Websites:
http://www.nbmedicine.org
http://www.sciencepub.net/nurse

Emails:
editor@sciencepub.net
nbmeditor@gmail.com

Biomedicine and Nursing

MARSLAND PRESS
Multidisciplinary Academic Journal Publisher

SGLT2 inhibitor empagliflozin monotherapy alleviates renal oxidative stress in albino wistar diabetic rats after myocardial infarction induction and its underlying mechanisms

Ahmed S. Ahmed (corresponding author)

Lecturer, Anatomy and embryology department, College of medicine, Tanta university.
Tanta, Egypt. Mail: Ahmedsahmed.tanta@gmail.com

Abstract: Introduction, Acute kidney injury (AKI) is a sudden insult of kidney that happens within a short period of time which is associated with poor prognosis in diabetic patients with myocardial infarction (MI). Subclinical AKI is a condition in which tubular damage biomarkers [Neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1(KIM-1)] are positive even in absence of elevated serum creatinine. Recent studies reported that SGLT-2 inhibitors could protect against subclinical AKI in diabetic patients by elevating the level of β-Hydroxybutyric acid (βOHB). The aim of this study is to examine the reno-protective potential of empagliflozin (EMPA) against MI associated AKI in diabetic rats. **Material and methods,** 80 Albino Wistar rats were divided into; (1) non diabetic sham group (CS), (2) non diabetic + myocardial infarction group (CM), (3) diabetic + myocardial infarction group (DM) and (4) diabetic + myocardial infarction + empagliflozin group (DME). At the end of experiment, blood samples and kidneys were collected for biochemical analysis histopathological and immunohistochemical studies. **Results,** after induction of myocardial infarction, there was a significant decrease in serum creatinine and NGAL levels in DME. After EMPA administration, mesangial matrix index and glomerular area were lowered in DME if compared to DM group. As a marker for tubular injury we used anti-NGAL and anti-KIM-1 immunohistochemistry. Strong positive reaction was noticed in DM group if compared to DME group which showed weak positive reaction. Levels of renal mRNAs [NGAL; KIM-1; Nox-2,4; TLR-2,4; MyD88; TNF- α and IL-1 β, 18] in DME group were reduced significantly if compared to DM group**. Conclusion,** Empagliflozin can protect against subclinical acute kidney injury in diabetic albino wistar rats after myocardial infarction induction which could improve the clinical outcome of SGLT-2 inhibitors in diabetic patients.
[Ahmed S. Ahmed.**SGLT2 inhibitor empagliflozin monotherapy alleviates renal oxidative stress in albino wistar diabetic rats after myocardial infarction induction and its underlying mechanisms.** *Biomedicine and Nursing* 2023; 9(3):63-76]. ISSN 2379-8211 (print); ISSN 2379-8203 (online). http://www.nbmedicine.org. 09. doi:10.7537/marsbnj090323.09.

Key words: SGLT2 inhibitor; Empagliflozin; Renal; Myocardial infarction; AKI

Introduction

Acute kidney injury (AKI) has emerged as a major health problem affecting millions of patients associated with decreased in their survival rate(1), it is defined as an abrupt insult affecting kidney functions that occurs within a few hours or a few days(2), it occurs in 37% of intensive care unit patients(3), and affects 2200 per million population(4). It was suggested that inflammation plays a key pillar in acute kidney injury (AKI) pathogenesis. Myocardial infarction (MI) is one of the risk factors that predispose to AKI mainly in type 2 diabetic patients (5).

Neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1(KIM-1) are considered novel kidney biomarkers for MI- associated AKI (6). Previous studies have reported that the diagnosis of clinical AKI is delayed by 48 hours compared to diagnosis of the condition identified by using NGAL (7). Subclinical acute kidney injury is characterized by normal (serum creatinine level & urine output) and elevated kidney biomarkers (NGAL and KIM-1) (8, 9). The mere increase in kidney injury markers can be a hallmark of kidney injury despite normal serum creatinine level (10, 11, 12).

Diabetes mellitus can facilitate the occurrence of AKI via oxidative stress pathway and activation of renal toll-like receptors responsible for the innate immune response regulation (13). To the best of our

knowledge it has been a vague fact whether hypoglycemic drugs could interfere with AKI in diabetic patients. Recent clinical trials reported the beneficial effect of Sodium-glucose co-transporter-2 (SGLT2) inhibitors on the kidney functions in diabetic patients and suggested that SGLT-2 inhibitors could protect against AKI in diabetic patients by elevating the level of β-Hydroxybutyric acid (βOHB) which favors the antioxidant state of the kidney tissue(14, 15, 16). But it remains a question, whether other SGLT-2 inhibitors could play a reno-protective role in cases of myocardial infarction (17, 18, 19). The aim of this study is to examine the reno-protective potential of empagliflozin (EMPA) against MI associated AKI in diabetic rats.

Material and methods

Animals:

80 Albino Wistar rats were used (Average weight 200 gm and average age 6 months). Animals were housed individually. Free access to chow and water was allowed.12 light/dark cycle was kept. By the help of air conditions, the temperature was kept 25º C. This research study was performed in accordance to national and institutional guidelines and approved by Research and Ethics Committee, Quality Assurance Unit, Faculty of Medicine, Tanta University, Egypt.

Experimental design:

40 rat were used for control groups, the remaining 40 rats received single intraperitoneal injection of streptozotocin (40 mg/kg b.w.) to induce diabetes, after one week, blood glucose (BG) measurement were done using Glutest-mint (Sanwa Kagaku Kenkyusho, Co. Ltd. , Japan) to ensure the diabetic condition of the rats (Note: Rat was considered diabetic if BG >300 mg/dl). Rats were divided into four groups (n=20); Control (non diabetic) sham group (CS), Control (non diabetic) + myocardial infarction group (CM), Diabetic + myocardial infarction group (DM) and Diabetic + myocardial infarction + empagliflozin group (DME). CS, CM and DM groups received vehicle, dimethyl sulfoxide (Sigma-Aldrich Chemie, Germany). DME group received empagliflozin (EMPA) (Sino pharm Chemical Reagent Co., Ltd., China) [Dosage 15 mg/kg b.w. daily by subcutaneous injection] for 28 days. At day 29, chow was removed from cages for 12 hours. Blood glucose measurements were done. β-Hydroxybutyric acid (βOHB) was assessed using Abbott Precision Xceed (Medaval Co., Ltd., Ireland), then myocardial infarction induction was performed under general anesthesia in accordance to (20). Briefly, rats were collected, anesthetized, intubated for operation (Note: Respiration was assisted by Rodent Ventilator, code 7025 purchased from Ugo Basile Co., Ltd., Italy. Thoracotomy was done, heart was expose, left coronary artery (marginal branch) (magnification) was ligated using 5-0 silk thread (company) Surgical incision was repaired. Food was restricted for 12 hours postoperative. Blood samples were collected, serum separated by centrifuge at 3000 rpm for biochemical analyses analysis and kept at -80 degree Celsius. All rats were sacrificed under anesthesia. Right kidneys were extracted, stored at -80ºC for biomedical studies. Left kidneys were extracted, fixed in 10% paraformaldehyde solution for histopathological and immunohistochemical studies. Methodology summary is presented in (Figure 1).

Biochemical analysis:

Serum NGAL and creatinine were determined by using ELISA Kit - LS-F2720 (LSBIO, USA) and ELISA Kit - OKEH02617 (Aviva Systems Biology, Corp., USA) respectively.

Histopathological examination:

Hematoxylin and eosin staining was done in accordance to (21). briefly, fresh kidney tissue was cut into 1cm3 cubes immediately after extraction from the rats. It was placed in fixative 10% paraformaldehyde solution and left for 48 hours then placed in tissue processing cassettes. By help of ascending grades of alcohol, kidney tissue is dehydrated to remove water and formalin traces from tissue then immersed in xylene to remove alcohol and facilitate paraffin wax infiltration into the tissue. Cassettes were placed on warm plates then kidney tissue was removed and immersed in paraffine blocks. After paraffine solidification, the blocks were cut into 5 µm thick sections by using manually operated rotary microtome CUT 4050 (4050F, R) (Microtec Laborgeräte GMBH, Germany). Tissue sections were placed on glass microscope slides, rehydrated, stained with hematoxylin (stains nuclei in blue) for 10 minutes and eosin (stains cytoplasm in red) for 10 seconds. The stained tissue sections were dehydrated again by ascending grades of alcohol for 10 minutes than covered by coverslip. Periodic Acid Schiff (PAS) staining was done in accordance to (22) using (PAS) Stain Kit (ab150680) (Abcam, United Kingdom). For Masson trichrome staining we used Trichrome Stain Kit (ab150686) (Abcam, United Kingdom) and protocol was followed. Three fields per section were analyzed by Image J 1.24 v. software.

Immunohistochemical examination:

Immunohistochemistry was done in accordance to (23). Briefly, paraffine embedded tissue sections were sliced (5 µm thick) and mounted to charged slides. Sections were deparaffinized and rehydrated by descending grades of alcohol. Endogenous peroxidase activity was quenched by placing the tissue sections in 3% hydrogen peroxide for 10 minutes. 200 µl of diluted 1ry antibody [Anti NGAL

antibody (Abcam, United Kingdom) dilution 1:100 and Anti KIM-1 antibody (Aviva Systems Biology, Corp., USA) dilution 1:20] were mounted to the tissue after dilution with antibody diluent as per manufacturer protocol (signal stain diluent). Slides were incubated overnight at 4ºC in a humidified chamber. In next morning, slides were washed by wash buffer for 3 minutes then covered with 2 drops of Signal Stain Boost Detection Reagent followed by incubation at room temperature in humidified chamber for 30 minutes.200 µl of SignalStain® DAB (Biocompare, USA) were applied to each section. After staining, slides were immersed in distilled water then counterstained with haematoxyline to stain nuclei in blue for better visualization. Coverslips were applied. Three fields per section were analyzed by Image J 1.24 v. software.

Total mRNA analysis:

Total RNA was isolated from renal tissues by using RNeasy Fibrous Tissue Mini Kit (Qiagen, USA). 30 mg of kidney tissue were lysed in a guanidine-isothiocyanate buffer. After dilution of the lysate, the sample was treated with proteinase K. Debris was pelleted by centrifugation. Ethanol was then added to the cleared lysate, and RNA was bound to the RNeasy silica membrane. Traces of DNA that may copurify were removed by a DNase treatment on the RNeasy spin column. DNase and any contaminants were washed away, and up to 100 µg high-quality total RNA is eluted in 30–100 µl RNase-free water. All assays were performed in duplicate and by the standard curve method using serial cDNA dilution. β-actin served as an internal control (24).

Statistical analysis:

SPSS software, 25 V. (SPSS Inc., USA) was used for data analysis, data were expressed in mean ± standard deviation and probability value was considered significant if <0.05. Tukey-Kramer test was used for groups comparison.

Results

Before myocardial infarction induction, blood glucose level was significantly ($p<0.05$) higher in DM group if compared to CS &CM groups. Upon administration of EMPA, blood glucose in DME group was significantly ($p<0.05$) lowered if compared to DM group. There was insignificant difference between blood βOHB level in CS, CM and DM groups but it increased significantly ($p<0.05$) in DME group if compared to DM group. After myocardial infarction induction, there was a significant ($p<0.05$) decrease in serum creatinine level in DM&DME groups if compared to CS &CM groups. Serum NGAL level was significantly ($p<0.05$) elevated in DM group if compared to CM group and it was significantly lowered in DME group if compared to DM group ($p<0.05$) (figure 2).

Examination of hematoxylin and eosin stained kidney sections did not reveal abnormal histopathological findings in glomeruli or tubules in the four groups (figure 3). Examination of PAS stained sections reveled that glomerular area was larger in DM group if compared to CS group. Mesangial matrix index was higher in DM group if compared to CS&CM groups. Upon EMPA administration, Mesangial matrix index was lowered in DME if compared to DM group (figure 4). Examination of Masson trichrome stained sections demonstrated no interstitial fibrosis in all groups (figure 5).

As a marker for tubular injury we used anti-NGAL and anti-KIM-1 immunohistochemistry. Negative reaction was noticed in CS&CM groups, significant ($p<0.05$) strong positive reaction was noticed in DM group if compared to DME group which showed weak positive reaction (figure 6,7).

Levels of NGAL mRNA and KIM-1 mRNA in the kidney tissue were significantly ($p<0.05$) elevated in DM group if compared to CS&CM groups. Renal tissue Nox-2,4 mRNAs levels in DME group were reduced significantly ($p<0.05$) if compared to DM group. While the levels of TLR-2,4 mRNAs were comparable in CS and CM groups. Renal tissue level of MyD88 mRNA, TNF- α mRNA and IL-1 β, 18 mRNAs were significantly ($p<0.05$) elevated in DM group if compared to CS&CM groups and were reduced significantly ($p<0.05$) in DME group if compared to DM group (Figure 8).

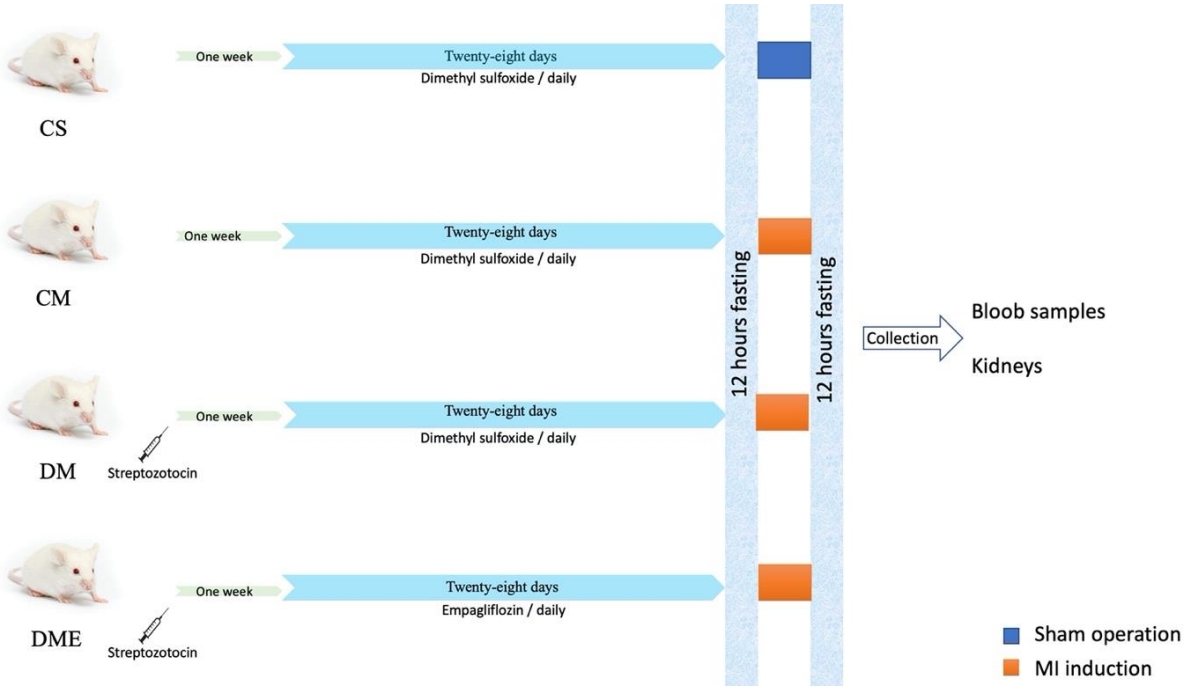

Fig. 1: Schematic representative of experimental study. Rats were divided into four groups; Control sham group (CS), Control + myocardial infarction group (CM), Diabetic + myocardial infarction group (DM) and Diabetic + myocardial infarction + empagliflozin group (DME).

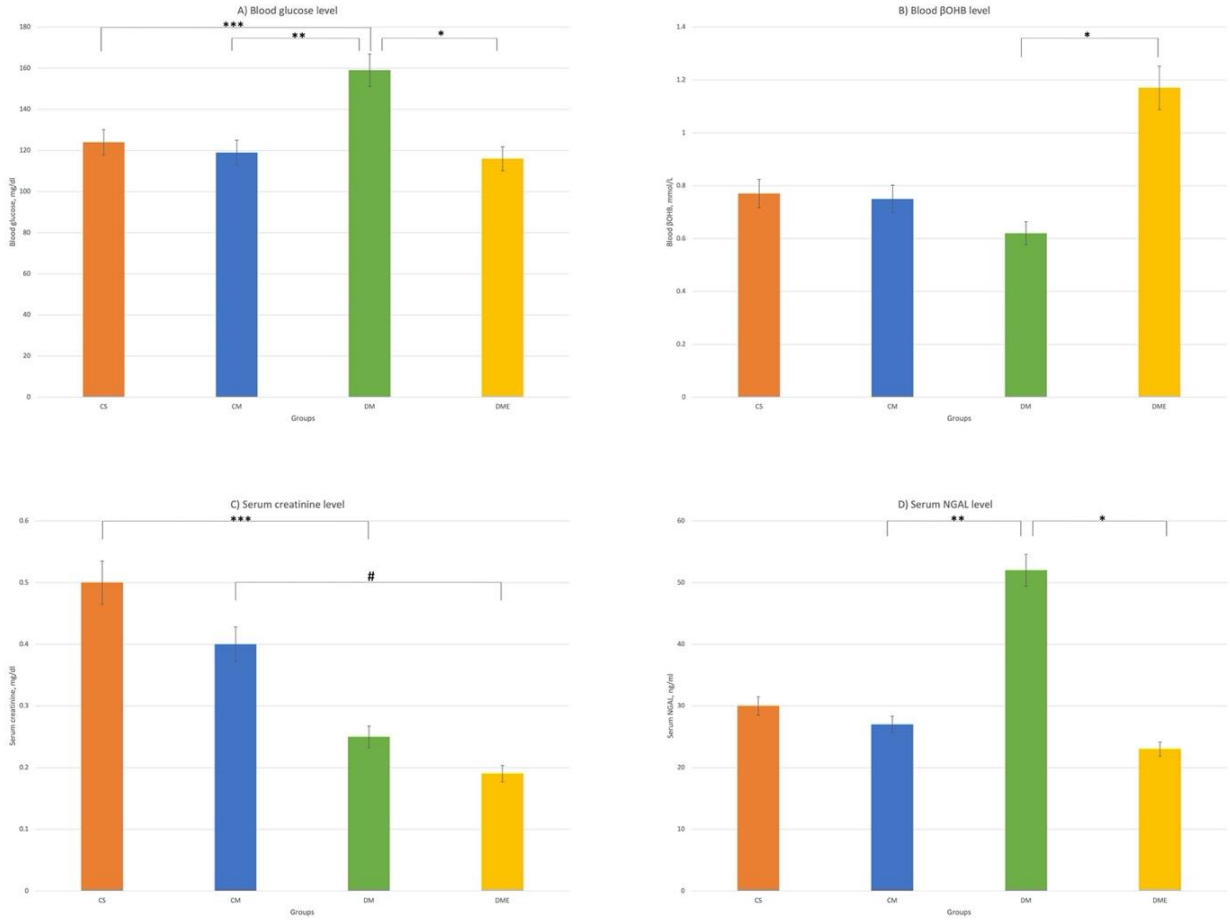

Fig. 2: Represents biochemical analysis of some blood parameters, before (a, b) and after (c, d) myocardial infarction induction. A) Represents blood glucose level which is significantly ($p<0.05$) higher in DM group if compared to CS &CM groups. Level in DME group is significantly ($p<0.05$) lowered if compared to DM group. B) Represents blood βOHB level, there is insignificant difference between CS, CM and DM groups, but level increases significantly ($p<0.05$) in DME group if compared to DM group. C) Represents serum creatinine level, there is a significant ($p<0.05$) decrease in serum creatinine level in DM&DME groups if compared to CS &CM groups. D) Represents serum NGAL level, significantly ($p<0.05$) elevated in DM group if compared to CM group and it is significantly lowered in DME group if compared to DM group ($p<0.05$). * Significant difference ($p<0.05$) between DM&DME groups. ** Significant difference ($p<0.05$) between DM&CM groups. *** Significant difference ($p<0.05$) between DM&CS groups. # Significant difference ($p<0.05$) between CM&DME groups. Data are presented as mean ± standard deviation.

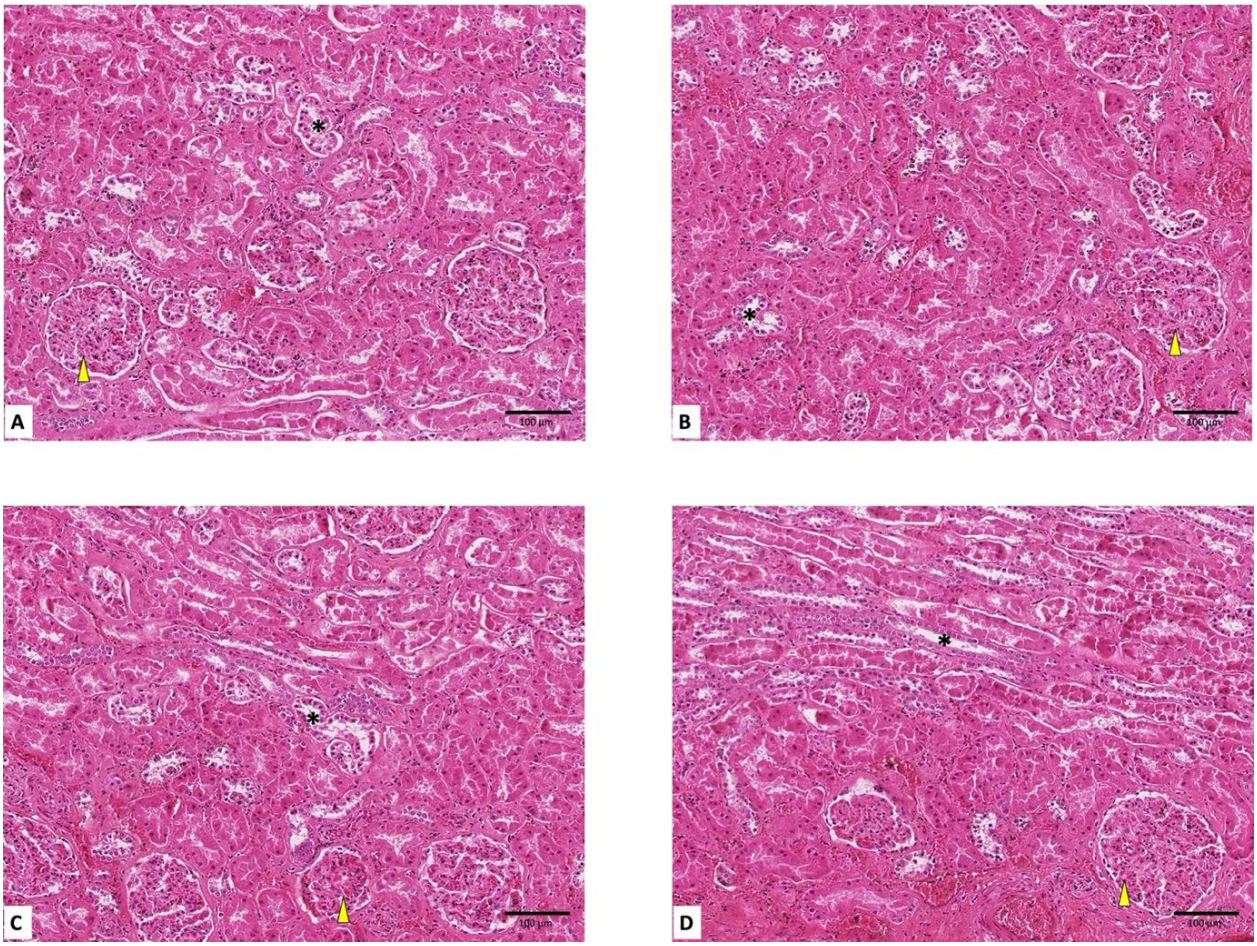

Fig. 3: Photomicrograph of kidney tissue stained with hematoxylin and eosin (X 1000), reveals no abnormal histopathological findings in glomeruli (Yellow arrow) or tubules (Asterisk) in the four groups. (A) Represents CS group, (B) Represents CM group, (C) Represents DM group and (D) Represents DME group.

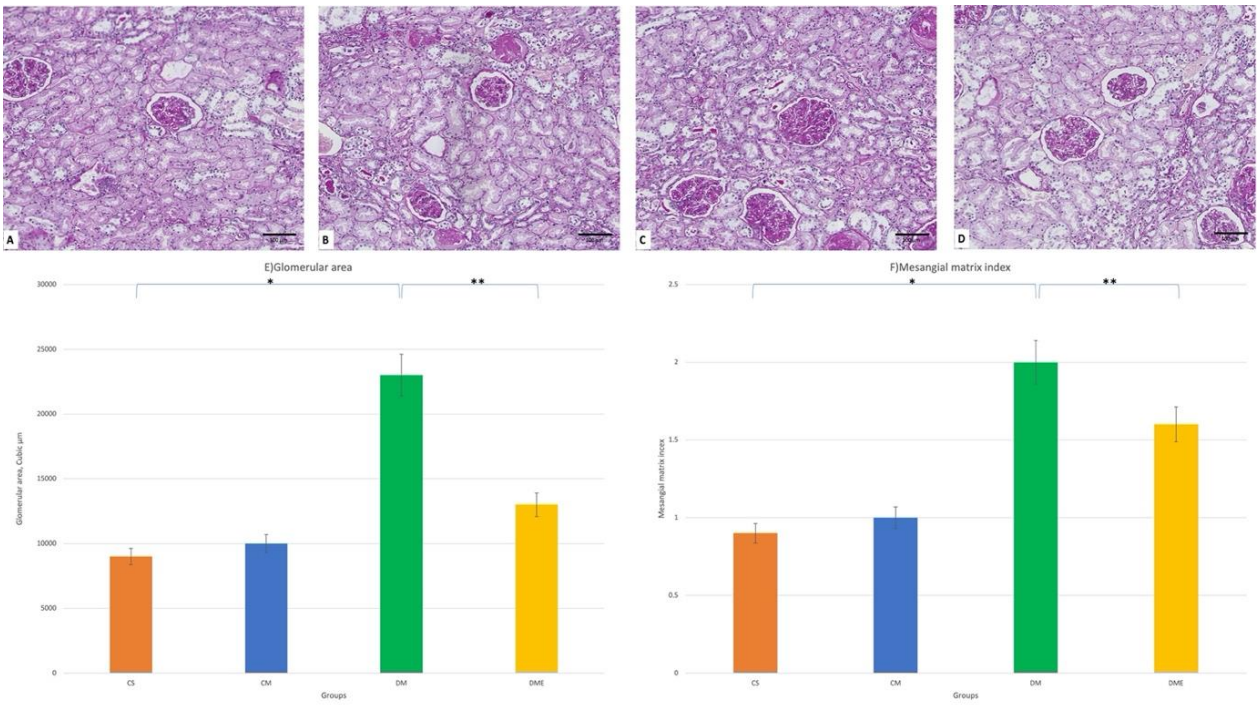

Fig. 4: (A-D) Photomicrograph of kidney tissue stained with PAS (X 1000), reveals that glomerular area is larger in DM group if compared to CS group. (A) Represents CS group, (B) Represents CM group, (C) Represents DM group and (D) Represents DME group. (E) Represents that glomerular area is significantly increased in DM group if compared to CS group. The glomerular area is significantly lowered in DME group if compared to DM group. * Significant difference ($p<0.05$) between DM&CS groups. ** Significant difference ($p<0.05$) between DME&DM groups. Data are presented as mean ± standard deviation. (F) Mesangial matrix index is higher in DM group if compared to CS&CM groups. Upon EMPA administration, Mesangial matrix index was lowered in DME if compared to DM group. * Significant difference ($p<0.05$) between DM&CS groups. ** Significant difference ($p<0.05$) between DME&DM groups. Data are presented as mean ± standard deviation.

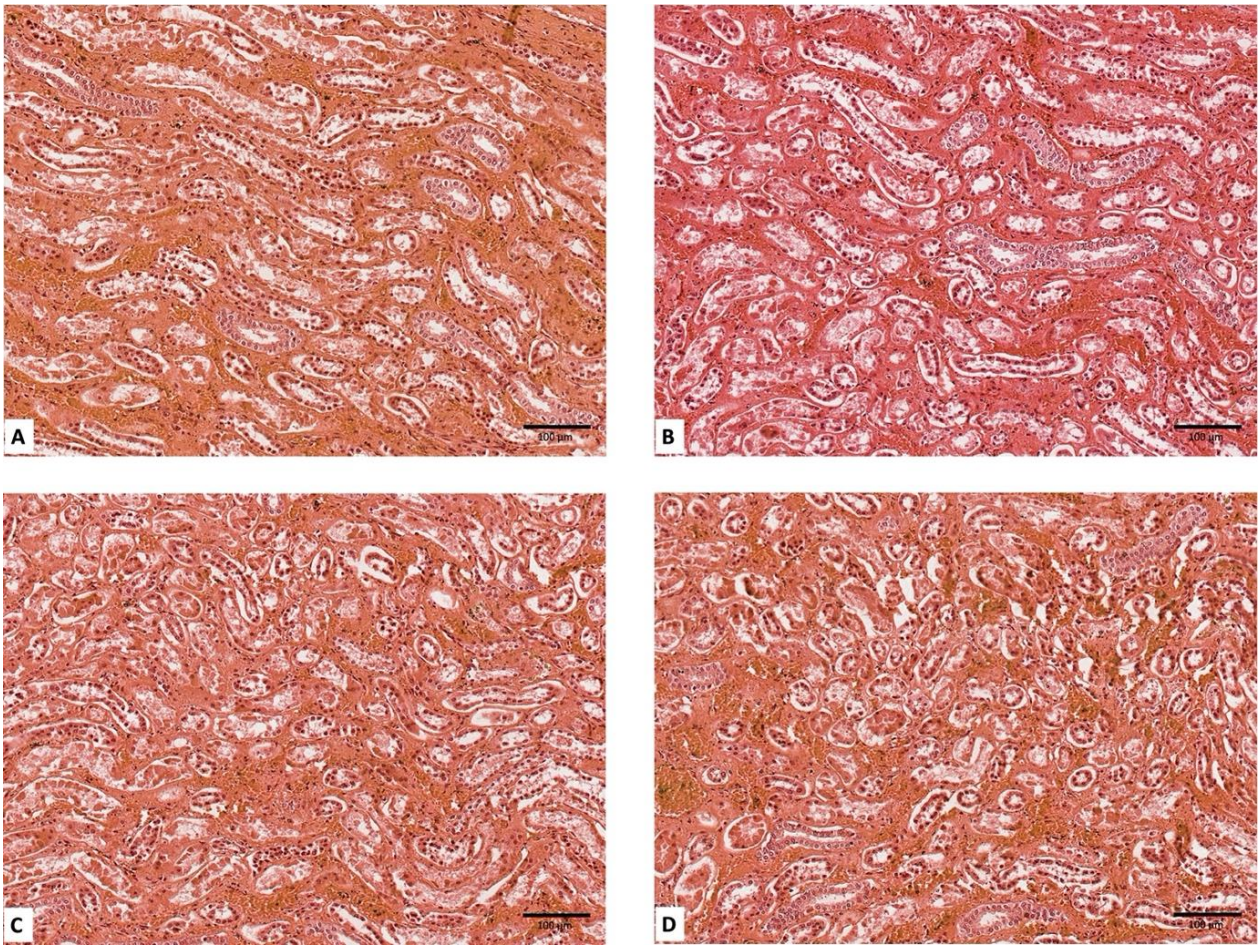

Fig. 5: Photomicrograph of kidney tissue stained with Masson trichrome (X 1000), demonstrates no interstitial fibrosis in all groups. (A) Represents CS group, (B) Represents CM group, (C) Represents DM group and (D) Represents DME group. No interstitial fibrosis is noticed in any group.

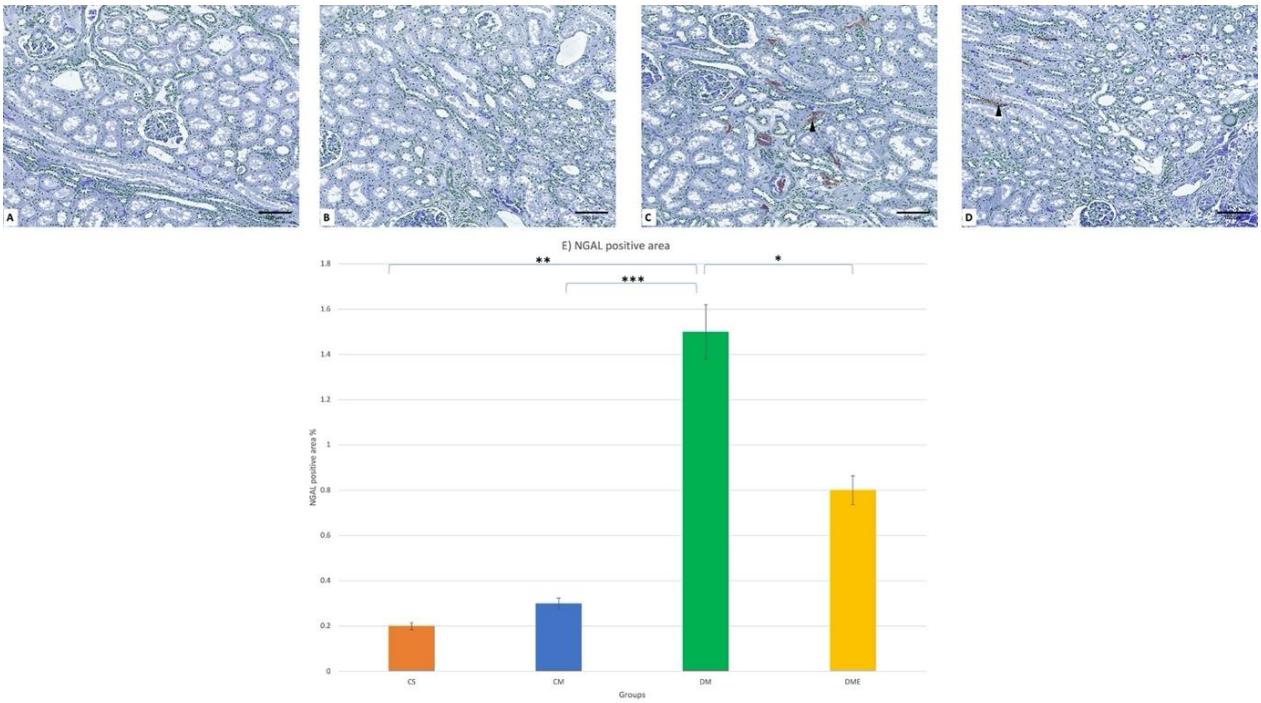

Fig. 6: (A-D) Photomicrographs of kidney tissue stained with anti-NGAL. (A) Represents CS group, (B) Represents CM group, (C) Represents DM group and (D) Represents DME group. Negative reaction was noticed in CS&CM groups, significant strong positive reaction was noticed in DM group if compared to DME group which showed weak positive reaction. (Note: Positive reaction is marked with black arrows).(E) Represents NGAL positive areas. * Significant difference ($p<0.05$) between DM&DME groups. ** Significant difference ($p<0.05$) between CS&DM groups. *** Significant difference ($p<0.05$) between CM&DM groups. Data are presented as mean ± standard deviation.

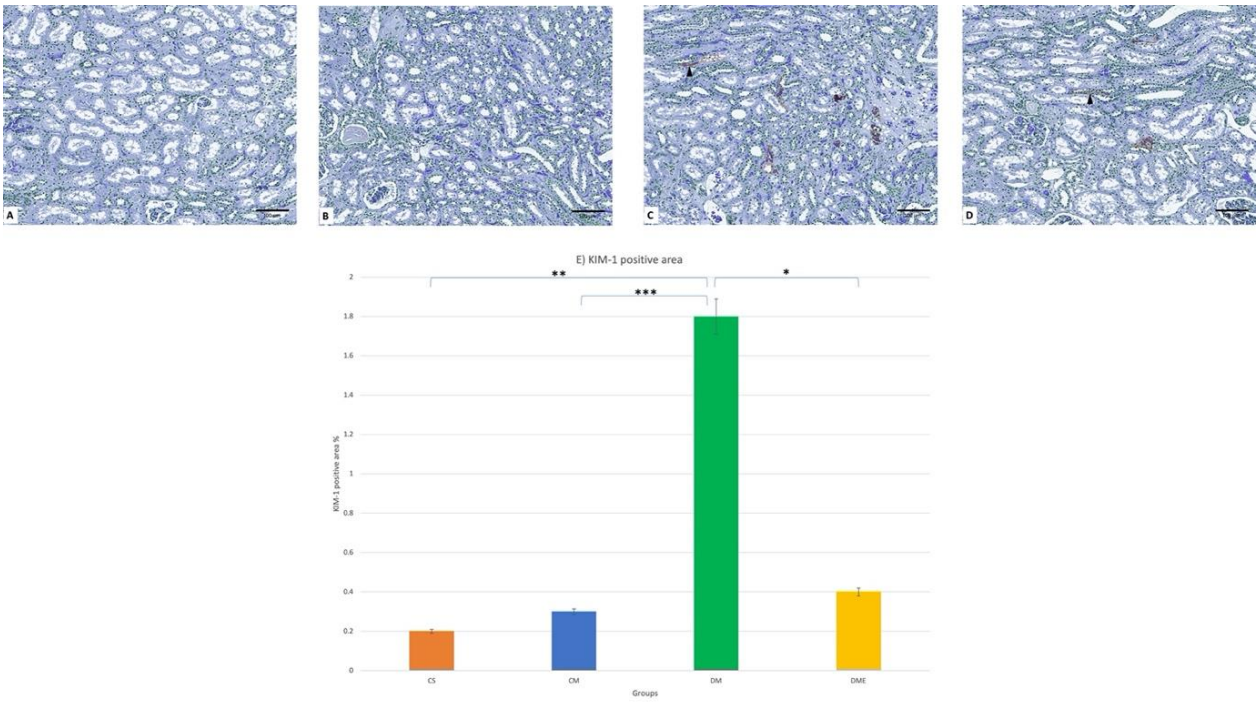

Fig. 7: (A-D) Photomicrographs of kidney tissue stained with anti-KIM-1. (A) Represents CS group, (B) Represents CM group, (C) Represents DM group and (D) Represents DME group. Negative reaction was noticed in CS&CM groups, significant strong positive reaction was noticed in DM group if compared to DME group which showed weak positive reaction. (Note: Positive reaction is marked with black arrows). (E) Represents KIM-1 positive areas. * Significant difference ($p<0.05$) between DM&DME groups. ** Significant difference ($p<0.05$) between CS&DM groups. *** Significant difference ($p<0.05$) between CM&DM groups. Data are presented as mean ± standard deviation.

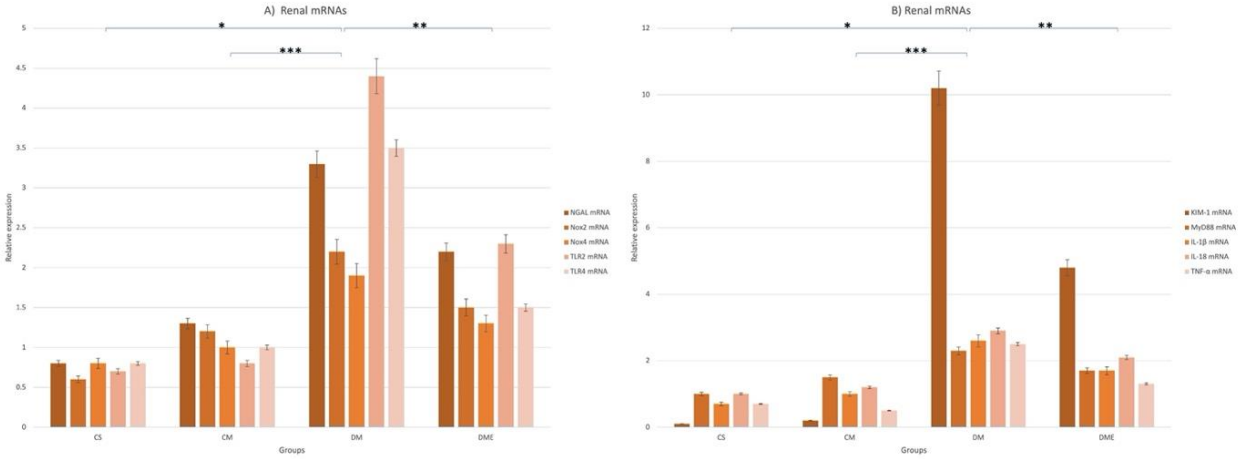

Fig. 8: (A) Represents renal tissue NGAL, Nox2, Nox4, TLR2 and TLR4 mRNAs. (B) Represents renal tissue KIM-1MyD88, IL-1β, IL-18 and TNF- α mRNAs. Levels of NGAL mRNA and KIM-1 mRNA in the kidney tissue are significantly ($p<0.05$) elevated in DM group if compared to CS&CM groups. Renal tissue Nox-2,4 mRNAs levels in DME group are reduced significantly ($p<0.05$) if compared to DM group. While the levels of TLR-2,4 mRNAs are comparable in CS and CM groups. Renal tissue level of MyD88 mRNA, TNF- α mRNA and IL-1 β, 18 mRNAs are significantly ($p<0.05$) elevated in DM group if compared to CS&CM groups and are reduced significantly ($p<0.05$) in DME group if compared to DM group. * Significant difference ($p<0.05$) between DM&CS groups. ** Significant difference ($p<0.05$) between DM&DME groups. *** Significant difference ($p<0.05$) between DM&CM groups. Data are presented as mean ± standard deviation.

Discussion

In our study, before infarction induction, blood glucose level was significantly higher in DM group in comparison with either CS or CM groups which is consistent with diabetic state of the rats in DM group. Blood glucose level in DME group was significantly lowered if compared to DM group as inhibition of SGLT2 blocks reuptake of glucose in the kidney tubules which results in increasing their concentration in macula densa and attenuate kidney hyperfiltration (25). Blood βOHB level increases significantly in DME group if compared to DM group, which provides evidence of the antioxidant properties of empagliflozin (26). After infarction induction, there was a significant decrease in serum creatinine level in DME groups, but Kataoka et al., reported that empagliflozin treatment for 1-2 months, did not affect serum creatinine concentration (27). Serum NGAL level, significantly elevated in DM group if compared to CM group and it was significantly lowered in DME group if compared to DM group which is consistent with both our results shown after total mRNA analysis in kidney tissue and Shang et al., who reported that NGAL is closely associated with AKI (28).

Examination of PAS stained sections reveled that glomerular area was larger in DM group if compared to CS group. The glomerular area and mesangial matrix index were significantly lowered in DME group if compared to DM group. Same results were reported by Albrecht et al., after administration of carnosine. He stated that carnosine treatment restored the glomerular ultrastructure without affecting podocyte number (29). As a marker for tubular injury we used anti-NGAL and anti-KIM-1 immunohistochemistry, strong positive reaction was noticed in DM group if compared to DME group which showed weak positive reaction. Assadi et al., reported that same biomarkers were elevated in patients with circulatory collapse (30). Oshima et al., reported that EMPA reduced oxidative stress in cases of myocardial infarction in diabetic state

which confirm the cardioprotective potential of EMPA (31).

After total mRNA analysis in kidney tissue we noticed that levels of NGAL mRNA and KIM-1 mRNA in the kidney tissue were significantly elevated in DM group if compared to CS&CM groups which is consistent with our immunohistochemistry results. Renal tissue Nox-2,4 mRNAs levels in DME group were reduced significantly if compared to DM group. Given that Nox-2,4 play a crucial role in kidney programmed cell death, our results provide an evidence of reno-protective potential of EMPA against AKI and could be linked to the antioxidant effect of EMPA (32, 33). Our study showed that, the levels of TLR-2,4 mRNAs were reduced significantly in DME group. Taking into account the role of TLR-2,4 in inflammation, apoptosis and tissue injury in several AKI models, our results emphasize the reno-protective role of EMPA (34, 35). After total mRNA analysis in kidney tissue we noticed that levels of MyD88 mRNA (plays a role in acute and chronic kidney injury), TNF- α mRNA (mediator of proximal tubular necrosis and apoptosis) and IL-1β(which is released in response to mitochondrial damage), IL-18 mRNAs (considered as the most promising biomarkers in prediction of AKI) were significantly elevated in DM group if compared to CS&CM groups and were reduced significantly in DME group if compared to DM group (36, 37, 38, 39).

Conclusions

In summary, SGLT2 inhibitor empagliflozin monotherapy protect against subclinical acute kidney injury in albino wistar diabetic rats after myocardial infarction induction which could improve the clinical outcome of SGLT-2 inhibitors in diabetic patients.

Competing interest disclaimer:

Author has declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the author and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the author.

References

[1]. 1- Levey AS, James MT. Acute Kidney Injury [published correction appears in Ann Intern Med. 2018 Jan 2;168(1):84]. Ann Intern Med. 167(9):ITC66-ITC80(2017).

[2]. 2- Farrar A. Acute Kidney Injury. Nurs Clin North Am. 53(4):499-510(2018).

[3]. 3- Gaião SM, Paiva JAOC. Biomarkers of renal recovery after acute kidney injury. Biomarcadores de recuperação renal após lesão renal aguda. Rev Bras Ter Intensiva. 29(3):373-381(2017).

[4]. 4- Fragasso T, Ricci Z, Goldstein SL. Pediatric Acute Kidney Injury. Contrib Nephrol. 193:113-126(2018).

[5]. 5- Vijayan M, Avendano M, Chinchilla KA, Jim B. Acute kidney injury in pregnancy. Curr Opin Crit Care. 25(6):580-590(2019).

[6]. 6- Ghasemi H, Einollahi B, Kheiripour N, Hosseini-Zijoud SR, Farhadian Nezhad M. Protective effects of curcumin on diabetic nephropathy via attenuation of kidney injury molecule 1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) expression and alleviation of oxidative stress in rats with type 1 diabetes. Iran J Basic Med Sci. 22(4):376-383(2019).

[7]. 7- George JA, Gounden V. Novel glomerular filtration markers. Adv Clin Chem. 88:91-119(2019).

[8]. 8- Seibert FS, Sitz M, Passfall J, et al. Prognostic Value of Urinary Calprotectin, NGAL and KIM-1 in Chronic Kidney Disease. Kidney Blood Press Res. 43(4):1255-1262(2018).

[9]. 9- George B, Joy MS, Aleksunes LM. Urinary protein biomarkers of kidney injury in patients receiving cisplatin chemotherapy. Exp Biol Med (Maywood). 243(3):272-282(2018).

[10]. 10- Westhoff JH, Seibert FS, Waldherr S, et al. Urinary calprotectin, kidney injury molecule-1, and neutrophil gelatinase-associated lipocalin for the prediction of adverse outcome in pediatric acute kidney injury. Eur J Pediatr. 176(6):745-755(2017).

[11]. 11- Wołyniec W, Ratkowski W, Urbański R, et al. Urinary Kidney Injury Molecule-1 but Not Urinary Neutrophil Gelatinase Associated Lipocalin Is Increased after Short Maximal Exercise. Nephron. 138(1):29-34(2018).

[12]. 12- Ding Y, Nie LM, Pang Y, et al. Composite urinary biomarkers to predict pathological tubulointerstitial lesions in lupus nephritis. Lupus. 27(11):1778-1789(2018).

[13]. 13- McMurray JJV, DeMets DL, Inzucchi SE, et al. A trial to evaluate the effect of the sodium-glucose co-transporter 2 inhibitor dapagliflozin on morbidity and mortality in patients with heart failure and reduced left ventricular ejection fraction (DAPA-HF). Eur J Heart Fail. 21(5):665-675(2019).

[14]. 14- Ghosh RK, Ghosh GC, Gupta M, et al. Sodium Glucose Co-transporter 2 Inhibitors and Heart Failure. Am J Cardiol. 124(11):1790-1796(2019).
[15]. 15- Donnan JR, Grandy CA, Chibrikov E, et al. Comparative safety of the sodium glucose co-transporter 2 (SGLT2) inhibitors: a systematic review and meta-analysis. BMJ Open. 9(1):e022577(2019).
[16]. 16- Zhao Y, Xu L, Tian D, et al. Effects of sodium-glucose co-transporter 2 (SGLT2) inhibitors on serum uric acid level: A meta-analysis of randomized controlled trials. Diabetes Obes Metab. 20(2):458-462(2018).
[17]. 17- Baruah MP, Makkar BM, Ghatnatti VB, Mandal K. Sodium Glucose Co-transporter-2 Inhibitor: Benefits beyond Glycemic Control. Indian J Endocrinol Metab. 23(1):140-149(2019).
[18]. 18- Usman MS, Siddiqi TJ, Memon MM, et al. Sodium-glucose co-transporter 2 inhibitors and cardiovascular outcomes: A systematic review and meta-analysis. Eur J Prev Cardiol. 25(5):495-502(2018).
[19]. 19- Thomson SC, Vallon V. Renal Effects of Sodium-Glucose Co-Transporter Inhibitors. Am J Cardiol. 124 Suppl 1:S28-S35(2019).
[20]. 20- Ozgun E, Ozgun GS, Usta U, Eskiocak S, Sut N, Gokmen SS. The effect of lipoic acid in the prevention of myocardial infarction in diabetic rats. Bratisl Lek Listy. 119(10):664-669(2018).
[21]. 21- Feldman AT, Wolfe D. Tissue processing and hematoxylin and eosin staining. Methods Mol Biol. 1180:31-43(2014).
[22]. 22- Fu DA, Campbell-Thompson M. Periodic Acid-Schiff Staining with Diastase. Methods Mol Biol. 1639:145-149(2017).
[23]. 23- Ramos-Vara JA. Principles and Methods of Immunohistochemistry. Methods Mol Biol. 1641:115-128(2017).
[24]. 24- Bockmeyer CL, Wittig J, Säuberlich K, et al. Recommendations for mRNA analysis of micro-dissected glomerular tufts from paraffin-embedded human kidney biopsy samples. BMC Mol Biol. 19(1):2(2018).
[25]. 25-Ishibashi Y, Matsui T, Yamagishi S. Tofogliflozin, A Highly Selective Inhibitor of SGLT2 Blocks Proinflammatory and Proapoptotic Effects of Glucose Overload on Proximal Tubular Cells Partly by Suppressing Oxidative Stress Generation. Horm Metab Res. 48(3):191-195(2016).
[26]. 26-Iannantuoni F, M de Marañon A, Diaz-Morales N, et al. The SGLT2 Inhibitor Empagliflozin Ameliorates the Inflammatory Profile in Type 2 Diabetic Patients and Promotes an Antioxidant Response in Leukocytes. J Clin Med. 8(11):1814(2019).
[27]. 27-Kataoka H, Yoshida Y. Enhancement of the serum chloride concentration by administration of sodium-glucose cotransporter-2 inhibitor and its mechanisms and clinical significance in type 2 diabetic patients: a pilot study. Diabetol Metab Syndr. 12:5(2020).
[28]. 28-Shang W, Wang Z. The Update of NGAL in Acute Kidney Injury. Curr Protein Pept Sci. 18(12):1211-1217(2017).
[29]. 29-Albrecht T, Schilperoort M, Zhang S, et al. Carnosine Attenuates the Development of both Type 2 Diabetes and Diabetic Nephropathy in BTBR ob/ob Mice. Sci Rep. 7:44492(2017).
[30]. 30-Assadi F, Sharbaf FG. Urine KIM-1 as a Potential Biomarker of Acute Renal Injury After Circulatory Collapse in Children. Pediatr Emerg Care. 35(2):104-107(2019).
[31]. 31-Oshima, H. et al. Empagliflozin, an SGLT2 Inhibitor, Reduced the Mortality Rate after Acute Myocardial Infarction with Modification of Cardiac Metabolomes and Antioxidants in Diabetic Rats. J. Pharmacol. Exp. Ther. 368, 524–534, (2019).
[32]. 32-Al-Harbi NO, Nadeem A, Ahmad SF, Alanazi MM, Aldossari AA, Alasmari F. Amelioration of sepsis-induced acute kidney injury through inhibition of inflammatory cytokines and oxidative stress in dendritic cells and neutrophils respectively in mice: Role of spleen tyrosine kinase signaling. Biochimie. 158:102-110(2019).
[33]. 33-Meng XM, Ren GL, Gao L, et al. NADPH oxidase 4 promotes cisplatin-induced acute kidney injury via ROS-mediated programmed cell death and inflammation. Lab Invest. 98(1):63-78 (2018).
[34]. 34-Shen Q, Zhang X, Li Q, et al. TLR2 protects cisplatin-induced acute kidney injury associated with autophagy via PI3K/Akt signaling pathway. J Cell Biochem. 120(3):4366-4374(2019).
[35]. 35-Tan X, Zheng X, Huang Z, Lin J, Xie C, Lin Y. Involvement of S100A8/A9-TLR4-NLRP3 Inflammasome Pathway in Contrast-Induced Acute Kidney Injury. Cell Physiol Biochem. 43(1):209-222(2017).
[36]. 36-Zhang LM, Liu JH, Xue CB, et al. Pharmacological inhibition of MyD88 homodimerization counteracts renal ischemia reperfusion-induced progressive renal injury in vivo and in vitro. Sci Rep. 6:26954(2016).
[37]. 37-Szeto HH, Liu S, Soong Y, et al. Mitochondria Protection after Acute Ischemia Prevents Prolonged Upregulation of IL-1β and

IL-18 and Arrests CKD. J Am Soc Nephrol. 28(5):1437-1449(2017).

[38]. 38-Gan J, Zhou X. Comparison of urine neutrophil gelatinase-associated lipocalin and interleukin-18 in prediction of acute kidney injury in adults. Medicine (Baltimore). 97(39):e12570(2018).

[39]. 39-Ozkok A, Edelstein CL. Pathophysiology of cisplatin-induced acute kidney injury. Biomed Res Int. 2014:967826(2014).

9/22/2023