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Nephroprotective potential effect of Canagliflozin in renal ischemia reperfusion injury in rat model: Role of Nrf2 pathway

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ABSTRACT

Renal ischemia reperfusion injury promotes tissue damage through inducing uncontrolled inflammation, oxidative stress and excessive renal tubular epithelial cell death. Nrf2 pathway is essential in protecting the kidney against renal IRI. Canagliflozin is a sodium glucose co-transporter 2 (SGLT2) inhibitor, Canagliflozin attenuated nephrotoxicity possibly through its anti-inflammatory and antioxidant effects and may confer reno-protective effects independently of its glycemic effects. To study the Nephroprotective potential effect of Canagliflozin renal ischemia reperfusion injury in rats model via modulation of Nrf2 pathway. In this study, adult male rats of Westar Albino type with 18-28 weeks in age and weighing 300-350 g were randomized into equal four groups (6 rats each group) as following: Sham group, Control group, Vehicle (DMSO) group, Canagliflozin treated group. Rats were subject to bilateral renal ischemia for 30 min by clamping and reperfusion for 2 hr. We conclude that Canagliflozin significantly decrease renal ischemia reperfusion injury in rat model through Nrf2 pathway via it is pleiotropic effects as anti-oxidant, anti-inflammatory and anti-apoptotic.

Key words: glycemic effects, canagliflozin, Nrf2 pathways

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INTRODUCTION

Renal ischemia reperfusion injury is results from localized or generalized impairment of oxygen and nutrient delivery and waste product eliminate from cells of the kidney (Le Dorze, 2009). Renal ischemia reperfusion injury is common cause of acute kidney injury (Ishani, Xue et al. 2009). The kidneys are particularly susceptible to ischemic injury and represents a challenge in various clinical disorders such as renal transplantation (Ellenberger, 2006, Hoste, 2006), renal artery angioplasty, sepsis, cardiopulmonary bypass and aortic bypass surgery, certain hypertensive states and by the action of vasoconstrictor drugs (Yun, Duan et al. 2009), hemorrhagic shock, partial nephrectomy (Anaya-Prado, 2002). Oxidative stress has been validated to contribute to the pathogenesis of renal IRI (Nath, 2000). Increased intracellular reactive oxygen species (ROS) was suggested to cause renal apoptosis of tubular epithelial cells (Havasi, 2011). Furthermore, oxidative stress could be included in kidney glomerular damage caused by several pro-inflammatory mediators that lead to (ROS) production, leukocyte activation and glomerular lesion (Sener, 2006). Nuclear factor kappa B (NF-KB) is transcription factor that controls many cellular processes including cell proliferation, immune and inflammatory responses, apoptosis, migration and differentiation (Ghosh and Hayden 2012). Nuclear factor-kappa B most frequently activated by a wide range of stimuli relevant to renal injury such as cytokines, metabolic stress, pathogen –associated damage and growth factor (Guijarro 2001). It has a complex role in kidney functions and cell cycle changes when cellular stress was provoked, ROS was produced and DNA damage occurred. In addition, NF-KB is involved in the genesis of various inflammatory diseases such as renal IRI, therefore NF-KB activation is extremely essential for inflammatory and oxidative stress pathways in renal IRI (Yan, 2015). Furthermore, NF-KB mediated inflammatory cytokine and chemokine such as TNF-α and interleukin -1β (Nguyen, 2007).Activation of NF-KB can also be affected by Nrf2 target genes such as HO-1(Iskander, 2006, Jun, 2006, Seldon, 2007). This suggests that there is crosslink between Nrf2, NF-KB and inflammation (Reuter, 2010). Nuclear factor elytroid 2-related factor 2(Nrf2) is a basic leucine zipper protein that regulates the expression of antioxidant protein that protect the organs against oxidative damage triggered via inflammation and injury (Gold, 2012). Consistent with it is function, highly expression of Nrf2 in the tissues that are regularly exposed to environmental and metabolic stresses including intestine, lungs, liver and kidneys, where stress-protection mechanisms are vital for the survival of individual cells and whole organ function. The actual stress-sensor is Kelch-like ECH-associated protein 1 (Keap1), which controls the Nrf2 protein level based on cellular stress conditions. Under normal conditions, Nrf2 is degraded by the Kelch-like ECH-associated protein 1 (Keap1)-dependent pathways (Peng, 2013). Keap1 binds to both Nrf2 and cullin (cul3) - based ubiquity E3 ligase complex, causing constant ubiquitination and proteasome degradation of Nrf2. Electrophilic modification of specific Keap1 cysteine, for instance, ROS or dietary photochemical, induces a conformational change in Keap1 preventing Nrf2 ubiquitination. As a result, Nrf2 protein will accumulate translocation to the nucleus and bind to ARE in the promoter of target genes such as hemeoxygenase (HO-1) (Kensler, 2007). Furthermore, Nrf2 has protective effect by blocking NF-KB signaling pathway (Jiang, 2014) . Therefore, Nrf2 has been proposed to the hub defense against oxidative stress in renal IRI (Shokeir, 2014). Canagliflozin is a potent, a sodium glucose co-transporter-2

inhibitor, administered orally, approved recently for treatment of patient with type 2 diabetic mellitus (Sha, 2011, Bode, 2013). Canagliflozin possesses a nephro-protective effect by decreasing Albuminuria and improvement the pathological changes in diabetic nephropathy in rat. Canagliflozin might serve as a new anti-inflammatory drug for acute and chronic inflammatory diseases; it is inhibit the production and release of interleukin -1β, interleukin-6 and TNF-α. These results showed that Canagliflozin might exert anti-inflammatory activity (Xu, Wang et al. 2018). Subsequently, Canagliflozin slowed renal disease progression independently of its glycemic effects (Heerspink, 2017).

MATERIALS AND METHODS

Preparation of animals

In this study, adult male rats of Westar albino type (weighted 300-350 g and aged 18-28 weeks), were purchased from Animal resource center in college of Veterinary medicine-Duhok University. The Animal Care and Research Committee of the University of Kufa approved all experiments. The animals were healthy, they were kept in the animal house of College of Science in Kufa University in temperature controlled (24°C ± 2°C) room with alternating 12-hr light/12-hr dark cycles and were allowed free access to water and diet. After 2weeks of acclimatization in quarantine room, the experiment started.

Ethical Statement

This study was done according to the Guide for the Care and Use of Laboratory Animals Association for Laboratory Animals Science. The Animal Care Committee approved all animal considerations and conventions. All rats sacrifice was performed under ketamine and xylazine mixture anesthesia.

Design of the study

In our experiment study rats were randomized into equal Four groups (6rat each group).Rats was subjected to bilateral renal ischemia for 30 min by clamping and reperfusion for 2 hours (Liu, 2018, Zhou, 2000) as following:

1. Sham group: Rats were undergoing same anesthetic and surgical procedure except for ischemia induction.
2. Control group: Rats were undergoing bilateral renal ischemia for 30 min and reperfusion for 2 hours.
3.Vehicle (DMSO) group for Canagliflozin: Rats were pretreated with (DMSO) by intra-peritoneal injection 30 min before ischemia reperfusion injury (Lim, Bell et al. 2019)and undergo bilateral renal ischemia for 30 min and reperfusion for 2 hours.
4. Canagliflozin treated group: Rats were pretreated with Canagliflozin (1mg/kg/rat) (Nair S, Wilding J, et al .2010) by intra-peritoneal injection 30 min before ischemia reperfusion injury and undergo bilateral renal ischemia for 30 min and reperfusion for 2 hours.

Preparation of Canagliflozin

Pure Canagliflozin powder was purchased from AK scientific, USA Company.

Chemical Names: (1S)-1, 5-anhydro-1-C- [3 - [5-(4-florophennyl) -2– thienyl] methyl] -4- methylphenyl]–D-glucitol hemihydrates

Molecular Formula: C24H25FO5S

Molecular weight: 444.52

Melting point: 103-109 °C

This product soluble in DMSO =85 mg/ml, Water 1.2 mg /ml, Ethanol =85 mg /ml according to AK scientific package insert.

Ischemia reperfusion model

Rats were weighed, anesthetized using an intra-peritoneal injection of ketamine in dose of 100 mg/kg and xylazine in dose of 10 mg/kg. Under sedation (5-10min), rats were placed on its back, fixed their limbs and tail with stickers to ensure their stability during surgery. Hair in the chest area was shaved and the skin disinfected. The reflexes were checked through pinching the tail and hind feet to be sure that the rats were sufficiently anesthetized. By making midline Laparotory incision to expose the abdomen and to expose both renal pedicles, the intestine was retracted. Using the bilateral model of ischemia, then the renal pedicles was isolated where both renal artery and vein were clamped using a non-traumatic micro vascular clamps were positioned around the renal pedicles (Mohammed, 2018), occlusion was confirmed by observing patched blanching of the entire kidney surface and change the color of a kidney from red to dark purple after several minutes. The total time of clamp was 30 minute (Shi, Lei et al. 2019)and during this procedure, 1ml normal saline was administered into abdomen, then the abdomen was covered using warm and moist gauze to keep animals well hydrated. After 30 min, the clamps were removed from pedicles permitting renal blood flow restoration which represent the beginning of the reperfusion phase. The kidney was returned back to its position, then the abdominal cavity incision was sutured in two layers using 3interrupted sutures(Wang, 2012). Post- euthanized by deep anesthesia (Najafi, 2014) and both blood and tissue samples were collected for analysis.

Collection and preparation of samples

Preparation of blood samples for measurement of renal function

At the end of the experiment, rats were anesthetized about (1-2ml) of blood was directly gathered from the heart. The sample of blood was placed in a plane tube at 37°C without anticoagulant, then it will centrifuged at 3000 rpm for 10 min, then serum obtained used for the determination of Urea and Creatinine (Mohammed T J ,et al .2018).

Preparation of Tissue for measurement Nrf2, HO-1, P-Akt, IL-1 β, NF-kB, NGAL and F2-Isoprostane

Renal section taken and homogenized with a high intensity ultrasonic liquid processor in 1:10 (w/v) phosphate buffered saline that contained 1% Triton X-100 and a protease inhibitor cocktail .The homogenate was centrifuged at 3000 rpm for 20 min at 4°C (Liu ,et al ,2018)(Matsuyama M, Funao K, et al 2008). The supernatant was collected for determination of Nrf2, HO-1, P-Akt, IL-1β, NF-kB, NGAL and F2-Isoprostane levels by Elisa technique.

Tissue sampling for histopathology Analysis and damage scores

The kidney tissue sample was fixed in 10% formalin, dehydrated in alcohol series, cleared in xylene and embedded in paraffin block. The tissue slide sections were cut about 5-µm thick horizontal and stain with H and E then sent to histopathology's for histological examination. After fixation, an investigator who was blinded to the experimental treatment groups performed an evaluation of scores. Tissue

sections were examined by light microscopy and graded for degeneration/necrosis (Xie, 2017, Zhou, 2017), using quantitative measurements for the assessing scoring system of tissue damage. The damage of tubule characterized as tubular epithelial swelling, loss of brush border, vacuolar degeneration, necrotic tubules, cast development; the degree of kidney injury was estimated at X40 magnification, the score of histological changes in the kidney were evaluated as previously described by the following Criteria (Yang, 2017, Ka, 2015, Waseem, 2008, Rossoni2004).

Score 0, represents normal
Score1, represent <25% of damage tubules
Score2, represent 25%-50% of damage tubules
Score3, represent 50% -75% of damage tubules
Score4, represent>75% of damage tubules

Statistical analysis

Statistical analyses were performed using SPSS 24.0 for windows. Data were express as mean ± SEM Analysis of Variance (ANOVA) was used for the multiple comparisons among all groups followed by post-hoc test using Bonferroni method. For histo-pathological renal changes, the Mann-Whitney U was used to assess the statistical significance of difference between two groups; the Kruskal Wallis test was used to assess the statistical significance of difference across multiple groups in total severity score. In all test, P<0.05 was considered statistically significant.

RESULTS

Effect of Canagliflozin on kidney markers following renal IRI

Effect on NF-KB

There was the statistically insignificant effect between Control and Vehicle groups P value 0.907. Control group causes significant increase in the mean ± SEM of tissue level of NF-KB (1951.17 ± 8.98 pg/ml, p value 0.001) when compared with Sham group (205.29 ± 33.08 pg/ml). Canagliflozin causes significant decrease in mean ± SEM of tissue level of NF-KB (845.96 ± 14.33 pg/ml, p value 0.001) compared with Control group as shown as shown in the figure (3.1).

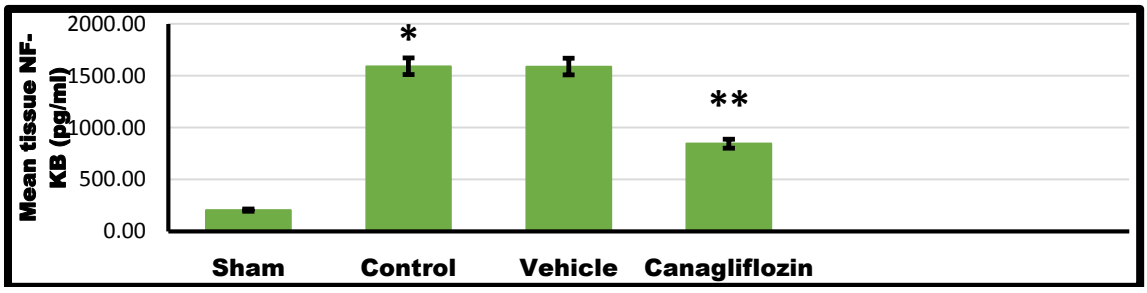

Figure (3.1).Bar chart showing the effect of Canagliflozin on tissue level of NF-kB following renal IRI, expressed as mean ± SEM pg/ml, n=6 in each group.

* P value < 0.05 Control when compared with the Sham group.
**P value < 0.05 Canagliflozin, when compared with Control group.

Effect on IL-1β

There was the statistically insignificant effect between Control and Vehicle groups P value 0.765. Control group causes significant increase in the mean ± SEM of tissue level of IL-1β (954.30 ± 4.86 pg/ml, p value 0.001) when compared with Sham group (126 ± 6.49 pg/ml). Canagliflozin causes significant decrease in mean ± SEM of tissue level of IL-1β (599.05 ± 7.83 pg / ml, p value 0.001) compared with Control groups shown in figure (3.2).

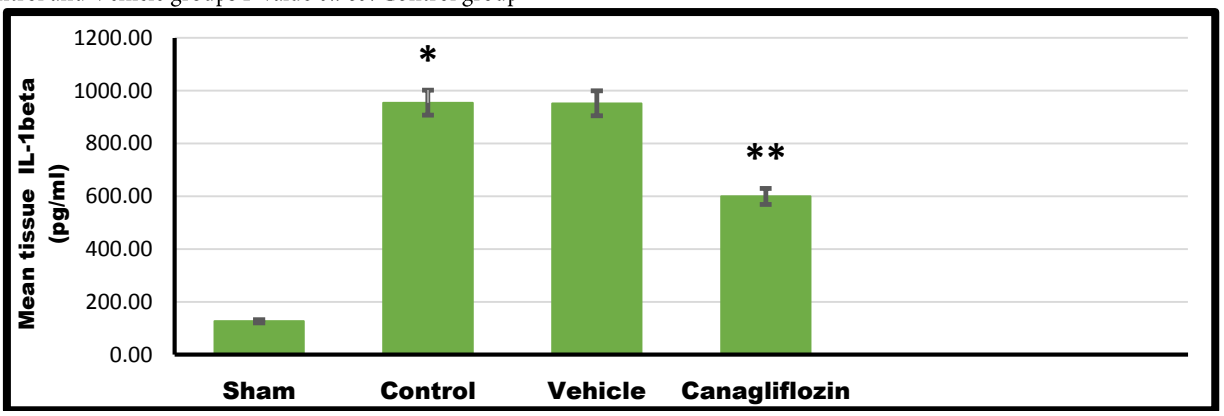

Figure (3.2). Bar chart showing the effect of Canagliflozin tissue level of IL-1β following renal IRI, expressed as mean ± SEM pg/ml, n=6 in each group

*P value <0.05 Control when compared with the Sham group,
** P value < 0.05Canagliflozin, when compared with Control group.

Effect on F2-isoprostane

There was the statistically insignificant difference between Control and Vehicle groups P value 0.782. Control group causes significant increase in the mean ± SEM of tissue level of F2-isoprostane (607.65 ± 5.01 pg/ml, p value 0.001) when compared with Sham group (78.92± 3.46 pg/ml).

Canagliflozin causes significant decrease in mean ± SEM of tissue level of F2-isoprostane (300.98 ± 3.83 pg / ml, p value 0.001) compared with Control group as shown in figure (3.3).

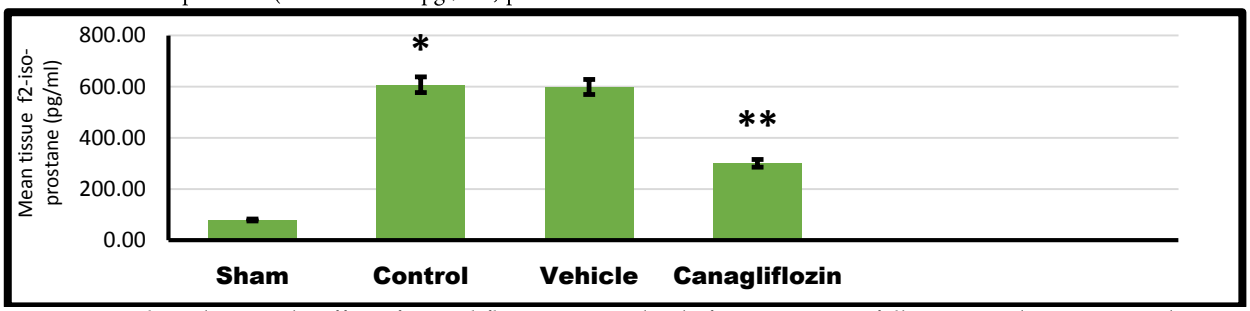

Figure 3.3.Bar chart showing the effect of Canagliflozin on tissue level of F2-isoprostane following renal IRI, expressed as mean ± SEM pg/ml, n=6 in each group

* P value <0.05 Control when compared with Sham group
** P value < 0.05Canagliflozin, when compared with Control group.

Effect on Nrf2

There was the statistically insignificant effect between Control and Vehicle groups P value 0.570.Control group causes significant increase in the mean ± SEM of tissue level of Nrf2 (434 ± 6.22 pg/ml, p value 0.001) when compared with Sham group (94.26±8.26 pg/ml). Canagliflozin causes significant increase in mean ± SEM of tissue level of Nrf2 (765.18 ± 2.97 pg/ml, p value 0.001) compared with Control group as shown in figure (3.4).

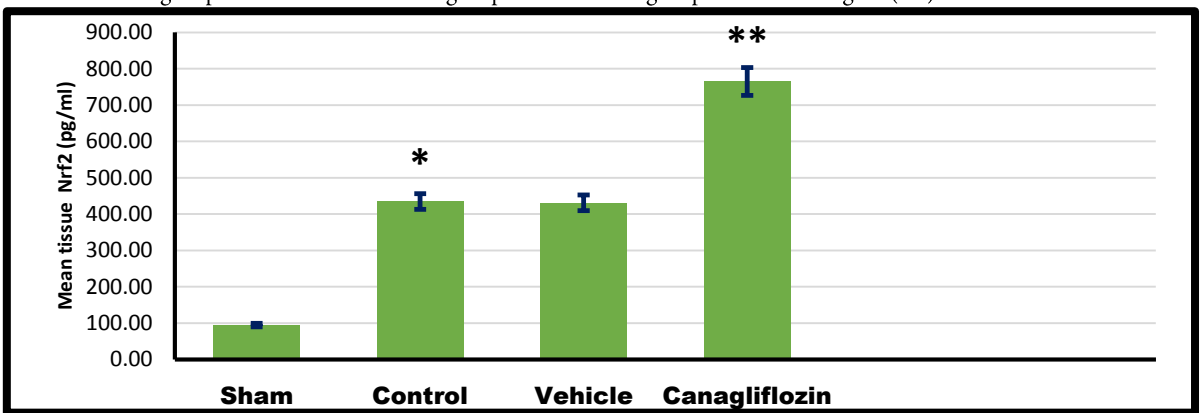

Figure (3.4). Bar chart showing the effect of Canagliflozin on tissue level ofNrf2 following renal IRI, expressed as mean ± SEM pg/ml, n=6 in each group

*P Control when compared with Sham group
**P value < 0.05P value < 0.05when Canagliflozin when compared with Control group.

Effect on HO-1

There was the statistically insignificant effect between Control and Vehicle groups P value 0.956. Control group causes significant increase in the mean ± SEM of tissue level of HO-1 (4.00 ± 0.016 pg/ml, p value 0.001) when compared with Sham group (0.53 ± 0.01 pg/ml). Canagliflozin causes significant increase in mean ± SEM of tissue level of HO-1 (7.39 ± 0.14 pg/ml, p value 0.001) compared with Control group as shown in figure 3.5.

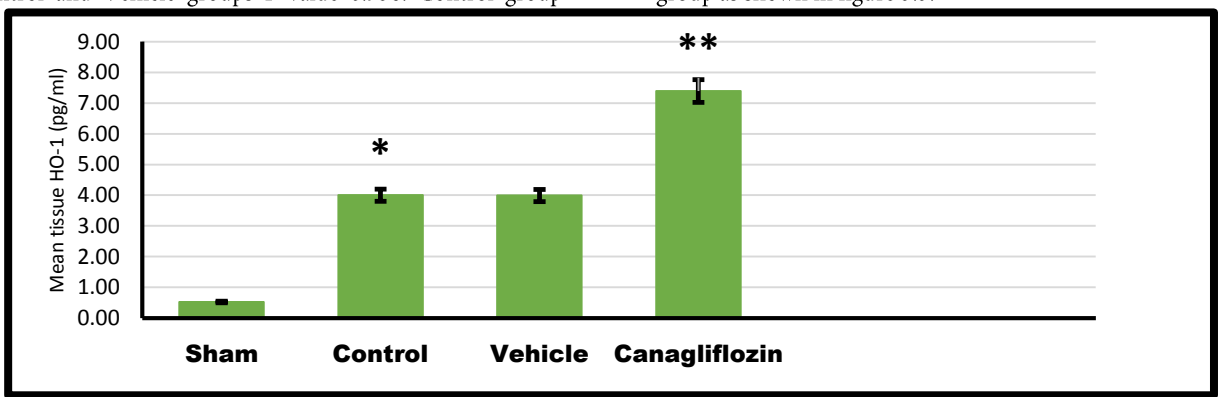

Figure (3.5). Bar chart showing the effect of Canagliflozin on tissue level of HO-1 following renal IRI , expressed as mean ± SEM pg/ml, n=6 in each group

* P value <0.05 Control when compared with the Sham group
**P value < 0.05Canagliflozinwhen compared with Control group.

Effect on P-Akt

There was the statistically insignificant effect between Control and Vehicle groups P value 0.987.Control group causes significant increase in the mean ± SEM of tissue level of P-Akt (296.23 ± 6.40 pg/ml, p value 0.001) when compared with Sham group (108.42 ± 3.42 pg/ml). Canagliflozin causes significant increase in mean ± SEM of tissue level of P-Akt (619.15 ± 5.27 pg/ml, p value 0.001) compared with Control group as shown in figure 3.6.

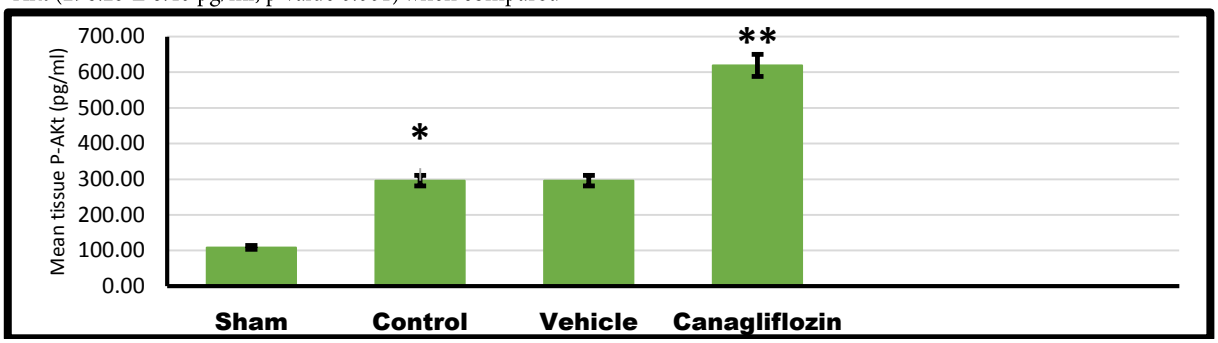

Figure (3.6). Bar chart showing the effect of Canagliflozin on tissue level of p-Akt following renal IRI, expressed as mean ± SEM pg/ml, n=6 in each group

* P value <0.05 Control when compared with the Sham group
**P value < 0.05Canagliflozin, when compared with Control group.

Effect on NGAL

There was the statistically insignificant effect between Control and Vehicle groups P value 0.951.Control group causes significant increase in the mean ± SEM of tissue level of NGAL (1477.32 ± 7.97 pg/ml, p value 0.001) when compared with Sham group (182.84 ± 23.86 pg/ml). Canagliflozin causes significant decrease in mean ± SEM of tissue level of NGAL (1047.86 ± 4.58 pg/ml, p value 0.001) compared with Control group as shown in figure 3.7.

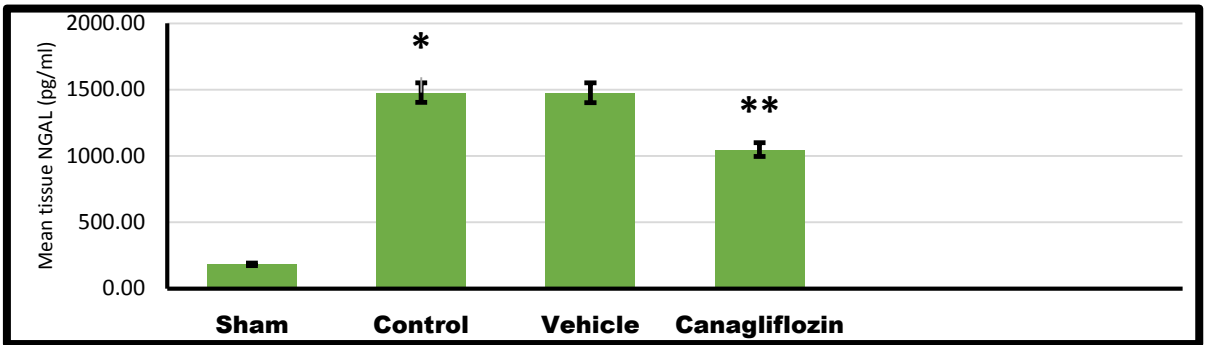

Figure (3.7). Bar chart showing the effect of Canagliflozin on tissue level of NGAL following renal IRI , expressed as mean ± SEM pg/ml , n=6 in each group

* P value <0.05 Control when compared with the Sham group
**P value < 0.05Canagliflozin, when compared with Control group.

Effect on blood Urea

There was the statistically insignificant difference between Control and Vehicle groups P value 0.565. Control group causes significant increase in the mean ± SEM of blood Urea level (91.1 ± 1.579 mg/dl, p value 0.001) when compared with Sham group (22.3± 0.714 mg/dl). Canagliflozin causes significant decrease in mean ± SEM of serum level of urea (55.5 ± 1.231 mg/dl, p value 0.001) compared with Control group as shown in figure 3.8.

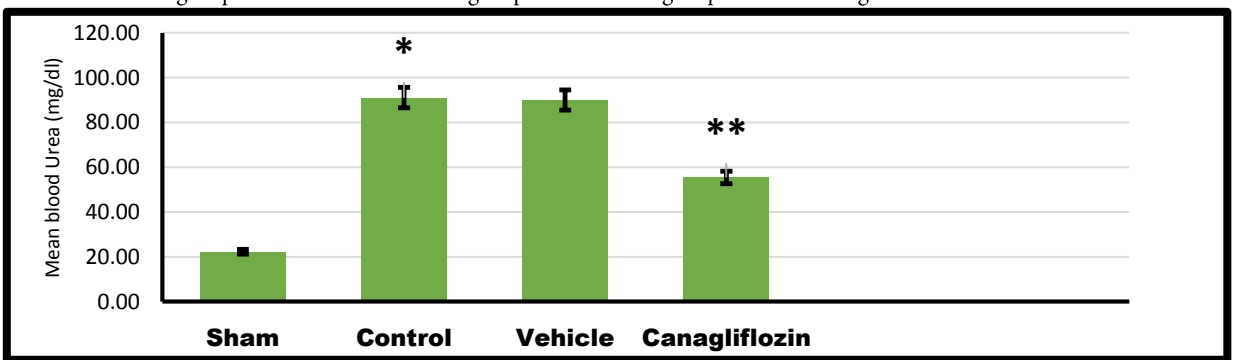

Figure (3.8). Bar chart showing the effect of Canagliflozin level of blood urea following renal IRI, expressed as mean ± SEM mg/dl, n=6 in each group

* P value <0.05 Control when compared with the Sham group
** P value < 0.05Canagliflozin, when compared with Control group.

Effect on serum Creatinine

There was the statistically insignificant effect between Control and Vehicle groups P value 0.09. Control group causes significant increase in the mean ± SEM of serum level of Creatinine (2.1 ± 0.047 mg/dl, p value 0.001) when compared with Sham group (0.6 ± 0.003 mg/dl). Canagliflozin causes significant decrease in mean ± SEM of serum level of creatinine (1.2± 0.047 mg/dl, p value 0.001) compared with Control group as shown in figure 3.9.

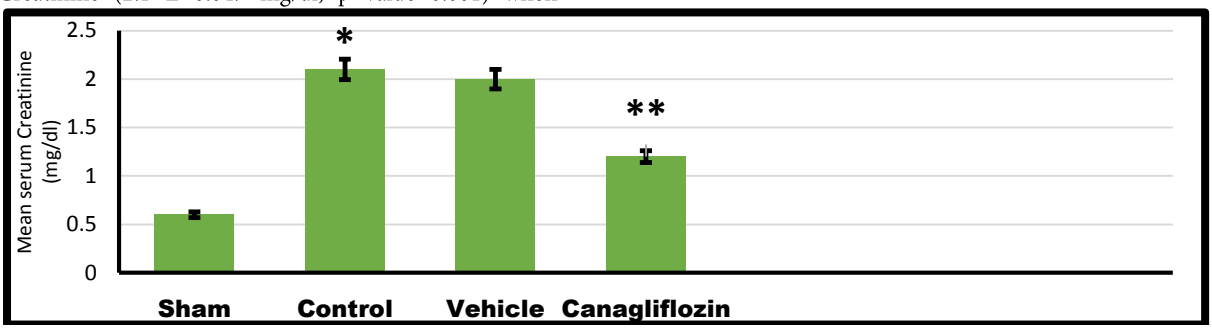

Figure (3.9). Bar chart showing the effect of Canagliflozin serum level of Creatinine following renal IRI, expressed as mean ± SEM pg/ml, n=6 in each group

* P value <0.05 Control when compared with the Sham group

** P value < 0.05Canagliflozin, when compared with Control group.

Histopathology finding

There was the statistically insignificant effect between Control and Vehicle P value >0.05. Figure 3.10, showed the histopathological score in the four experiment groups, Renal IRI causing significant tissue damage (p value 0.001) when compared with Sham group. Figure (3.11) showed normal renal tubule without inducing IRI. (3.12)(3.13) showed significant tissue damage caused by IRI, figure (3.14) showed how pretreatment with Canagliflozin caused significant reduction renal tissue injury.

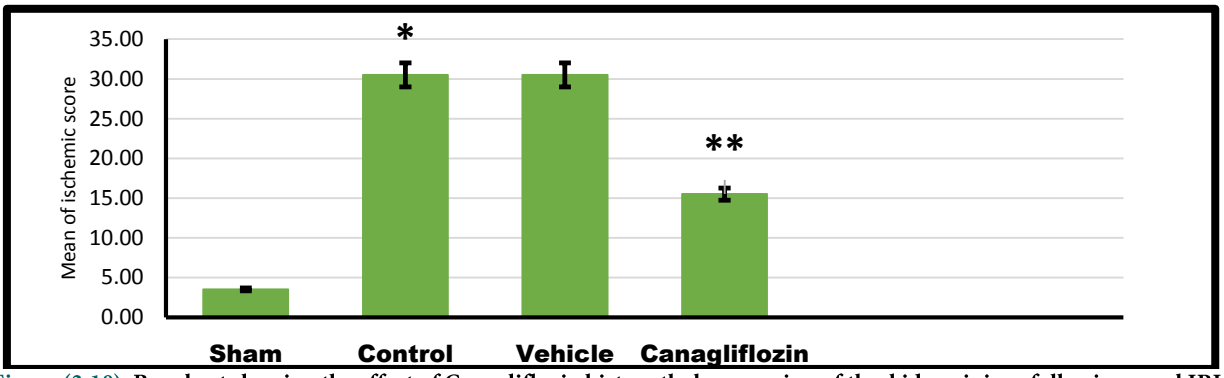

Figure (3.10). Bar chart showing the effect of Canagliflozin histopathology scoring of the kidney injury following renal IRI, expressed as mean ± SEM pg/ml, n=6 in each group

* P value <0.05 Control when compared with the Sham group

** P value < 0.05 Canagliflozin, when compared with Control group.

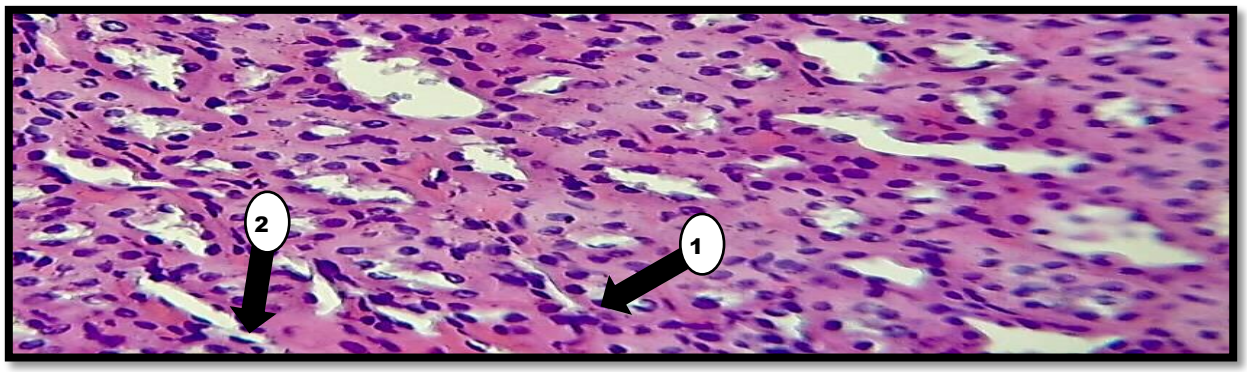

Figure (3.11). Photomicrograph of the renal section for the Sham groups shows the normal renal tubules. The section stained H and E and magnification at (X40), illustrating severity score1 as marked (1) and (2) normal renal tubules.

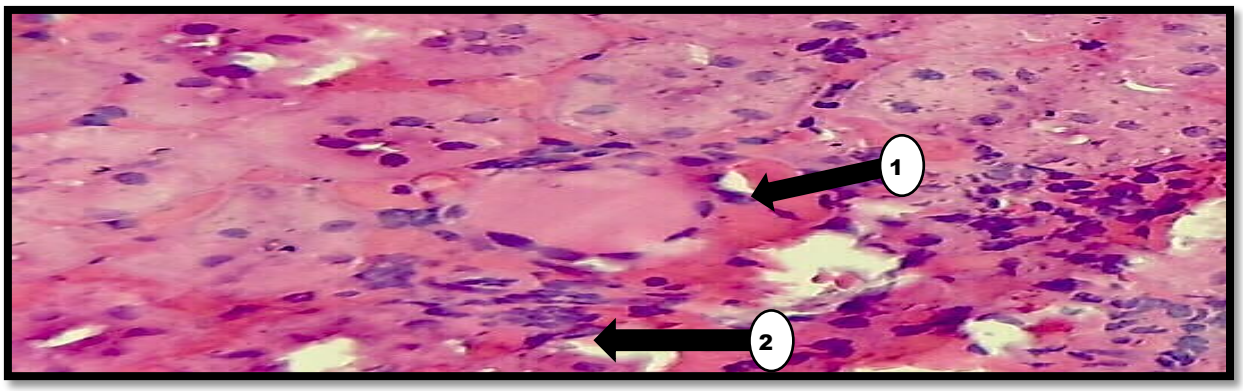

Figure (3.12). Photomicrograph of the renal section for the Control group showed ischemic changes including scattered individual cells with cellular swelling, loss of brush border, eosinophilic cast and karryolysis.

The section stained with H and E and magnification at (X40), illustrating severity score3as marked (1) tubular cellular swelling (2) eosinophilic cast formation and (3) karryolysis.

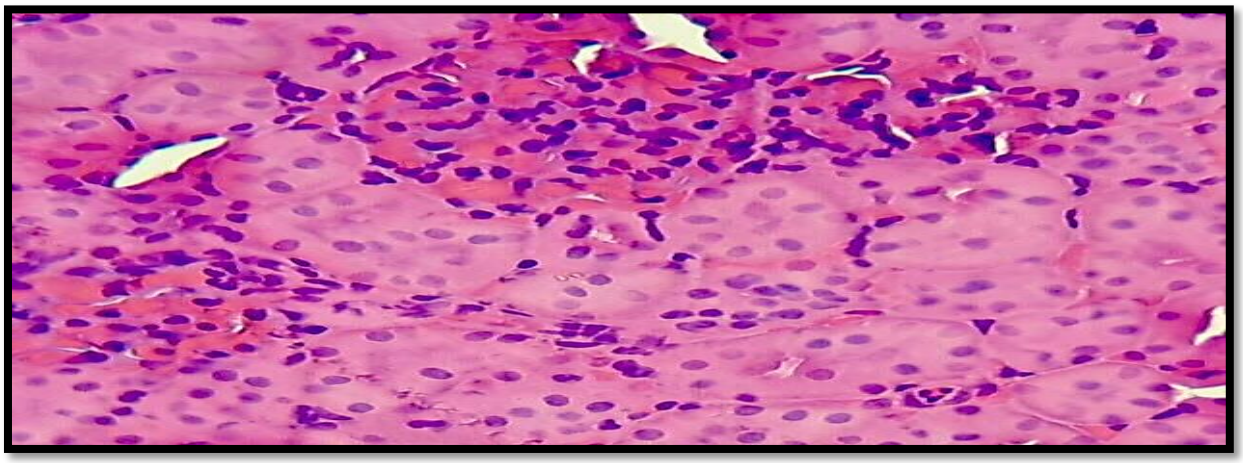

Figure (3.13). Photomicrograph of the renal section for the Vehicle group showed ischemic changes including scattered individual cells with cellular vacuolization, loss of brush border, eosinophilic cast formation and pyknotic nuclei. The section stained with H and E and magnification at (X40), illustrating severity score3 as marked (1) tubular cellular swelling.

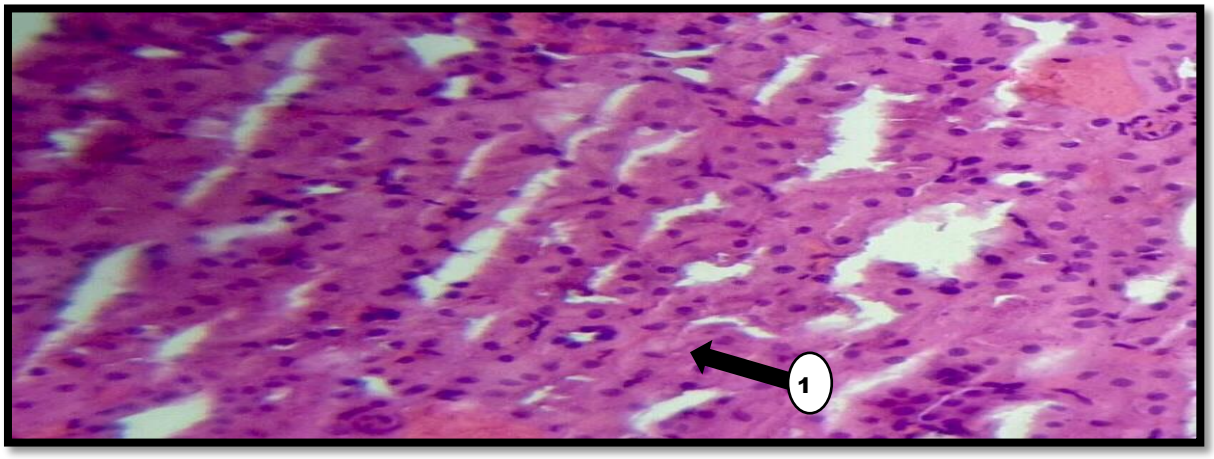

Figure (3.15).Photomicrograph of the renal section for Canagliflozin group showed less scattered individual cells with less cellular swelling, weak eosinophilic cytoplasm without pyknotic nuclei. The section stained with H and E and magnification at (x40), illustrating severity score1as marked (1) and (2) normal renal tubules.

DISCUSSION

Renal ischemia/reperfusion injury is a major cause of AKI (Xie, 2015), it is characterized by reduced blood flow and organ oxygenation (Bonventre, 2011, Malek 2015). Ischemia / reperfusion injury causes several morphological and biochemical alterations including high calcium and intracellular sodium, inflammation, oxidative stress, intracellular ATP depletion, fibrosis, tubular and glomerular damage and cell death by apoptosis or necrosis (Wu, 2018, Farag, 2015). Renal IRI induced apoptosis and necrosis of tubular epithelial cells are the main causes that result in acute

renal failure (Wang, 2011). Reperfusion may be even more harmful than ischemic injury. Through the reperfusion, ROS cause endothelial damage, high micro vascular permeability, produce tissue edema, activate adhesion molecules, release cytokines and lead to systemic inflammatory response (Huang, 2006, Xia, 2016, Gueler, 2007), we estimated the protective effect of Canagliflozin, U-50488H and their combination against experimental renal IRI.

Effect of renal IRI on inflammatory mediators (IL-1β and NF-KB)

This research approved that there is significant increase in renal tissue levels of inflammatory mediatorsIL-1β and NF-KB (P<0.05) when compared with the Sham group after IRI. Mounting study has suggested that inflammation is a hallmark of AKI (Salvadori, 2015). The concentrations of inflammatory markers in kidney tissues of the ischemia/reperfusion group were considerably higher due to increasing Neutrophils infiltration and oxidative stress (Jin, 2010, Eltzschig, 2011). Interluekine-1β is essential pro-inflammatory mediators in kidney ischemia, which produce a number of injurious changes in proximal tubular epithelial cells (ElSabbahy, 2011). Elevation level of NF-KB in ischemia/reperfusion group, when rats subjected to 30 min ischemia and reperfusion for 2 hours(Liu, 2018)and NF-KB possess critical role in the pathogenesis of renal ischemia/reperfusion injury (Cao, 2010). Nuclear factor-Kappa B is transcription factor activated by cytokines and chemokine after acute kidney injury (Sanz, 2010).

Effect of Canagliflozin inflammatory mediators (IL-1β and NF-KB)

The exiting study was found that there is significant decrease in renal tissue levels of IL-1β and NF-KB, (p<0.05) for Canagliflozin pretreated group as compared to Control group. Those findings suggest that Canagliflozin exhibited anti-inflammatory protective role against renal IRI damage. The occurrence of acute kidney injury tended to be decreased, or at least not increased in SGLT2 inhibitor treated subjects in the CANVAS program (Neal, 2017, Xu, 2018) supported the finding that Canagliflozin markedly decreased pro inflammatory cytokines including IL-1β and TNF-α, also according to previous data suggested that Canagliflozin could suppress vascular inflammatory signaling, which is associated with the development of cardiovascular disease (Mancini, Boyd et al. 2018). Previous experimental studies showing that in the renal tissues of animals treated with SGLT2 inhibitors, the marker of inflammation NF-KB reduced (Tahara, Takasu et al. 2017).To the best of our knowledge, there is no data available about the effect of Canagliflozin on NF-KB in renal ischemia/reperfusion injury.

Effect of renal IRI on oxidative stress marker F2-isoprostane

In this study the renal tissue level of f2-isoprostane was significantly increase (p<0.05)in the Control group as compared with the Sham group. Renal dysfunction is frequently is associated with oxidative stress, as levels of different markers including F2-isoprostane and malonyldialdehyde are increased in patients with vary degree of kidney function including patients with ESRD (Karamouzis, 2008, Ferretti, 2008). Elevated level of synthesis F2-isoprostane is inversely associated with GFR. Therefore, F2-isoprostane level is marker of oxidative stress, increased significantly early through the progression of CKD (Dounousi, 2006). The outcome within the study is associated with other studies that indicated that the level ofF2-isoprostane has been increased in renal IRI group (Carlström, 2010).

Effect of Canagliflozin on oxidative stress marker F2-isoprostane

In this study, we observed that pretreatment with Canagliflozin significantly decreased tissue level of F2-isoprostane in renal IRI (p<0.05) when compared with Control group. In a previous study, indicated that antioxidant effects of Canagliflozin by decreased level of F2-isoprostane (Abdelrahman, 2019).

Effect of Nrf2 and HO-1 on renal IRI

In this study, the renal tissue level of Nrf2 and HO-1 was significantly increased (p<0.05) in the Control group as compared with the Sham group. This outcome is consistent with previous study suggested that the Nrf2 pathway is important in protection against IRI (Choi, 2014). Liu, 2009 reported that deficiency of Nrf2 caused worsened ischemic kidney injury in animal model. The protective role of Nrf2 has shown in lowering renal and possibly even systemic inflammation among hem dialysis patients (Shokeir, 2014). Heme oxygenase-1 is induced via several stimuli and considered as a sensitive indicator of cellular stress. Up-regulation of HO-1 is adaptive mechanism that protects cells from stress such as hypoxia, ischemia and inflammation (Abraham, 2008).

Effect of Canagliflozin on Nrf2 and HO-1

Canagliflozin pretreated group showed a significant increase in tissue level of Nrf2 and HO-1 (p<0.05) when compared with Control group**.**

To the best of our knowledge, there is no previous study to investigate the effect of Canagliflozin on Nrf2 and HO-1 in renal ischemia/reperfusion injury in animal model, the results of our study about the effect of Canagliflozin on Nrf2 and HO-1 is probably due to the anti-inflammatory and anti-oxidant effect.

Effect of P-Akton renal IRI

In this study the renal tissue level of P-Akt was significantly increase (P<0.05) in the Control group as compared with the Sham group after IRI. Previous research demonstrated that the phosphorylated protein kinase B which was a cell survival regulation pathway could protect the kidney of rat from apoptosis via enhancing antioxidant capacity and reducing apoptotic protein content **(Liu, Ma et al. 2012)**. Phosphorylation of Akt was increased after ischemia/reperfusion of kidney.

Effect of Canagliflozin on P-Akt

Our results indicated that pretreatment with Canagliflozin significantly increase tissue level of P-Akt in renal IRI (P<0.05) when compared with Control group. This Concomitant with previous study that found a significantly increased Akt phosphorylation in Canagliflozin treated infracted heart through myocardial ischemia/reperfusion (Sayour, 2019).To the best of our knowledge, there is no data available about the effect of Canagliflozin on P-Akt in regional renal ischemia/reperfusion injury.

Effect of renal IR on renal Injury marker NGAL

The new marker has entered recently is NGAL, shown to be most expressed protein in the kidney a short time before Creatinine after ischemic injury. Therefore, renal function can be monitored using NGAL biomarker. After acute kidney injury NGAL protein is early identified as one of the earliest markers of renal damage after ischemic injury in animal models (Ozbilgin, 2016, Mishra, 2005).In this study the renal tissue level of NGAL was significantly increase ($p<0.05$) in the Control group as compared with the Sham group after IRI. This result is consistent with other studies that predicated NGAL has been introduced as a biomarker of AKI (Singer, 2013). Neutrophils gelatinase- associated lipocalinis produced by injured tubular epithelial cells (Mishra, 2003).Elevated NGAL level was already by 10 min ischemia and was further elevated significantly from 10 min to more severe 20 min or 30 min renal ischemia. There is evidence that NGAL detects renal injury before kidney function impairment in clinical trials (Haase, 2011).

Effect of Canagliflozin on NGAL

Pretreatment with Canagliflozin causes significant reduced tissue level of NGAL ($P<0.05$) when compared with Control group. This outcome is linked with many studies that demonstrated that Canagliflozin significantly improved most structural and functional features of the affected kidney by quantify the degree of renal damage via used several traditional indicators such as Urea and Creatinine as well as a novel biomarker NGAL 2019).

Effect of renal IRI on renal function parameters (Urea and Creatinine)

The experimental study confirmed that the Control group causes significant increase ($P<0.05$) in blood Urea and serum Creatinine levels when compared with Sham group. These observations were in agreement with others studies (Salahshoor, 2019, Kohansal, 2019, Kianian, 2019). Blood Urea and serum creatinine levels are the most commonly used markers in evaluating renal function. Significantly increased Urea and Creatinine levels are typical indicators of acute kidney injury (Molina, 2005, Visnagri, 2015, Kaya, 2015). A swift change in serum Creatinine is largely frequent sign of AKI. Due to excess of Creatinine, acute inflammatory edema and tubular necrosis formation are accompanied by significant changes in the incidence of cellular proliferation. There is ample reports concerning reduce in GFR of ischemia/reperfusion in rats model because of remarkable increase in blood Urea and serum Creatinine levels which are in accordance the earlier findings (Korkmaz 2010).

Effect of Canagliflozin on renal function parameters (Urea and Creatinine)

In this study, Canagliflozin significantly lower the levels of blood Urea and serum Creatinine ($P<0.05$) when compared with control group. Those finding demonstrated preservation of renal function. Our results are in accordance with many studies that demonstrated this effects (Badreldin, 2019). The effect of Canagliflozin on intra-renal hemodynamic are likely crucial contributor to the useful effects of it on kidney function (Cherney, 2014), lowering of intra-glomerular pressure manifests clinically as an acute reduction in GFR and Albuminuria, both of which have been demonstrated to occur with Canagliflozin (Perkovic, 2013). This study is also supported by recent outcome trials showing that Canagliflozin stabilized kidney function (Neal, 2017).

Effect of renal IRI on renal parenchyma

The total severity scores of the sections of Control group was significantly higher than that of Sham group($P< 0.05$), After renal IRI histological examination of sections from Control group exhibit more tissue injuries including disruption of normal kidney architecture with marked glomerular damage and congestion, inflammatory cell infiltration, epithelial atrophy and cell desquamation in the tubules, loss of brush border, hyaline cast formation, interstitial expansion, tubular dilation, vacuolar degeneration and edema of the tubules (Kinra, 2019).

Effect of Canagliflozin on renal parenchyma

Treatment of rat with Canagliflozin lowered kidney injury dramatically compared with Control group ($P<0.05$) and the total severity score mean of this group confirmed a slight kidney injury. The prevailing study verified that Canagliflozin, which administered before renal IRI, caused ameliorated renal injury during histopathological parameters. The results are in settlement with other study showed that Canagliflozin at tenu at edhistopathological changes by it is protective effect due to anti-inflammatory and antioxidant effects (Abdelrahman, 2019).

CONCLUSION

From the overall results, we conclude that Canagliflozin significantly decrease renal ischemia reperfusion injury in rat model through Nrf2 pathway via it is pleiotropic effects as anti-oxidant, anti-inflammatory and anti-apoptotic.

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