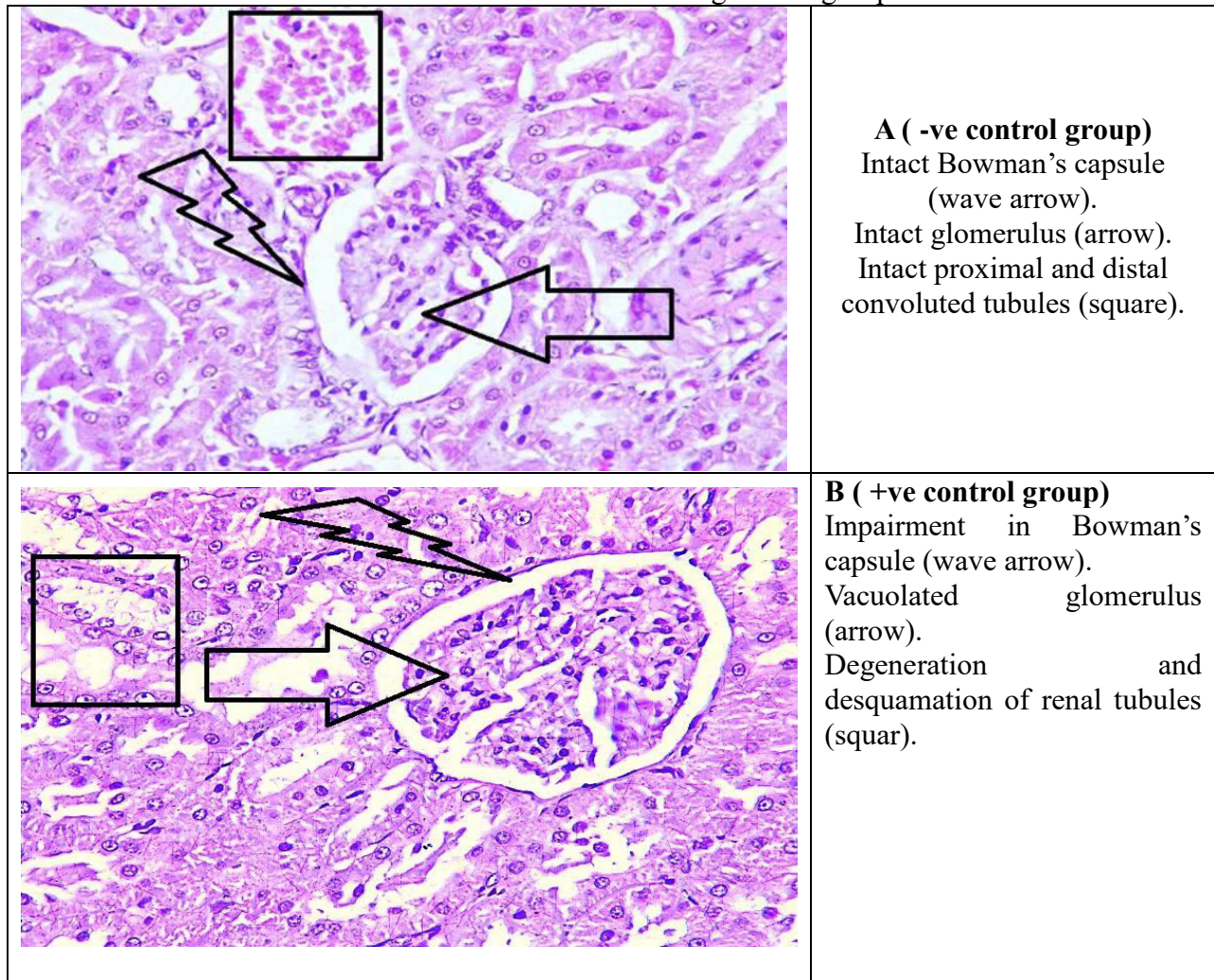**Table 4.Tukey test for comparing between the groups regarding analyzed data.**

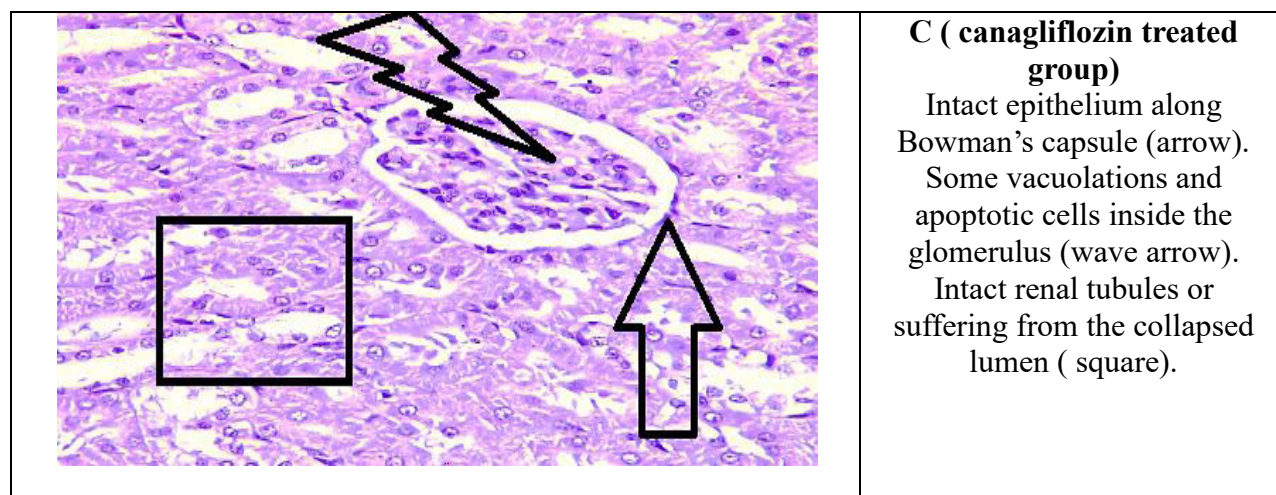| Variables		Negative control versus positive control	Negative control versus Canagliflozin	Positive control versus Canagliflozin
Creatinine	LSD	- 0.5	- 0.21	0.29
	p-value	0.058	0.342	0.262
Urea	LSD	- 10.4	**- 15.9**	- 5.4
	p-value	0.194	**0.045**	0.502
Weight at the start	LSD	8.3	10.2	- 1.91
	p-value	0.395	0.262	0.849
Weight (before STZ induction)	LSD	**28.4**	**38.1**	9.7
	p-value	**0.022**	**0.003**	0.386
Weight at the end	LSD	**- 90.7**	**-68.4**	22.3
	p-value	**0.003**	**0.01**	0.374
Blood glucose (before TTT)	LSD	**- 272.8**	**- 404.5**	**- 131.7**
	p-value	**< 0.001**	**< 0.001**	**0.001**
Blood glucose (after TTT)	LSD	**-209.5**	**- 155.2**	**54.3**
	p-value	**< 0.001**	**< 0.001**	**0.001**

LSD: Least significance difference; S: p-value < 0.05 is considered significant.; HS: p-value < 0.001 is considered highly significant.; NS: p-value > 0.05 is considered non-significant.

As regard blood glucose, there was high significant lower (AP-value < 0.001) between – ve control and + ve control groups. And (AP-value < 0.001) between – ve control and Canagliflozin groups. A statistically significant difference (AP-value = 0.001) between + ve control and Canagliflozin groups, before treatment. There was high significant decrease (AP-value < 0.001) between the – ve control and + ve control groups. While (AP-value < 0.001) between – ve control and canagliflozin groups. A statistically significant difference (AP-value = 0.001) between + ve control and canagliflozin groups, after treatment **Table 4.**

Regarding Serum Createnine, there was insignificant difference (AP-value = 0.058) between the – ve control and + ve control groups. No statistically significant difference (AP-value = 0.342) between – ve control and canagliflozin groups. No statistically significant difference (AP-value = 0.262) between the + ve control and canagliflozin groups **Table 4.**

Pointing to blood urea, insignificant decrease (AP-value = 0.194) between the – ve control and + ve control groups. A significant lower (AP-value = 0.045) between – ve control and canagliflozin groups. No significant lower (AP-value = 0.502) between + ve control and canagliflozin groups **Table 4.**

	A (-ve control group) Intact Bowman's capsule (wave arrow). Intact glomerulus (arrow). Intact proximal and distal convoluted tubules (square).
	B (+ve control group) Impairment in Bowman's capsule (wave arrow). Vacuolated glomerulus (arrow). Degeneration and desquamation of renal tubules (squar).

C (canagliflozin treated group)
Intact epithelium along Bowman's capsule (arrow). Some vacuolations and apoptotic cells inside the glomerulus (wave arrow). Intact renal tubules or suffering from the collapsed lumen (square).

Fig.6. Photomicrographs displayed the histopathological variations in the renal cortex area of kidney tissue sections between investigated groups

Histopathological results

(a) Section from the Negative Control group demonstrating normal histologic structure of renal cortex, proximal and distal convoluted tubules, as well as intact capsula glomeruli **(Fig. 6a). (b) Section from Positive control group** showing serious renal damage including atrophy in some renal corpuscle with dilated interglomerular space, impairment in Bowman's capsule, vacuolated glomerulus, severe vascular congestion and obvious increase in interstitial inflammatory cells infiltration. Furthermore, most renal tubules exhibited degeneration with apoptotic lining cells and others with desquamation of epithelial lining **(Fig. 6b). (c) The section from the Canagliflozin Group** revealed a marked decrease of the renal damage. The renal cortex presented with scarce vacuolations and some apoptotic cells inside the glomerulus, with intact epithelium along Bowman's capsule. Renal tubules emerged either intact or suffering from the collapsed lumen, sloughing of epithelium with pyknotic lining cells. Also, strong vascular congestion is still noticed **(Fig. 6c).**

Discussion

Diabetic nephropathy is one of the most prevalent side effects of diabetes mellitus and can result in chronic renal failure. Dialysis is used by over one third of individuals with chronic renal failure. Chronic kidney disease patients are more likely to acquire cardiovascular disease, particularly if they already had diabetes mellitus or cardiovascular disease prior to starting SGLT-2 inhibitor therapy for their DM **(Gadah *et al*., 2020).** One of the SGLT2 inhibitors, which are a novel class of anti-diabetic drugs that alter renal glucose reabsorption, is canagliflozin. Numerous clinical and experimental researches have shown that SGLT2 inhibitors can lower HbA1c and blood glucose levels without affecting insulin sensitivity or pancreatic beta-cell function. The consequences of SGLT2 inhibition on kidney structure and function, however, remain unclarified **(Ali *et al*.,2016).**

Our streptozotocin-induced diabetic rat models were used to examine canagliflozin's preventive effects on the onset and progression of DN and to assess the drug's therapeutic response. Our type II

diabetic rat model with nephropathy demonstrated a significant increase in blood glucose, body weight, and consequently, impairment in kidney functions and renal histopathological picture, which was consistent with already established findings by **Shimaa et al. (2021).**

Patients with T2DM who take SGLT2 inhibitors lose weight without affecting total body mass or skeletal muscle. So, according to body weight and blood glucose levels, the diabetic canagliflozine-treated group in this study showed a reduction which was previously ascertained by **Yi-Chou et al. (2020).**

The serum creatinine ratio was shown to decrease in the current investigation, but neither serum creatinine nor urea levels were statistically significant in the canagliflozin-treated group, contradicting findings made by **Shimaa et al. (2021).**

Our study demonstrated a substantial decrease in albuminuria and urinary albumin/creatinine ratio in the diabetic canagliflozin-treated group compared to renal function impairment in the diabetic rats, which was consistent with findings made by **Ali et al. (2016).**

Increased urine glucose excretion and the emergence of UTIs are related. Both urinary glucose excretion and pus cells in urine increased in the group receiving canagliflozin in this trial, which was consistent with findings previously established by **Masanori et al. (2014).**

In addition, our histopathological analysis revealed changes in the kidney with DN, including increased mesangial matrix, thickening of the tubular and glomerular basement membrane, impairment in Bowman's capsule, vacuolated glomerulus, and severe vascular congestion, which was consistent with what **Mohamed et al. (2020)** had already demonstrated.

The acute tubular damage features are gradually lessened in the canagliflozin-treated group. However, renal cortex showed some apoptotic cells in the glomerulus, intact epithelium along Bowman's capsule, and renal tubules that were either intact or had collapsed lumens, which was consistent with what **Mohamed et al. (2020)** had already documented.

Conclusion

Eventually, canagliflozin created a significantly downregulation in body weight, blood glucose level, renal damage and serum creatinine. However, no improvement in serum urea. So, we need to increase the dose of canagliflozin, increase sample size or adjunctive therapy to arrive at the suspected renoprotective role.

List of abbreviations

CKD; Diabetic kidney disease, DN; Diabetic nephropathy, SGLT2; sodium glucose cotransporter type 2 inhibitors, HbA1C; Glycated hemoglobin A1c, STZ; Streptozotocin, IP; Intraperitoneal.

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