Special Issue:

Veterinary Medicine between Sustainable Development and Public Health to Confront Global Changes

Renoprotective Impact of Dapagliflozin and Mulberry Extracts Toward Fr-STZ Induced Diabetic Nephropathy in Rats: Biochemical and Molecular Aspects

Emad M. Gad[1], Haidy G. Abdel-Rahman[2], Mohy Eldin Abd-El-Fattah[1], Merna M. Kamal[1], Ahmed Shaker Eltahan[3], Amina A. Dessouki[3]*

[1]Department of Organic Chemistry, Faculty of Science, Suez Canal University, Ismailia 41522, Egypt; [2]Department of Clinical Pathology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia 41522, Egypt; [3]Department of Pathology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia 41522, Egypt.

Abstract | Among the most typical reasons of end-stage renal disease (ESRD) is diabetic nephropathy (DN), which is also rated as a major microvascular complication of diabetes mellitus. The prevalence of diabetic nephropathy is more than 40% of diabetic patients. Even with current treatments, many individuals with DN progress to end-stage renal disease and needing dialysis or kidney transplantation. So,the development of new prevention and treatment strategies of diabetic nephropathy is required. The existent study looked at the impact of dapagliflozin, mulberry fruit and leaves extracts and their combination on the kidney of diabetic rats. To induce diabetic nephropathy, experimental rats were supplied with 10% fructose (Fr) in drinking water for the first two weeks. Each Fr-fed animal received an intraperitoneal injection of a low single dose of STZ (40 mg/kg) after being fasted for the whole night. Sixty albino rats were separated into six equivalent groups. Group I control rats administrated 0.5ml distilled water by gavage for six weeks, group II untreated diabetic rats, group III–VI are diabetic groups; received dapagliflozin (1 mg/Kg daily) by gavage for 6 weeks, mulberry fruit extract (300mg/kg b.wt) by gavage, mulberry leaves extract (250 mg/kg b.wt) by gavage and combination of DAPA, MFE and MLE, respectively for 6 weeks. Untreated diabetic rats exhibited considerable rise in serum glucose, urea, creatinine, KIM-1, β2-MG, TNF-α, and TGβ1 levels compared to control rats, while treated diabetic ones manifested significant decrease in these measures in contrast to the untreated diabetic rats. Also, renal tissue IL-6, NF-κB and NADPH oxidase manifested significant ($P \leq 0.05$) increase in untreated diabetic rats, while treated groups revealed significant decline in comparison to the untreated one. DAPA and mulberry fruit and leaves extracts optimized IL-10 and renin expression in renal tissue. Histopathological picture of kidney, revealed significant ($P \leq 0.05$) improvement in rats received DAPA and mulberry extracts compared to untreated diabetic rats. It could be concluded that, DAPA, mulberry fruits and leaves extracts alleviated diabetic nephropathy complications due to their powerful antioxidant and anti-inflammatory effects. Therefore, combining these ingredients in a supplement may be promising for modulating diabetic nephropathy.

Keywords| Diabetic nephropathy, Dapagliflozin, Mulberry, Glucose, Kidney, interleukins, RT-PCR, western blot, inflammation, oxidative stress, SGLT2 inhibitors.

Received | June 02, 2024; **Accepted** | August 21, 2024; **Published** | September 12, 2024

***Correspondence** | Dr. Amina A. Dessouki, Department of Pathology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia 41522, Egypt; **Email:** aminadessouki@vet.suez.edu.eg

Citation | Gad EM, Abdel-Rahman HG, Abd-El-Fattah ME, Kamal MM, Eltahan AS, Dessouki AA (2024). Renoprotective impact of dapagliflozin and mulberry extracts toward fr-stz induced diabetic nephropathy in rats: Biochemical and molecular aspects. Adv. Anim. Vet. Sci. 12(s1): 112-126.

DOI | https://dx.doi.org/10.17582/journal.aavs/2024/12.s1.112.126

ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

INTRODUCTION

Diabetes mellitus is a chronic metabolic condition brought on by cellular insulin receptors resistance or insufficient insulin secretion. It is characterized by raised glucose level with disturbance of fat, carbohydrate and protein metabolism (Harikumar *et al*., 2014). Among the important microvascular complications of diabetes is diabetic nephropathy (DN), which is considered the dominant principle of end-stage renal disease (ESRD) (Forbes and Cooper, 2013). Despite the fact that diabetic nephropathy has generally been regarded as a nonimmune condition, increasing evidence now suggests that the onset and advance of the condition are greatly influenced by inflammatory and immune strategies (Mora and Navarro, 2006; Tuttle, 2005). As a result, processes related to diabetic nephropathy involve a variety of cells, including macrophages, monocytes and leukocytes (Chow *et al*., 2004; Galkina and Ley, 2006), and many other particles, like growth factors (IL-6, TNF-α, TGF-β1) Pantsulaia (2006) and nuclear factors (NF-κB) (Schmid *et al*., 2006).

Nowadays, sodium-glucose cotransporter 2 (SGLT2) inhibitor as Dapagliflozin (DAPA), which is an innovative oral diabetes medication that increases renal glucose excretion and inhibits reabsorption of glucose by the kidney, thus lowering plasma levels of glucose without increasing body weight (Sayed *et al*., 2020). SGLT2 inhibitors are useful in cases with pancreatic dysfunction or insulin resistance. In experimental models, it was found that dapagliflozin decreased lipid metabolism, reduced inflammation, reduced oxidative stress, and fibrosis, and improved insulin resistance, hence prevented the progress of nephropathy (Kojima *et al*., 2015; Terami *et al*., 2014). SGLT-2Is were the first choice for kidney protection in T2DM and CKD American Diabetes Association Professional Practice Committee, 2022. In addition, Use of SGLT2 inhibitors was associated with lower mortality (Yang *et al*., 2022). Also, SGLT-2Is signifcantly reduced kidney and cardiovascular risk in T2DM and CKD, (Yang *et al*., 2023). Many new medications are in development but dapaglifozin could be the major turning factor in managing T2DM (Maksud *et al*., 2024).

Natural compounds were found to have potential benefits with no adverse side effects, and considered a valuable source of biologically active substances (Zhang and Ma, 2018). Black mulberry fruits and leaves belong to Morus family; they are deemed a favorable source of phenolic compounds that safeguard against a variety of human diseases (Juranic and Zizak, 2005; Shukitt-Hale *et al*., 2008). Mulberry extract has a potent effect against kidney diseases through improvement of biochemical parameters reflecting renal functions and displayed lesser glycogen accumulation on histopathology in diabetic rats (Hassanalilou *et al*., 2017). Researchers discovered that the alkaloids present in mulberry leaves have a powerful impact on lowering blood glucose levels by inhibiting α-glucosidase, increasing insulin sensitivity, and controlling oxidative stress in the body (Ajebli *et al*., 2021). The medicinal parts of mulberry include leaf, twig, root bark, and fruit, all of which have remarkable hypoglycemic effects (Liu *et al*., 2023). Mulberry leaf water extract attenuated T2D in HFHS diet and STZ-treated mice by suppressing LPS, eCBs and gut microbiota pathway (Du *et al*., 2024).

This study hypothesized that dapagliflozin and mulberry extract may have a protective effect against diabetic nephropathy in STZ induced diabetic rats through regulation of IL 10 the anti-inflammatory cytokine and subsequent inhibition of the pro-fibrotic pathways. Also controlling hyperglycemia leading to downregulation of renin which plays a crucial role in diabetic nephropathy complications.

MATERIALS AND METHODS

Rats and Habitation

The study was held utilizing 60 male Albino rats of weight; 180 to 250g brought from pharmaceutical Pharco organization, Cairo, Egypt. Rats were maintained for 1 week to minimize non-specific stress and were brought up at Animal House of Faculty of Veterinary Medicine, Suez Canal University. They were placed in cages with a 12-hour light/dark cycle and ambient temperatures of 20° C and 55 to 60% relative humidity. Water was provided freely. Rats were fed on a commercial basal diet. All animals were randomized to treatments to minimize potential bias. The research ethics committee of the Faculty of Veterinary Medicine at Suez Canal University (Ismailia, Egypt) approved the experimental protocol and issued it an approval number (2023016).

Chemicals

Streptozotocin (STZ) purchased from Sigma-Aldrich, USA, was broken up in a newly processed cold sodium citrate buffer solution (0.1 mol/L, pH = 4.5). Dapagliflozin powder was purchased from AstraZeneca pharmaceutical corporation (Egypt) and was suspended in distilled water. Fructose and glucose were purchased from Loba Chemie company, Mumbai, India. Ethanol was purchased from Perfect chemical company, Mumbai, India.

Induction of Diabetic Nephropathy

For the first two weeks, experimental rats other than the control ones were given a 10% solution of fructose (Fr) *ad libitum* in drinking water, while normal drinking water was provided to the control rats *ad libitum*. Every one of the of the Fr-fed rats got a single STZ (40 mg/kg) intraperitoneal

(i.p.) injection after being fasted for the entire night according to the method of Wilson and Islam, 2012 whereas, 10% fructose and 40 mg/kg STZ dose proved to be the best dose for induction of diabetes with low mortality. To prevent acute hypoglycemia, rats were administered a solution of glucose (10% w/v) for the first 24 hours of diabetes induction. Control rats received 1 ml of citrate buffer. Confirmation of diabetes was done after 72 h of STZ injection, the blood was taken from the tail vein, and Accu-Chek Active (Roche Diagnostics GnbHD-68298 Mannheim, Germany) was used to measure blood glucose levels. Diabetic animals were chosen because their non-fasting levels of blood glucose exceeded 300 mg/dl (Srinivasan *et al*., 2005).

Experimentation Protocol

Sixty rats were kept separate into six equal groups of ten each, in such a way:

Group I (control) and group II (Diabetic not treated): Rats were administered 0.5ml distilled water by stomach tube daily. Group III (DAPA): diabetic rats received dapagliflozin (1 mg/Kg) as described by Oraby *et al*. (2019) for four weeks (two weeks after induction of diabetes). Group IV (MFE): diabetic rats treated with mulberry fruit extract (300mg/kg b.wt) as described by Abouzed *et al*. (2020). Group V (MLE): diabetic rats treated with mulberry leaves extract (250 mg/kg b.wt) as Hassanalilou *et al*. (2017). Group VI (Mix): diabetic rats treated with DAPA + MFE+ MLE at half doses administered in groups III, IV & V. Treatments were administered daily in the morning, using stomach tube for six weeks. Fruits and leaves of mulberry extracts were given from the first day of fructose solution administration, while dapagliflozin was given after STZ injection for diabetes induction.

Blood samples were taken using micro capillary tubes from the retro-orbital plexus at the completion of the experiment, which lasted six weeks, and serum was extracted by centrifugation at 3000 rpm for 15 minutes. Until analysis, serum samples were kept at –20 °C. The kidneys were quickly separated. For histopathological examination, each rat had its right kidney fixed in 10% formalin. For qRT-PCR and western blot analysis, the left kidney was cleaned with ice-cold saline, dried, quickly frozen in liquid nitrogen, and kept at –80 °C.

Serum Biochemical Assay

The enzymatic colorimetric technique was used to estimate the concentration of glucose in the blood, employing glucose commercial kits (CliniChem company, Hungary) as represented by Trinder (1969). Serum level of urea was determined by enzymatic (UV) method, using urea kits (CliniChem company, Hungary) according to Talke and Schubert (1965). Serum level of creatinine was estimated by colorimetric method, alkaline picrate method (Jaffé), using creatinine kits (CliniChem company, Hungary) as described by Bartels *et al*. (1972). OxiSelect™ Rat Kim-1 ELISA Kit (CELL BIOLABS, INC. Company, United States) was used to measure the serum level of kidney injury molecule-1 (KIM-1), according to Han *et al*. (2002). Serum β2-microglobulin (β2-MG) level was determined using Rat-β2-Microglobulin ELISA kit (KAMIYA BIOMEDICAL Company, United States), according to Viau *et al*. (1986). Serum level of advanced glycation end products (AGEs) was assessed applying Rat AGEs ELISA kit (CUSABIO Company, United States). Serum tumor necrosis factor (TNF-α) amount was estimated by Rat TNF-α ELISA kit (KAMIYA BIOMEDICAL Company, United States). Rat TGF-β1 ELISA kit (My BioSource company, United States) was used to estimate transforming growth factor–β1 (TGF-β1) level in the serum, as outlined by Kropf *et al*. (1997).

Renal Tissue Biochemical Assay

Interleukin-6 (IL-6) level in renal tissue, was assessed by Rat IL-6 ELISA Kit (KAMIYA BIOMEDICAL Company, United States). Rat NF-κB ELISA Kit (CUSABIO Company, United States) was applied to assess the nuclear factor-kappa B (NF-κB) level in renal tissue. Renal NADPH oxidase was measured by Rat NADPH Oxidase ELISA Kit (Bioassay Technology Laboratory Company, United Kingdom).

Quantitative real time–polymerase chain reaction (qRT-PCR) assessment of IL-10 gene in renal tissue: The SensiFASTTM SYBR® Hi-ROX One-Step Kit was purchased from Bioline, a median life science company, UK, and carried out as described by the manufacturer's protocol. The yield of total RNA obtained was determined spectrophotometrically at 260 nm.

Gene symbol	Primer sequence from 5′- 3′	Gene bank accession number
β-actin	F: TCCGTCGCCGGTCCACACCC R: TCACCAACTGGGACGATATG	NM_031144.3
Sirt-1	F: GGCTCAGCACTGCTATGTTGCC R: AGCATGTGGGTCTGGCTGACTG	XM_032915519.1

Analysis of the expression of renin protein in renal tissue by Western blot: The Ready PrepTM protein extraction kit (total protein) was utilized under the directions provided by the manufacturer. Every sample of the homogenized tissues from each group was supplemented with a protein extraction kit. Bio basic Inc. (Markham, Ontario, L3R 8T4 Canada) provided the Bradford Protein Assay Kit (SK3041) for quantitative protein analysis.

Histopathological Screening

At the completion of the experiment, the right kidney of the rats was taken out and fixed instantly in 10% neutral buffered formalin. The samples were placed in paraffin blocks and cut into sections that were 5µm thick. Microscopic examination was conducted on the sections, after being dyed with periodic Acid-Schiff (PAS) and hematoxylin and eosin (H&E) stains.

Semiquantitative Scoring

Lesion scores of renal lesions were performed according to Gibson-Corley *et al*. (2013). Lesions in 10 fields selected randomly from each slide for each animal and inspected blindly then the mean was calculated. Lesions Score scale; 0 denotes no change in the tissue; 1 denotes <10%; 2 denotes 11-25%; 3 denotes 26-45%; 4 denotes 46-75% and 5=76-100%.

Statistical Assay

Homogeneity of variables were checked for normality using the Shapiro–Wilk test at 0.05 level. Variables were nonsignificant (Shapiro–Wilk test<0.05), and data were parametric. Differences between groups were checked using ANOVA and Duncan's post hoc test at p≤0.05.

One-way ANOVA and Duncan's post hoc test, SPSS version 20 for Windows (SPSS Inc., Chicago, IL) was applied for all statistical analyses of the obtained data (Mean ± SE) Levesque, 2005. It was presumed that there was statistical significance among the groups if the P value was ≤ 0.05.

RESULTS AND DISCUSSION

Serum Glucose Concentration and Biochemical Markers of Kidney Function

Serum glucose, urea and creatinine levels revealed considerable (*P≤0.05*) rise in untreated diabetic group related to the control and treated diabetic ones (DAPA, MFE, MLE and mix groups). On the other hand, these parameters were significantly (*P≤0.05*) decreased in the treated diabetic groups relevant to the untreated diabetic one, with best results recorded in MLE and Mix groups Furthermore, there was no notable change in creatinine level between control and mix group (Table 1).

Some Serum Kidney Injury Markers

At the termination of the experiment, the levels of KIM-1, β2- MG, TGF-β1, AGEs and TNF-α in serum revealed substantial (*P≤0.05*) increment in untreated diabetic rats in comparison with the control ones. While, diabetic groups supplemented with DAPA, MFE, MLE and mix of DAPA, MFE and MLE showed considerable (*P≤0.05*) decline in these analytes in contrast to untreated diabetic one. Significant variation was recorded between treated groups with each other in KIM-1, β2- MG, TGF-β1, AGEs and TNF-α levels. Treated group with mix of DAPA, MFE and MLE showed significant (*P≤0.05*) drop in β2- MG serum level in contrast to the control group. Furthermore, the mix group rats declared significant (*P≤0.05*) minimization in the serum values of all above mentioned markers in comparison with the DAPA rats (Table 2).

Table 1: Effect of DAPA, MFE and MLE on serum glucose and kidney function biomarkers of diabetic rats.

Parameter	Experimental groups					
	Control	Diabetic	DAPA	MFE	MLE	Mix
Glucose (mg/dl)	120.1^{f} ±0.95	281.7^{a} ±2.48	227.8^{c} ±3.61	236.5^{b} ±2.62	210.6^{d} ±2.59	156.5^{e} ±2.81
Urea (mg/dl)	15.01^{e} ±0.26	42.45^{a} ±0.34	34.22^{b} ±0.93	33.58^{b} ±0.76	23.92^{c} ±0.71	17.82^{d} ±0.18
Cr (mg/dl)	0.43^{d} ±0.005	0.89^{a} ±0.02	0.59^{b} ±0.01	0.61^{b} ±0.01	0.51^{c} ±0.01	0.43^{d} ±0.01

Each value represents mean ± SE (n=5)
Means in the same row with various superscript alphabets differ significantly when *P was ≤0.05*
[a,] P=0.03; [b,] P=0.04,

Table 2: Effect of DAPA, MFE and MLE on some serum kidney injury markers of diabetic rats.

Parameter	Experimental groups					
	Control	Diabetic	DAPA	MFE	MLE	Mix
KIM-1 (ng/ml)	2.49^{f} ±0.01	5.36^{a} ±0.005	3.19^{d} ±0.04	3.82^{b} ±0.09	3.33^{c} ±0.01	2.72^{e} ±0.01
β2- MG (ng/ml)	2.63^{e} ±0.02	5.82^{a} ±0.01	3.12^{d} ±0.009	4.11^{b} ±0.006	3.68^{c} ±0.01	2.47^{f} ±0.02
AGEs (µg/ml)	15.00^{f} ±0.24	67.01^{a} ±0.46	31.50^{d} ±0.29	44.92^{b} ±0.65	38.13^{c} ±0.32	24.99^{e} ±0.42
TNF-α (pg/ml)	8.57^{f} ±0.06	44.08^{a} ±0.47	20.94^{d} ±0.54	35.68^{b} ±1.06	25.09^{c} ±0.10	16.12^{e} ±0.07
TGF-β1 (pg/ml)	63.73^{f} ±0.90	251.51^{a} ±1.51	110.34^{d} ±1.16	179.45^{b} ±1.03	142.51^{c} ±1.64	85.03^{e} ±0.53

Each value represents mean ± SE (n=5)
Means in the same row with various superscript alphabets differ significantly when *P was ≤0.05*

Renal Tissue Injury Markers

At 6 weeks of the experimental period, IL-6, NF-κB and NADPH oxidase exhibited considerably (*P≤0.05*) elevated levels in renal tissues of untreated diabetic rats as to control rats. But diabetic groups treated with DAPA, MFE, MLE and mix manifested notable (*P≤0.05*) decrement in their levels in comparison to the untreated diabetic rats. There was significant variance among the treated groups regarding the renal tissue concentrations of NADPH oxidase, NF-κB and IL-6. where best results were recorded in DAPA and Mix groups. However, treated diabetic rats with mix of DAPA, MFE and MLE revealed substantial

Table 3: Effect of DAPA, MFE and MLE on renal tissue injury markers of diabetic rats.

Parameter	Experimental groups					
	Control	Diabetic	DAPA	MFE	MLE	Mix
IL-6 (pg/ml)	$71.29^{f}\pm0.51$	$241.34^{a}\pm1.60$	$122.16^{d}\pm1.31$	$178.31^{b}\pm1.71$	$150.60^{c}\pm0.99$	$87.55^{e}\pm1.57$
NF-κB (pg/ml)	$6.63^{f}\pm0.02$	$18.21^{a}\pm0.16$	$10.91^{d}\pm0.16$	$15.32^{b}\pm0.20$	$12.23^{c}\pm0.20$	$8.09^{e}\pm0.09$
NADPH- oxidase (ng/ml)	$1.74^{f}\pm0.02$	$4.61^{a}\pm0.006$	$3.17^{d}\pm0.03$	$4.05^{b}\pm0.03$	$3.63^{c}\pm0.03$	$2.10^{e}\pm0.02$

Each value represents the mean ± SE (n=5)

Means in the same row with various superscript alphabets differ significantly when *P was ≤0.05*

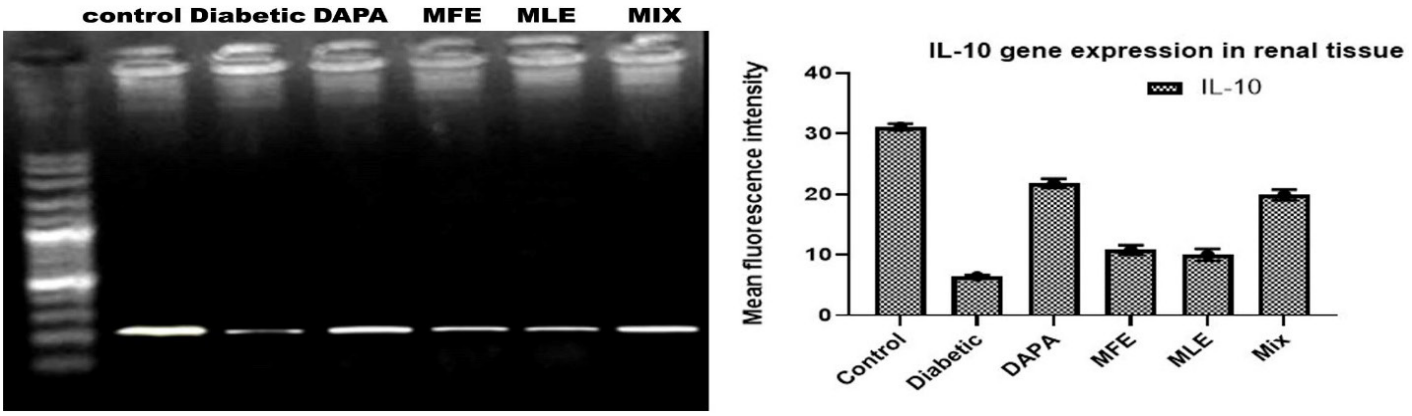

Figure 1: The mRNA levels of renal tissue IL-10 gene was detected and relatively quantified by RT-qPCR. PCR products seen by electrophoresis on agarose gel in all studied groups. Lane M: DNA ladder, Lane 1: control, Lane 2: diabetic, Lane 3: DAPA, Lane 4: MFE, Lane 5: MLE and Lane 6: Mix group.

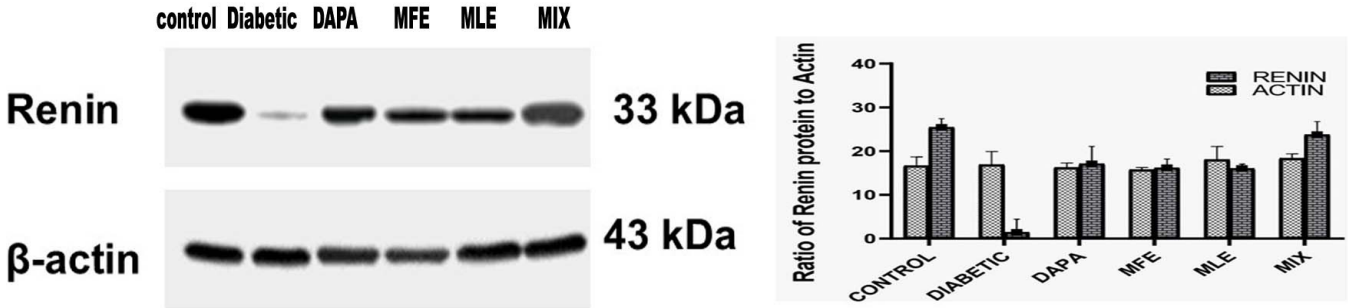

Figure 2: The renal tissue renin protein levels was detected by western blotting. Relative level of Renin protein was quantitated by densitometry and normalized to actin levels. Shown are representative immunoblots from two independent experiments with similar results.

(*P≤0.05*) decrease in NF-κB and IL-6 levels related to DAPA rats (Table 3).

qRT-PCR of IL-10 gene in renal tissue: In the present study, IL-10 gene expression in renal tissue was diminished in untreated diabetic group related to the control one and treated groups. DAPA and mix treated groups manifested increment in IL-10 gene expression compared to MFE and MLE treated groups (Figure 1).

Western blot of renin protein expression in renal tissue: At 6 weeks of the experiment, the untreated diabetic group showed decrement in renin protein expression in correspondence to control group and treated diabetic groups. Mix diabetic treated group showed improvement in renin protein expression compared to other treated diabetic groups. There was no variation between DAPA, MFE and MLE treated diabetic groups in renin protein expression (Figure 2).

Renal Histopathological Results

As shown in Figure 3, the kidney sections stained with H&E revealed that nephrons in control rats were normal and had proximal and distal renal tubules as well as glomeruli. The cells of the proximal renal tubules had a nucleus that was either round or oval, and their cytoplasm was uniform, cuboidal, and eosinophilic, with prominent brush borders. The cells in the distal renal tubules of the diabetic rats were swollen, had outstanding cell boundaries, a condensed nucleus and clear cytoplasm. The swollen

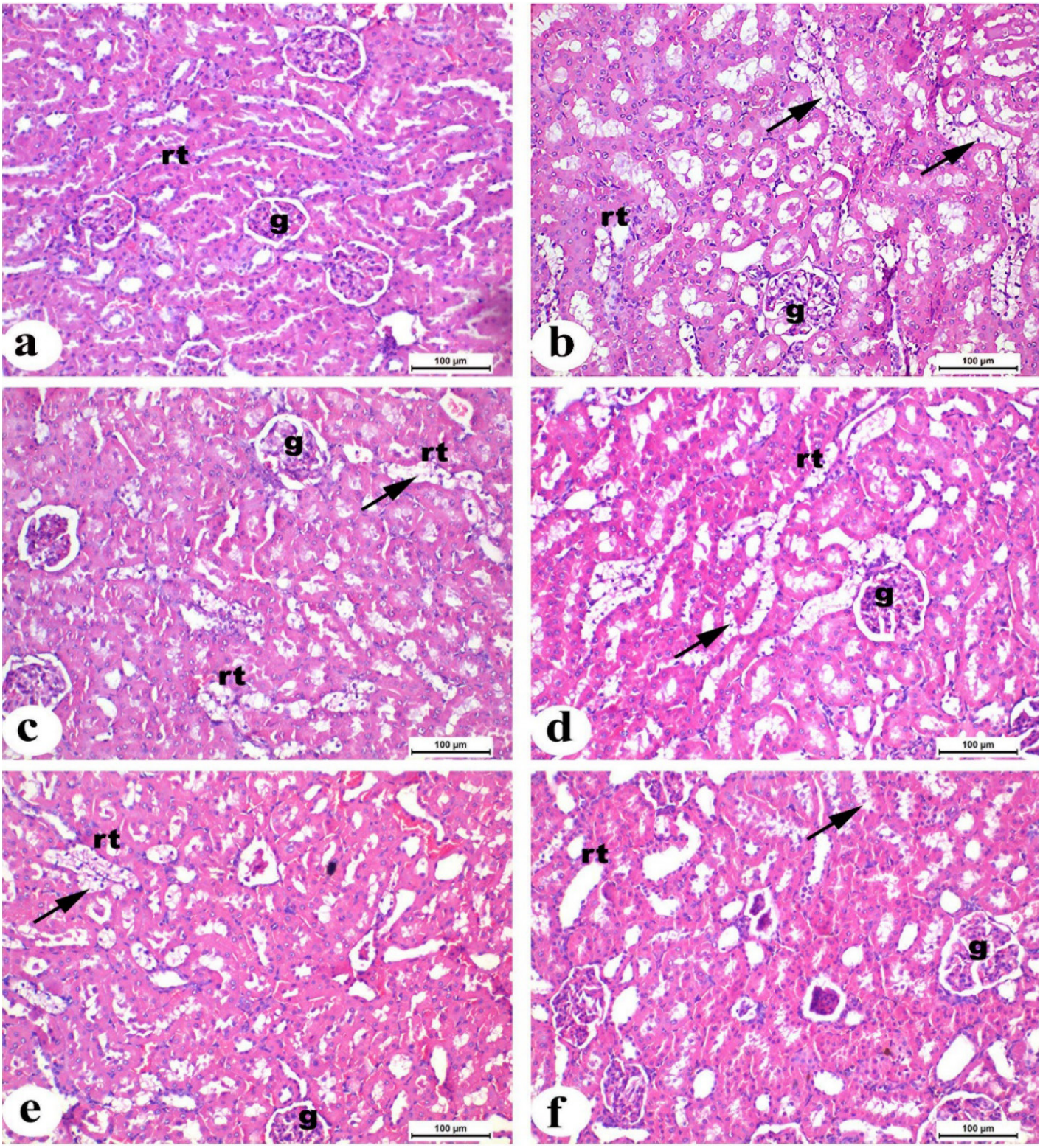

Figure 3: Shows histopathological findings of the kidney sections of diabetic rats in various groups (a-f). **(a)** control group, displaying typical glomeruli and renal tubular epithelium. **(b)** untreated diabetic group, showing vacuolated tubular epithelium and loss of brush borders of the renal tubules along with glycogen infiltration of renal tubular epithelium. **(c)** dapagliflozin group, the renal tubules and glomeruli are nearly normal, and intratubular capillary congestion is minimal. **(d)** mulberry fruit extract (MFE) group, showing renal glomeruli with thin-walled capillary tuft. **(e)** & **(f)** mulberry leaves extract (MLE) & mix group respectively, showing normal proximal renal tubules with nuclei that were either round or oval and homogeneous, cuboidal, eosinophilic cytoplasm with intact brush borders. H&E staining. X 400. Bar (100 µm). Glomeruli (g), renal tubule (rt), Glycogen infiltration (arrows)

cells of diabetic rats contained glycogen deposits. Diabetic treated group with dapagliflozin (DAPA) revealed mild to moderate improvement in kidney tissue through reduction of mesangial expansion, thickening of capillary tufts and hyalinosis. While, diabetic rats treated with mulberry fruit extract (MFE), sections in the kidney tissue revealed renal glomeruli with thin-walled capillary along with slightly dilated Bowman's space, normal renal epithelial cells with intact basement membranes and preserved brush borders. Diabetic treated groups with mulberry leaves extract (MLE) and mix (DAPA, MFE and MLE) showed normal proximal renal tubules with nuclei that were either round or oval and homogeneous, cuboidal, eosinophilic cytoplasm with distinct brush borders. Additionally, they demonstrated fatty degeneration of renal convoluted tubules and a slight accumulation of glycogen.

As for kidney tissues stained with PAS (Figure 4), glycogen infiltration in the renal tubule epithelial cells was not seen in control animals. Glycogen infiltration was observed in the distal tubules' epithelial cells in untreated diabetic group. The distal tubules' epithelial cells of diabetic treated group with dapagliflozin were free from glycogen. Diabetic treated group with mulberry fruit extract (MFE) revealed glycogen infiltration in the epithelial cells of distal tubules. Diabetic treated group with mulberry leaves extract (MLE) & mix groups demonstrating that glycogen is not present in the distal tubules' epithelial cells.

Semiquantitative Scoring of Tubular Injury

Injury of the kidney was quantified and shown in Figure 5, statistical analysis of renal lesions score exhibited significantly higher scores in rats treated with STZ in comparison with the control group. Meanwhile, DAPA and MFE&MLE treated rats showed significant declines in the renal lesions score, compared with rats in the STZ group. Moreover, mix group restored a fairly normal morphological appearance of the kidney (Figure 5).

Long term of diabetes represents a threat to the patient's kidney known as Diabetes nephropathy (DN), which is a major diabetic complication caused by hyperglycemia, which damages the nerves and blood vessels. DN is still the most noted explanation of end stage renal disease (ESRD) worldwide (Boer *et al*., 2011).

Kidney damage was obvious in the current study, hyperglycemia and elevated creatinine and urea serum levels were recorded in untreated diabetic group. Greater protein catabolism is linked to a higher urea concentration in diabetic rats. The elevated serum levels of urea and creatinine were clear indicators of impaired kidney function, which lead to nephrotoxicity due to induction of STZ (Ronco *et al*., 2010), as at least half of the kidney nephrons must be damaged for the serum creatinine level to increase. Also, Ceriello *et al*. (2000) illustrated a favorable link between nephropathy advancement and hyperglycemia. As, it was recorded that diabetic rats' renal tubules accumulate glycogen in form of clusters by virtue of hyperglycemia (Nannipieri *et al*., 2001) that was confirmed by our histopathological inspection of kidney tissue.

Contrarily, treated rats with DAPA displayed enhancement in renal function parameters compared to untreated diabetic animals, by reason of the prohibition of endoplasmic reticulum stress, inflammation, apoptosis and fibrosis, and due to reduction of lipids accumulation in the diabetic rats' kidneys (Jaikumkao *et al*., 2018). This was in harmony with Thomas, 2014, who denoted the possible anti-inflammatory and antioxidant effects of DAPA through decreasing the proximal tubule's reabsorption of glucose and sodium. Thus, reabsorption of glucose from the glomerular filtrate is controlled by SGLT2, which seems to be the main transporter involved in renal glucose transport, therefore reducing hyperglycemia and promoting renal glucose excretion would be achieved by inhibiting SGLT2 by dapagliflozin (Han *et al*., 2008). Over and above, proximal tubular cells that are exposed to high glucose levels have altered functions, such as elevated production of reactive oxygen species and advanced glycation end products, as well as increased expression of pro-inflammatory cytokines, growth factors, and profibrotic mediators. The onset and advancement of diabetic nephropathy have been linked to each of them. Since SGLT2 is the primary mechanism of glucose entry into the tubular cell, which mediates the induction of these pathways, inhibiting SGLT2 would seem to be a helpful strategy for lowering intracellular exposure to glucose in the proximal tubule and, thus, its detrimental effects there (Thomas, 2014).

Furthermore, Diabetic rats treated with mulberry fruit and leaves extract and mix showed an improvement in serum glucose, urea and creatinine levels. Similarly, (Abouzed *et al*., 2020) stated that the black mulberry fruit extract ameliorated the renal function biomarkers in diabetic rats. Mulberry extract might advantageously affect diabetic confusions, like renal dysfunction, by keeping up with blood glucose homeostasis in diabetic rats. Rutin, as a major polyphenol in the MFE, has a hypoglycemic impact because it increases the action of the insulin-dependent receptor kinase, which activates the pathway for insulin signaling and causes an increase in glucose absorption and GLUT4 translocation (Hsu *et al*., 2014; Min *et al*., 2020) demonstrated that increased antioxidant enzyme action in the pancreas and kidneys was linked to mulberry's antioxidant properties. These results also revealed that rats treated with mulberry leaves showed significant improvement in renal function tests compared to mulberry fruits

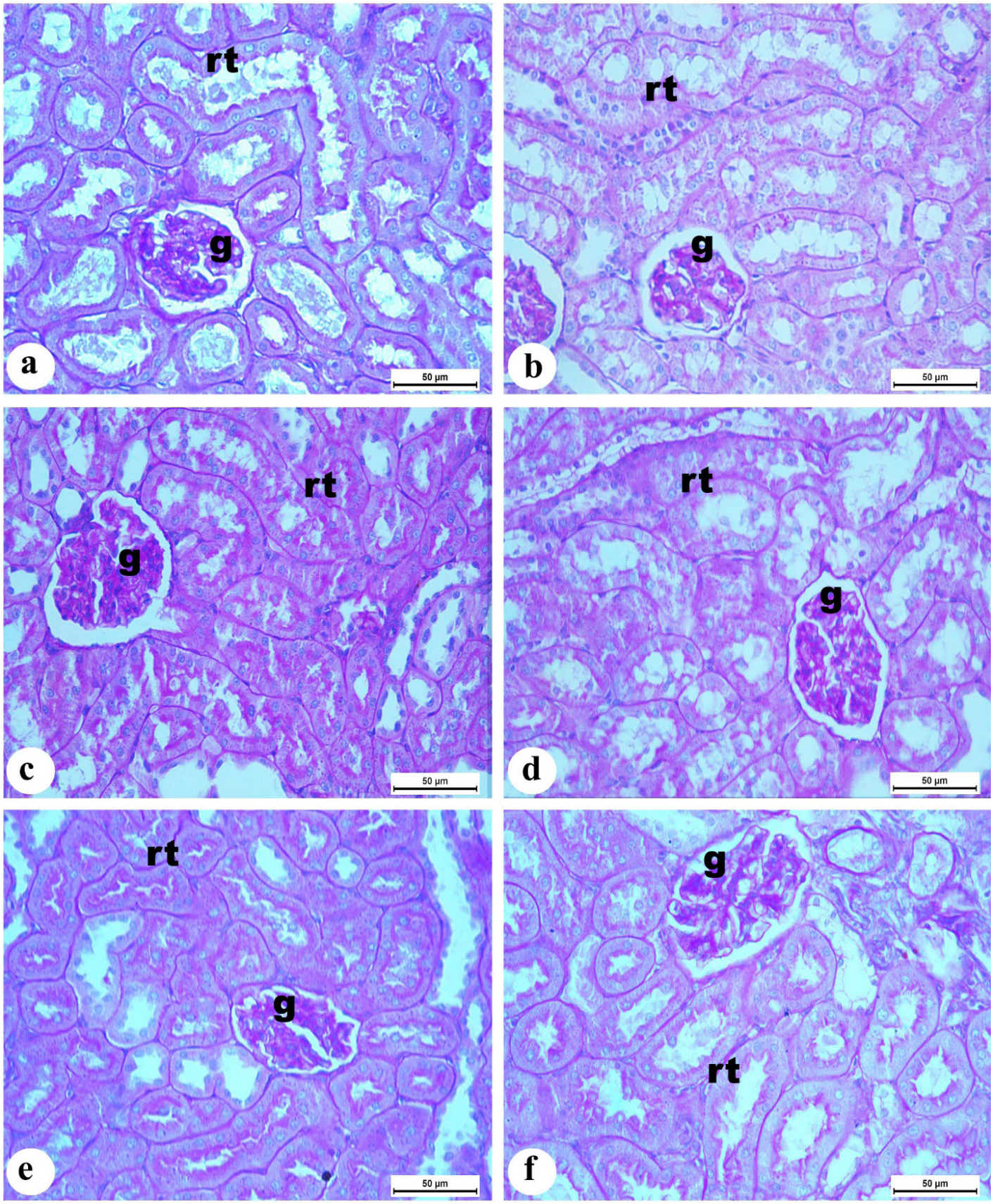

Figure 4: Shows histopathological findings of the kidney sections of diabetic rats in different groups (a-f). Control group **(a)** showing no glycogen infiltration in renal tubules' epithelial cells. Untreated diabetic group **(b)**, showing glycogen infiltration in the distal tubules' epithelial cells. Dapagliflozin group **(c),** the epithelial cells of distal tubules were free from glycogen. Mulberry fruit extract (MFE) group **(d),** showing glycogen infiltration in the distal tubules' epithelial cells. Mulberry leaves extract (MLE) and mix group [**(e)** and **(f)**] respectively, demonstrating that the distal tubules' epithelial cells do not contain any glycogen. PAS staining, Bar (50 µm). Glomeruli (g), renal tubule (rt).

group because of the high chlorogenic acid content, which has anti-inflammatory and antioxidant characteristics and can show the development of DN by modifying the Nrf2/HO-1 and NF-κB pathways (Bao *et al*., 2018).

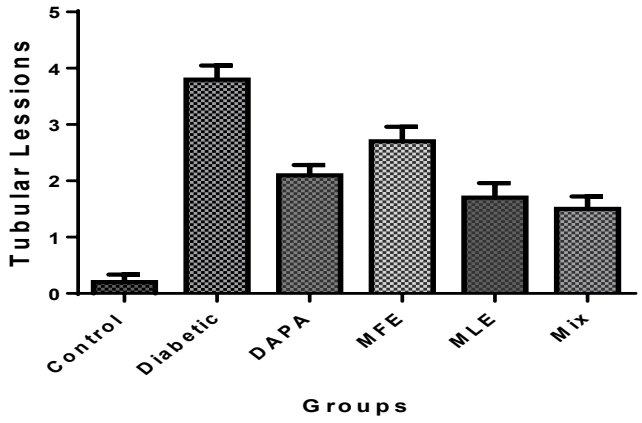

Figure 5: Semiquantitative scoring of tubular injury as described in the Methods.
* P < 0.001 vs other groups. # P < 0.05 vs cisplatin treated group (n = 5).

The anti–diabetic effect of mulberry leaves is due to the presence of 1-Deoxynojirimycin which inhibits the α-glucosidase activity, decreases serum insulin and glucose levels and improves carbohydrate metabolism (Bai *et al*., 2021).

The trans-membrane protein, Kidney injury molecule (KIM-1) has been identified as a particular biomarker for the early detection of acute and chronic kidney impairment in laboratory animals (Sabbisetti *et al*., 2013). Beta-2-microglobulin (β2-MG) has been used to determine the glomerular filtration rate as it is eliminated by the kidney (Amighi *et al*., 2011). Serum KIM-1 and β2-MG levels showed notable rise in diabetic untreated animals related to control animals. This could be attributed to injection of STZ which increases the free radicals that damage the proximal tubule of the kidney. The present results are analogous to (Humphreys *et al*., 2013) who stated that mice's tubule-interstitial fibrosis was caused by chronic KIM-1 expression. It has been demonstrated that when renal function deteriorates, the serum concentration of β2-microglobulin increased before the concentration of serum creatinine. However, β2-MG level in serum has been recommended as a superior indicator of glomerular filtration rate than creatinine level in serum (Bianchi *et al*., 2001). Where, serum globulin β2-MG is of low molecular weight, has high stability and can be filtered by the glomerular filtration membrane before being absorbed by the proximal convoluted tubule. Increased serum β2-MG levels in T2DM patients frequently signify that the patient's glomerular filtration function is impaired (Kim *et al*., 2020). This is also in accord with (Kamal *et al*., 2021).

The existent findings revealed that diabetic animals treated with DAPA showed significant decrement in KIM-1 level. Similar results were recorded by (Dekkers *et al*., 2018) where excretion of inflammatory marker IL-6 and proximal tubular marker KIM-1 in urine is reduced after 6 weeks of dapagliflozin medication. Since KIM-1 is a particular marker for hypoxia damage to proximal tubular cells due to high oxygen demand for glucose and sodium reabsorption in case of diabetes, but dapagliflozin lessens the damage to these cells (Lin *et al*., 2014). KIM-1 levels might be an indicator of novel treatments like SGLT2 inhibitors that protect the kidneys. Where, SGLT2 inhibitors can minimize proximal tubular damage in diabetic kidney disease by lowering the need for re-absorptive activity thus improving the tubular hypoxia (Liu *et al*., 2021). Also, MFE, MLE and mix groups showed significant decline in KIM-1 level correlated to the untreated diabetic one. This reduction could be due to antioxidant activity of mulberry extract where numerous researches had proven the antioxidant effect of black mulberry with different methods, including DPPH assay (Chen *et al*., 2016; Souza *et al*., 2018; Wang *et al*., 2018) that came in the same line with the present results. Additionally, several flavonoids and phenolic compounds isolated from black mulberry extracts demonstrated potent anti-diabetic effects via α-glucosidase inhibition (Xu *et al*., 2020) thus reducing glucose level in the blood which leads to reduction of KIM-1 level.

Serum levels of AGEs, TGF-β1 and TNF-α were substantially raised in untreated diabetic group in comparison with the control one. AGEs are glycated proteins or lipids by the action of high glucose level (Goldin *et al*., 2006). One of the harmful impacts that non-enzymatic alteration of plasma proteins may have, is the formation of oxygen free radicals (Negre-Salvayre *et al*., 2009). Glycation may alter the cell function, where the target protein and lipid get denatured and lose their functionality, organopathy results from the tissue buildup of AGEs, cell receptor-mediated signaling pathways are activated, and oxidative and carbonyl stresses are produced (Yonekura *et al*., 2005). It was previously recorded that, DN patients have higher levels of serum TNF-α and TNF receptors, which are indicators of renal deterioration and the likelihood of ESRD (Niewczas *et al*., 2012). TNF-α is a pleiotropic cytokine that promotes pro-inflammatory mediators, apoptosis, and necroptosis via activating TNF receptors (Vassalli, 1992). On the contrary, diabetic rats received DAPA showed significant decline in serum AGEs, TNF-α and TGF-β1 in contrast to rats in diabetic group. As previously demonstrated by Moses *et al*., 2014 that type 2 diabetes patients may have raised SGLT-2 proteins levels, which raise the glucose renal threshold and aggravate hyperglycemia giving rise to renal damage and diabetic nephropathy. Therefore, oral administration of SGLT-2 inhibitors prevent the

glucose reabsorption in order to manage blood sugar levels (Yao *et al*., 2018) and reduce renal tubule fibrosis and inflammatory response (Komala *et al*., 2013). It was previously believed that the predominant driving force behind the increased expression of transforming growth factor β (TGF-β) is tubular glucose reabsorption through SGLT2 due to hyperglycemia, which involved renal proximal tubular growth that in turn gives rise to nephropathy and renal fibrosis. Emaglifozin, a SGLT2 inhibitor, has declared its ability to reduce renal hypertrophy linked to experimental diabetes by inhibiting glucose reabsorption (Vallon *et al*., 2014). Over and above, (Yuan *et al*., 2023) observed that IL-1β, TNF-α and IL-6 were reduced by dapagliflozin, which in turn reduced kidney fibrosis and inflammation. Mulberry leaves demonstrated anti-inflammatory effects by means of IL-6, TNF-α and IL-1β prohibition which in turn suppress NF-κB, which is implicated in inflammation brought on by macrophage activation (Park *et al*., 2013).

Transforming growth factor β1 (TGF-β1) is a versatile cytokine that regulates a variety of biological processes, including apoptosis (Wiener *et al*., 2014), cell division (Barcellos-Hoff and Cucinotta, 2014) and immunity (Stephen *et al*., 2014), plus patients with diabetes mellitus have higher levels of TGF-β1 mRNA and protein expression in their kidneys (Yamamoto *et al*., 1993). As a result of TGF-β1 promoting extracellular matrix formation and mesangium cell hypertrophy, the rate of glomerular filtration is reduced, and chronic renal failure results. As well known that, diabetes decreases the glucose availability, the metabolic switch from glycolysis to oxidative phosphorylation takes place by utilizing more fatty acids as a source of energy (Chang *et al*., 2016). Therefore, the mitochondrial electron transport chain stimulates the three fundamental hyperglycemic deterioration pathways - generation of AGEs, polyol pathway motivation and protein C kinase stimulation - and also boosts superoxide production (Nishikawa *et al*., 2000). Additionally, excessive hyperglycemia triggers reactive oxygen species (ROS) through NADPH oxidase, mitochondrial electron transport chain and protein C kinase (Lee *et al*., 2003).

It has been documented that, the primary ROS generator in the vasculature is NADPH oxidase (Bendall *et al*., 2007). As well, through NADPH oxidase, AGEs can exactly motivate the ROS genesis (Wautier *et al*., 2001).

Our results declared that, untreated diabetic group was considerably greater than the diabetic treated groups and the control one regarding renal tissue IL-6, NADPH-oxidase and NF-κB. It has been discovered that a high glucose level led to the provocation of NF- κB and the stimulation of pro-inflammatory cytokines (Lin *et al*., 2012). AGEs ligated to particular receptors (RAGEs) can also trigger NF-κB stimulation (Wendt *et al*., 2003). Yet, a NF-κB-dependent pathway increased IL-6 expression in podocytes subjected to raised levels of glucose (Brasier, 2010). Similarly, Yang *et al*. (2022) verified that hyperglycemia may trigger the kidney's immune response via the TLR2/Myd88/NF-κB signaling pathway, which would cause diabetic kidney disease to develop.

To the contrary, diabetic rats received dapagliflozin manifested significant reduction in renal NF-κB and IL-6 expression in correspondence to untreated diabetic rats. In addition to suppressing NF-κB DNA binding action induced by extreme glucose, the SGLT2 inhibitor reduced IL6 expression (Panchapakesan *et al*., 2013). The current study noted DAPA's useful impacts on inhibition of renal NF-κB in diabetic rats, with subsequent diminution of IL-6 generation. Besides, Yao *et al*. (2018) studied the reno-protective impact of DAPA on human diabetic nephropathy via inhibiting NF-κB pathway.

Moreover, MFE, MLE and mix groups significantly diminished renal NF-κB and IL-6 related to the untreated diabetic group. These outcomes could be coming from the presence of flavonoids and chlorogenic acid, which regulate NF-κB expression and prevent inflammation-related oxidative stress. Additionally, they are crucial in the inflammatory cytokine signaling process (Oliveira *et al*., 2016). Moreover, Chen *et al*. (2016) said that the anti-inflammatory properties of total flavonoids of mulberry may be related to their antioxidant capacity.

Renal NADPH oxidase was diminished in DAPA group in correspondence to the untreated diabetic one. The conservative outcome of dapagliflozin has been related to glucose level normalization, loss of body weight, natriuresis and the fall of intra-glomerular pressure (Górriz *et al*., 2020; Vergara *et al*., 2019). It was similar to Terami *et al*. (2014) who found that dapagliflozin may lessen oxidative strain by inhibiting the output of ROS in the kidneys of mice that are derived from Nox4. Moreover, Liu *et al*. (2010) Concluded that the inhibition of AGEs/RAGE and the prohibition of NADPH oxidase and NF-κB stimulation, are linked to the influence of pioglitazone on insulin resistance in rats consuming fructose.

Moreover, renal NADPH oxidase was significantly decreased in rats supplied with MFE, MLE and mix groups corresponded to the untreated diabetic one. It could be accounted for the existence of anthocyanins in the extract of mulberry fruit, which are outstanding antioxidants that are more capable for hunting free radicals (Du *et al*., 2008). Also, the presence of flavonoids and phenolic compounds which are the largest phytochemical molecules owning a strong antioxidant action (Huyut *et al*., 2017; Li *et al*.,

2014). It is similar to Ji *et al.* (2019) who demonstrated that mulberry leaves inhibited the activity of NADPH oxidase.

Both the development of chronic renal failure and normal renal physiology are significantly influenced by IL-10. In a healthy adult kidney, the primary local source of IL-10 is mesangial cells (Sinuani *et al.*, 2006). Mesangial cells that have been activated begin to secrete a lot of chemokines, cytokines and extracellular matrix proteins which affect the renal IL-10 expression. In turn, these factors have affect the mesangial cells and mediate interactions with blood inflammatory cells and endothelial and epithelial tubular cells (Schlöndorff and Banas, 2009). In the existing study, the untreated diabetic group showed inhibition in renal IL-10 gene expression compared to control group. It was parallel to Summers *et al.* (2011), who reported that the histological changes and decreased renal function are both achieved by inhibition of IL-10. But, DAPA and mix treated rats exhibited raised IL-10 renal expression which explained the anti-inflammatory effect of DAPA. Also, Yuan *et al.* (2023) recorded elevated levels of IL-10 in diabetic mice treated with DAPA.

The earliest glomerular hemodynamic disruption in DN is largely mediated by the renin-angiotensin system (RAS). The kidney's juxtaglomerular cells produce the proteolytic enzyme renin. Angiotensinogen is converted by renin into angiotensin I, that is transformed into angiotensin II (Ang II) by the action of angiotensin-converting enzyme (ACE) (Paul *et al.*, 2006). Angiotensin II is another important protein that controls the expression and synthesis of cytokines that are implicated in various processes like cell proliferation, inflammation, fibrosis, and death (Ruiz-Ortega *et al.*, 2001; Sadoshima, 2000). In the existing study, renal renin protein expression was decreased in the untreated diabetic group. While, diabetic treated groups with DAPA, MFE, MLE and their mix showed enhancement in renal IL-10 and renin expression when compared to untreated diabetic group. Dapagliflozin increases renal IL-10 and renin expression as a result of histopathological changes in kidney which declared nearly normal renal glomeruli and renal tubules. Similar to Yang *et al.* (2022), who found an improvement in renal histopathological findings in diabetic rats treated with dapagliflozin.

Moreover, renal tissue of diabetic rats supplied with mulberry extracts revealed regular proximal renal tubules with nuclei that were either round or oval and cuboidal cytoplasm with prominent brush borders that increase the expression of renal IL-10 and renin in the current study. It was similar to Zhang *et al.* (2019) who confirmed the effect of mulberry components on renal tissue.

CONCLUSIONS AND RECOMMENDATIONS

Administration of DAPA, MFE and MLE extracts ameliorated the diabetic complications on the kidney via depression of serum glucose, urea, creatinine, KIM-1, β2-MG, TNF-α, and TGβ1 levels and renal tissue levels of IL-6, NF-κB and NADPH oxidase together with improvement in IL-10 and renin expression in renal tissues. The best reno-protective action was noticed in a mixture group followed by MLE group then MFE one. Finally, combining these ingredients might be successful in reducing the risks of diabetic nephropathy.

The results analyzed in this study are based on rat diabetes models; however, as humans and animals have different physiologies, metabolisms, and other characteristics, more investigation is required to confirm the efficacy of mulberry in diabetes patients. Furthermore, the long-term effects of SGLT-2 inhibition on kidney tissue and inflammatory biomarkers were not examined in this investigation, which presents further limitations. Lastly, more research is needed to guarantee the constant quality and concentration of mulberry extracts, which will deepen the conversation about real-world use.

NOVELTY STATEMENT

Using Mulberry leaves and fruits extracts and their combination on diabetic nephropathy in albino rats.

AUTHOR'S CONTRIBUTIONS

The study's idea and design were created by [Mohy Eldin Abd-El-Fattah, Amina A. Dessouki and Haidy G. Abdel-Rahman]. Execution of the experimental work and clinical data gathering were done by [Merna M. Kamal] as well as initial writing of the study, while [Haidy G. Abdel-Rahman] supervised data gathering and analysis. [Ahmed Shaker] helped in IL-10 gene and renin protein expression in renal tissue. [Emad Gad, Haidy G. Abdel-Rahman and Amina A. Dessouki] revised the findings and helped interpret them. The final manuscript has been read by all authors and has their approval.

Ethics Approval

The research ethics committee of the Faculty of Veterinary Medicine at Suez Canal University (Ismailia, Egypt) approved the experimental protocol and issued it an approval number (2023016).

Conflict of Interest

The authors have declared no conflict of interest.

REFERENCES

Abouzed, T.K., Sadek, K.M., Ghazy, E.W., Abdo, W., Kassab, M. A., Hago, S., Abdel-Wahab, S., Mahrous, E.A., Abdel-Sattar, E., Assar, D.H. Black mulberry fruit extract alleviates streptozotocin-induced diabetic nephropathy in rats: targeting TNF-α inflammatory pathway. Journal of Pharmacy and Pharmacology, 2020; 72 (11): 1615-1628. https://doi.org/10.1111/jphp.13338

Ajebli, M., Khan, H., Eddouks, M. Natural Alkaloids and Diabetes Mellitus: A Review. Endocrine, metabolic and immune disorders drug targets, 2021; 21(1): 111-130. https://doi.org/10.2174/1871530320666200821124817

American Diabetes Association Professional Practice Committee. Addendum. 11. Chronic Kidney Disease and Risk Management,2022; Standards of Medical Care in Diabetes—2022. Diabetes Care, 2022; 45 (Suppl. 1) 45(9): S175–S184. https://doi.org/10.2337/dc22-ad08a

Amighi, J., Hoke, M., Mlekusch, W., Schlager, O., Exner, M., Haumer, M., Pernicka, E., Koppensteine, R., Minar, E., Rumpold, H., Schillinger, M., Wagner, O. Beta 2 microglobulin and the risk for cardiovascular events in patient with asymptomatic carotid atherosclerosis. Stroke, 2011; 42(7): 1826-1833. https://doi.org/10.1161/STROKEAHA.110.600312

Bai, H., Jiang, W., Wang, X., Hu, N., Liu, L., Li, X., Xie, Y., Wang, S. Component changes of mulberry leaf tea processed with honey and its application to in vitro and in vivo models of diabetes. Food additives & contaminants. part A, chemistry, analysis, control, exposure &risk assess, 2021; 38(11): 1840-1852. https://doi.org/10.1080/19440049.2021.1953709

Bao, L., Li, J., Zha, D., Zhang, L., Gao, P., Yao, T., Wu, X. Chlorogenic acid prevents diabetic nephropathy by inhibiting oxidative stress and inflammation through modulation of the Nrf2/HO-1 and NF-κB pathways. International immunopharmacology, 2018; 54: 245-253. https://doi.org/10.1016/j.intimp.2017.11.021

Barcellos-Hoff, M. H., Cucinotta, F. A. New tricks for an old fox: impact of TGFβ on the DNA damage response and genomic stability. Science signaling, 2014; 7: 341, re5. https://doi.org/10.1126/scisignal.2005474

Bartels, H., Böhmer, M., Heierli, C. Serum creatinine determination without protein precipitation. Clinica chimica acta; International Journal of Clinical Chemistry, 1972; 37: 193-197. https://doi.org/10.1016/0009-8981(72)90432-9

Bendall, J. K., Rinze, R., Adlam, D., Tatham, A. L., Bono, J., Channon, K. M. Endothelial Nox2 overexpression potentiates vascular oxidative stress and hemodynamic response to angiotensin II: studies in endothelial-targeted Nox2 transgenic mice. Circulation Research, 2007; 100(7): 1016-1025. https://doi.org/10.1161/01.RES.0000263381.83835.7b

Bianchi, C., Donadio, C., Tramonti, G., Consani, C., Lorusso, P., Rossi, G. Reappraisal of serum beta2-microglobulin as marker of GFR. Renal failure, 2001; 23(3-4): 419-429. https://doi.org/10.1081/JDI-100104725

Boer, D. I. H., Rue, T. C., Hall, Y. N., Heagerty, P. J., Weiss, N. S., Himmelfarb, J. Temporal trends in the prevalence of diabetic kidney disease in the United States. Jama, 2011; 305(24): 2532-2539. https://doi.org/10.1001/jama.2011.861

Brasier, A. R. The nuclear factor-kappaB-interleukin-6 signalling pathway mediating vascular inflammation. Cardiovascular research, 2010; 86(2): 211-218. https://doi.org/10.1093/cvr/cvq076

Ceriello, A., Morocutti, A., Mercuri, F., Quagliaro, L., Moro, M., Damante, G., Viberti, G. C. Defective intracellular antioxidant enzyme production in type 1 diabetic patients with nephropathy. Diabetes, 2000; 49(12): 2170-2177. https://doi.org/10.2337/diabetes.49.12.2170

Chang, A. S., Hathaway, C. K., Smithies, O., Kakoki, M. Transforming growth factor-β1 and diabetic nephropathy. Am J Physiol Renal Physiol, 2016; 310(8): F689-f696. https://doi.org/10.1152/ajprenal.00502.2015

Chen, H., Pu, J., Liu, D., Yu, W., Shao, Y., Yang, G., Xiang, Z., He, N. Anti-Inflammatory and Antinociceptive Properties of Flavonoids from the Fruits of Black Mulberry (Morus nigra L.). PloS one, 2016; 11(4): e0153080. https://doi.org/10.1371/journal.pone.0153080

Chow, F., Ozols, E., Nikolic-Paterson, D. J., Atkins, R. C., Tesch, G. H. Macrophages in mouse type 2 diabetic nephropathy: correlation with diabetic state and progressive renal injury. Kidney international, 2004; 65(1): 116-128. https://doi.org/10.1111/j.1523-1755.2004.00367.x

Dekkers, C.J., Petrykiv, S., Laverman, G. D., Cherney, D. Z., Gansevoort, R. T., Heerspink, H. J. L. Effects of the SGLT-2 inhibitor dapagliflozin on glomerular and tubular injury markers. Diabetes Obes Metab, 2018; 20(8): 1988-1993. https://doi.org/10.1111/dom.13301

Du, Q., Zheng, J., Xu, Y. Composition of anthocyanins in mulberry and their antioxidant activity. Journal of Food Composition and Analysis, 2008; 21(5): 390-395. https://doi.org/10.1016/j.jfca.2008.02.007

Du, Y., Zhang, R., Zheng, X. X., Zhao, Y. L., Chen, Y. L., Ji, S., Guo, M. Z, Tang, D. Q. Mulberry (Morus alba L.) leaf water extract attenuates type 2 diabetes mellitus by regulating gut microbiota dysbiosis, lipopolysaccharide elevation and endocannabinoid system disorder. Journal of Ethnopharmacology, 2024; 323: 117681. https://doi.org/10.1016/j.jep.2023.117681

Forbes, J.M., Cooper, M.E.. Mechanisms of diabetic complications. Physiological reviews, 2013; 93(1): 137-188. https://doi.org/10.1152/physrev.00045.2011

Galkina, E., Ley, K. Leukocyte recruitment and vascular injury in diabetic nephropathy. J Am Soc Nephrol, 2006; 17(2): 368-377. https://doi.org/10.1681/ASN.2005080859

Gibson-Corley, K.N., Olivier, A.K., Meyerholz, D.K. Principles for valid histopathologic scoring in research. Veterinary Pathology, 2013; 50: 1007-15. https://doi.org/10.1177/0300985813485099

Goldin, A., Beckman, J. A., Schmidt, A. M., Creager, M. A. Advanced Glycation End Products. Circulation, 2006; 114(6): 597-605. https://doi.org/10.1161/CIRCULATIONAHA.106.621854

Górriz, J. L., Navarro-González, J. F., Ortiz, A., Vergara, A., Nuñez, J., Jacobs-Cachá, C., Martínez-Castelao, A., Soler ,M. J. Sodium-glucose cotransporter 2 inhibition: towards an indication to treat diabetic kidney disease. Nephrology Dialysis Transplantation, 2020; 35(Supplement_1): i13-i23. https://doi.org/10.1093/ndt/gfz237

Han, S., Hagan, D. L., Taylor, J. R., Xin, L., Meng, W., Biller, S. A., Wetterau, J. R., Washburn, W. N., Whaley, J. M. Dapagliflozin, a selective SGLT2 inhibitor, improves glucose homeostasis in normal and diabetic rats. Diabetes, 2008; 57(6): 1723- 9. https://doi.org/10.2337/db07-1472

Han, W. K., Bailly, V., Abichandani, R., Thadhani, R., Bonventre, J. V. Kidney Injury Molecule-1 (KIM-1): a novel biomarker

for human renal proximal tubule injury. Kidney international, 2002; 62(1): 237-244. https://doi.org/10.1046/j.1523-1755.2002.00433.x

Harikumar, K., Kishore, K. B., Hemalatha, G. J., Bharath, K. M., Saky, L. S. F. A Review on Diabetes Mellitus. International Journal of Novel Trends in Pharmaceutical Sciences, 2014; 4(6): 201-217.

Hassanalilou, T., Payahoo, L., Shahabi, P., Abbasi, M. M. The protective effects of Morus nigra L. leaves on the kidney function tests and histological structures in streptozotocin-induced diabetic rats. Biomedical Research, 2017; 28(14): 6113-6118.

Hsu, C. Y., Shih, H. Y., Chia, Y. C., Lee, C. H., Ashida, H., Lai, Y. K., Weng, C. F. Rutin potentiates insulin receptor kinase to enhance insulin-dependent glucose transporter 4 translocation. Molecular nutrition & food research, 2014; 58(6):1168-1176. https://doi.org/10.1002/mnfr.201300691

Humphreys, B. D., Xu, F., Sabbisetti, V., Grgic, I., Naini, S. M., Wang, N., Chen, G., Xiao, S., Patel, D., Henderson ,J. M., Ichimura, T., Mou, S., Soeung, S., McMahon, A. P., Kuchroo, V. K., Bonventre, J. V. Chronic epithelial kidney injury molecule-1 expression causes murine kidney fibrosis. J Clin Invest, 2013; 123(9):4023-4035. https://doi.org/10.1172/JCI45361

Huyut, Z., Beydemir, Ş., Gülçin, İ. Antioxidant and Antiradical Properties of Selected Flavonoids and Phenolic Compounds. Biochemistry Research International, 2017; 7616791. https://doi.org/10.1155/2017/7616791

Jaikumkao, K., Pongchaidecha, A., Chueakula, N., Thongnak, O., Wanchai, K., Chatsudthipong, V., Chattipakorn, N., Lungkaphin, A. Dapagliflozin, a sodium-glucose co-transporter-2 inhibitor, slows the progression of renal complications through the suppression of renal inflammation, endoplasmic reticulum stress and apoptosis in prediabetic rats. Diabetes, Obesity and Metabolism, 2018; 20(11):2617-2626. https://doi.org/10.1111/dom.13441

Ji, T., Su, S. L., Zhu, Y., Guo, J. M., Qian, D. W., Tang, Y. P., Duan, J.A. The mechanism of mulberry leaves against renal tubular interstitial fibrosis through ERK1/2 signaling pathway was predicted by network pharmacology and validated in human tubular epithelial cells. Phytotherapy research, 2019; PTR 33(8): 2044-2055. https://doi.org/10.1002/ptr.6390

Juranic, Z., Zizak, Z. Biological activities of berries: from antioxidant capacity to anti-cancer effects. BioFactors (Oxford, England), 2005; 23(4): 207-211. https://doi.org/10.1002/biof.5520230405

Kamal, M., Apu, S., kamal, A., Alam, I., kamal, A. M., Rahman, A.K.M. S. Serum Beta- 2 Microglobulin is a Reliable Biomarker to Predict Diabetic Nephropathy. Archives of Clinical and Biomedical Research, 2021; 05:724-736. https://doi.org/10.26502/acbr.50170197

Kim, H., Wang, D., Chalmers, J., Jun, M., Zoungas, S., Marre, M., Hamet, P., Harrap, S., Mancia, G., Poulter, N.R., Cooper, M. E., Woodward, M., Selvin, E., Rebholz, C. M. Alternative kidney filtration markers and the risk of major macrovascular and microvascular events, and all-cause mortality in individuals with type 2 diabetes in the Advance trial. J Diabetes, 2020;12(12): 929-941. https://doi.org/10.1111/1753-0407.13083

Kojima, N., Williams, J. M., Slaughter, T.N., Kato, S., Takahashi, T., Miyata, N., Roman, R.J. Renoprotective effects of combined SGLT2 and ACE inhibitor therapy in diabetic Dahl S rats. Physiological reports, 2015; 3(7): e12436. https://doi.org/10.14814/phy2.12436

Komala, M.G., Panchapakesan, U., Pollock, C. Sodium glucose cotransporter 2 and the diabetic kidney. Current opinion in nephrology and hypertension, 2013; 22(1): 113-119. https://doi.org/10.1097/MNH.0b013e32835a17ae

Kropf, J., Schurek, J. O., Wollner, A., Gressner, A. M. Immunological measurement of transforming growth factor-beta 1 (TGF-β1) in blood; assay development and comparison. Clinical chemistry, 1997; 43(10): 1965-1974. https://doi.org/10.1093/clinchem/43.10.1965

Lee, H. B., Yu, M.R., Yang, Y., Jiang, Z., Ha, H. Reactive oxygen species-regulated signaling pathways in diabetic nephropathy. Journal of the American Society of Nephrology, 2003;14(suppl 3): S241-S245. https://doi.org/10.1097/01.ASN.0000077410.66390.0F

Levesque, R. 2005: SPSS programming and data management: a guide for SPSS and SAS users.(Spss).

Li, A.N., Li, S., Zhang, Y.J., Xu, X.R., Chen, Y.M., Li, H.B. Resources and biological activities of natural polyphenols. Nutrients, 2014; 6(12): 6020-6047. https://doi.org/10.3390/nu6126020

Lin, M., Yiu, W. H., Wu, H. J., Chan, L.Y. Y., Leung, J.C.K., Au, W. S., Chan, K.W., Lai, K.N., Tang, S.C.W. Toll-like receptor 4 promotes tubular inflammation in diabetic nephropathy. J Am Soc Nephrol, 2012; 23(1): 86-102. https://doi.org/10.1681/ASN.2010111210

Lin, Q., Chen, Y., Lv, J., Zhang, H., Tang, J., Gunaratnam, L., Li, X., and Yang, L. Kidney injury molecule-1 expression in IgA nephropathy and its correlation with hypoxia and tubulointerstitial inflammation. Am J Physiol Renal Physiol, 2014; 306: F885-895. https://doi.org/10.1152/ajprenal.00331.2013

Liu, C. H., Liu, F., Xiong, L. Medicinal parts of mulberry (leaf, twig, root bark, and fruit) and compounds thereof are excellent traditional Chinese medicines and foods for diabetes mellitus. Journal of Functional Foods, 2023;106: 105619. https://doi.org/10.1016/j.jff.2023.105619

Liu, H., Sridhar, V.S., Lovblom, L.E., Lytvyn, Y., Burger, D., Burns, K., Brinc, D., Lawler, P.R., Cherney, D.Z.I. Markers of Kidney Injury, Inflammation, and Fibrosis Associated With Ertugliflozin in Patients With CKD and Diabetes. Kidney International Reports, 2021; 6(8): 2095-2104. https://doi.org/10.1016/j.ekir.2021.05.022

Liu, X., Luo, D., Zheng, M., Hao, Y., Hou, L., Zhang, S. Effect of pioglitazone on insulin resistance in fructose-drinking rats correlates with AGEs/RAGE inhibition and block of NADPH oxidase and NF kappa B activation. European journal of pharmacology, 2010; 629(1-3): 153-158. https://doi.org/10.1016/j.ejphar.2009.11.059

Maksud, N., Bera, S., Naim, M. J., Alam, O. Dapagliflozin: A new hope for the therapeutic treatment of type 2 diabetes mellitus. European Journal of Medicinal Chemistry Reports, 2024; 100167. https://doi.org/10.1016/j.ejmcr.2024.100167

Min, A.Y., Yoo, J.M., Sok, D.E., Kim, M.R. Mulberry Fruit Prevents Diabetes and Diabetic Dementia by Regulation of Blood Glucose through Upregulation of Antioxidative Activities and CREB/BDNF Pathway in Alloxan-Induced Diabetic Mice. Oxidative Medicine and Cellular Longevity, 2020; 1298691. https://doi.org/10.1155/2020/1298691

Mora, C., Navarro, J.F. Inflammation and diabetic nephropathy. Current diabetes reports, 2006; 6(6): 463-468. https://doi.org/10.1007/s11892-006-0080-1

Moses, R. G., Colagiuri, S., Pollock, C. SGLT2 inhibitors: New

medicines for addressing unmet needs in type 2 diabetes. The Australasian medical journal, 2014; 7(10): 405. https://doi.org/10.4066/AMJ.2014.2181

Nannipieri, M., Lanfranchi, A., Santerini, D., Catalano, C., Van de Werve, G., Ferrannini, E. Influence of long-term diabetes on renal glycogen metabolism in the rat. Nephron, 2001; 87(1): 50-57. https://doi.org/10.1159/000045884

Negre-Salvayre, A., Salvayre, R., Augé, N., Pamplona, R., Portero-Otín, M . Hyperglycemia and glycation in diabetic complications. Antioxidants & redox signaling, 2009; 11(12): 3071-3109. https://doi.org/10.1089/ars.2009.2484

Niewczas, M. A., Gohda, T., Skupien, J., Smiles, A. M., Walker, W. H., Rosetti, F., Cullere, X., Eckfeldt, J. H., Doria, A., Mayadas, T. N., Warram, J. H., Krolewski, A.S . Circulating TNF receptors 1 and 2 predict ESRD in type 2 diabetes. J Am Soc Nephrol, 2012; 23(3): 507-515. https://doi.org/10.1681/ASN.2011060627

Nishikawa, T., Edelstein, D., Du, X.L., Yamagishi, S., Matsumura, T., Kaneda, Y., Yorek, M.A., Beebe, D., Oates, P.J., Hammes, H.P., Giardino, I., Brownlee, M. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature, 2000; 404(6779): 787-790. https://doi.org/10.1038/35008121

Oliveira, A.M., Nascimento, M.F., Ferreira, M.R., Feijó de Moura, D., Dos Santos Souza, T.G., Cavalcante da Silva, G., Henrique da Silva, E.R., Guedes, P. P.M., Paloma, L. P, Gonçalves da Silva, T., Lira, S. L.A., Cristiano Aparecido, C. C.A., Antônia de Souza, I., Napoleão, T.H. Evaluation of acute toxicity, genotoxicity and inhibitory effect on acute inflammation of an ethanol extract of Morus alba L. (Moraceae) in mice. Journal of ethnopharmacology, 2016;194: 162-168. https://doi.org/10.1016/j.jep.2016.09.004

Oraby, M.A., El-Yamany, M.F., Safar, M.M., Assaf, N., Ghoneim, H.A. Dapagliflozin attenuates early markers of diabetic nephropathy in fructose-streptozotocin-induced diabetes in rats. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, 2019; 109: 910-920. https://doi.org/10.1016/j.biopha.2018.10.100

Panchapakesan, U., Pegg, K., Gross, S., Komala, M.G., Mudaliar, H., Forbes, J., Pollock, C., Mather, A. Effects of SGLT2 Inhibition in Human Kidney Proximal Tubular Cells—Renoprotection in Diabetic Nephropathy? PloS one, 2013; 8(2): e54442. https://doi.org/10.1371/journal.pone.0054442

Pantsulaia, T. Role of TGF-beta in pathogenesis of diabetic nephropathy. Georgian medical news, 2006; 131: 13-18.

Park, E., Lee, S.M., Lee, J.E., Kim, J.H. Anti-inflammatory activity of mulberry leaf extract through inhibition of NF-κB. Journal of Functional Foods, 2013; 5(1):178-186. https://doi.org/10.1016/j.jff.2012.10.002

Paul, M., Poyan, M. A., Kreutz, R. Physiology of local renin-angiotensin systems. Physiological reviews, 2006; 86(3): 747-803. https://doi.org/10.1152/physrev.00036.2005

Ronco, C., Grammaticopoulos, S., Rosner, M., De Cal, M., Soni, S., Lentini, P., Piccinni, P. Oliguria, Creatinine and Other Biomarkers of Acute Kidney Injury. Contributions to nephrology, 2010; 164: 118-127. https://doi.org/10.1159/000313725

Ruiz-Ortega, M., Lorenzo, O., Suzuki, Y., Rupérez, M., Egido, J. Proinflammatory actions of angiotensins, 2001;10(3): 321-329. https://doi.org/10.1097/00041552-200105000-00005

Sabbisetti, V.S., Ito, K., Wang, C., Yang, L., Mefferd, S.C., Bonventre, J.V. Novel assays for detection of urinary KIM-1 in mouse models of kidney injury. Toxicological sciences : an official journal of the Society of Toxicology, 2013; 131(1):13-25. https://doi.org/10.1093/toxsci/kfs268

Sadoshima, J. Cytokine actions of angiotensin II. Circulation Research, 2000; 86(12):1187-1189. https://doi.org/10.1161/01.RES.86.12.1187

Sayed, N., Abdalla, O., Kilany, O., Dessouki, A., Yoshida, T., Sasaki, K., Shimoda, M. Effect of dapagliflozin alone and in combination with insulin in a rat model of type 1 diabetes. The Journal of veterinary medical science, 2020; 82(4): 467-474. https://doi.org/10.1292/jvms.19-0450

Schlöndorff , D., Banas, B. The mesangial cell revisited: no cell is an island. J Am Soc Nephrol, 2009; 20: 1179-1187. https://doi.org/10.1681/ASN.2008050549

Schmid, H., Boucherot, A., Yasuda, Y., Henger, A., Brunner, B., Eichinger, F., Nitsche, A., Kiss, E., Bleich, M., Gröne, H.J., Nelson, P.J., Schlöndorff, D., Cohen, C.D., Kretzler, M. Modular Activation of Nuclear Factor-κB Transcriptional Programs in Human Diabetic Nephropathy. Diabetes, 2006; 55(11):2993-3003. https://doi.org/10.2337/db06-0477

Shukitt-Hale, B., Lau, F.C., Joseph, J.A. Berry fruit supplementation and the aging brain. Journal of agricultural and food chemistry, 2008; 56(3): 636-641. https://doi.org/10.1021/jf072505f

Sinuani, I., Averbukh, Z., Gitelman, I., Weissgarten, J. Mesangial cells initiate compensatory renal tubular hypertrophy via IL-10-induced TGF-beta secretion: effect of the immunomodulator AS101 on this process. Am J Physiol Renal Physiol, 2006; 291(2):F384-394. https://doi.org/10.1152/ajprenal.00418.2005

Souza, G.R., Oliveira-Junior, R.G., Diniz, T.C., Branco, A., Lima-Saraiva, S. R. G., Guimarães, A.L., Oliveira, A.P., Pacheco, A.G. M., Silva, M.G., Moraes-Filho, M.O., Costa, M.P., Pessoa, C.Ó., Almeida, J.R.G.S. Assessment of the antibacterial, cytotoxic and antioxidant activities of Morus nigra L. (Moraceae). Brazilian journal of biology = Revista brasleira de biologia, 2018; 78,(2):248-254. https://doi.org/10.1590/1519-6984.05316

Srinivasan, K., Viswanad, B., Asrat, L., Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. Pharmacological Research, 2005; 52(4): 313-320. https://doi.org/10.1016/j.phrs.2005.05.004

Stephen, T.L., Rutkowski, M.R., Allegrezza, M.J., Puchalt, A.P., Amelia, J., Tesone, A.J., Svoronos, N., Nguyen, J.M., Sarmin, F., Borowsky, M.E., Tchou, J., Conejo-Garcia, J.R. Transforming growth factor β-mediated suppression of antitumor T cells requires FoxP1 transcription factor expression. Immunity, 2014; 41(3): 427-439. https://doi.org/10.1016/j.immuni.2014.08.012

Summers, S.A., Phoon, R.K., Odobasic, D., Dewage, L., Kitching, A.R., Holdsworth, S.R. Signal transducer and activation of transcription 6 (STAT6) regulates T helper type 1 (Th1) and Th17 nephritogenic immunity in experimental crescentic glomerulonephritis. Clinical and experimental immunology, 2011; 166(2): 227-234. https://doi.org/10.1111/j.1365-2249.2011.04437.x

Talke, H., Schubert, G.E. Enzymatic urea determination in the blood and serum in the warburg optical test. Klinische Wochenschrift, 1965; 43: 174-175. https://doi.org/10.1007/BF01484513

Terami, N., Ogawa, D., Tachibana, H., Hatanaka, T., Wada, J., Nakatsuka, A., Eguchi, J., Horiguchi, C.S., Nishii, N., Yamada, H., Takei, K., Makino, H. Long-term treatment

with the sodium glucose cotransporter 2 inhibitor, dapagliflozin, ameliorates glucose homeostasis and diabetic nephropathy in db/db mice. PloS one, 2014; 9(6): e100777. https://doi.org/10.1371/journal.pone.0100777

Thomas, M.C. Renal effects of dapagliflozin in patients with type 2 diabetes. Therapeutic advances in endocrinology and metabolism, 2014; 5(3): 53-61. https://doi.org/10.1177/2042018814544153

Trinder, P. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. Journal of clinical pathology, 1969; 22(2):158-161. https://doi.org/10.1136/jcp.22.2.158

Tuttle, K.R. Linking metabolism and immunology: diabetic nephropathy is an inflammatory disease. J Am Soc Nephrol, 2005; 16(6): 1537-1538. https://doi.org/10.1681/ASN.2005040393

Vallon, V., Gerasimova, M., Rose, M. A., Masuda, T., Satrriano, J., Mayoux, E., Koepsell, H., Thomson, S.C., Rieg, T. SGLT2 inhibitor empagliflozin reduces renal growth and albuminuria in proportion to hyperglycemia and prevents glomerular hyperfiltration in diabetic Akita mice. Am J Physiol Renal Physiol, 2014; 306(2): F 194- 204. https://doi.org/10.1152/ajprenal.00520.2013

Vassalli, P. The pathophysiology of tumor necrosis factors. Annual review of immunology, 1992; 10: 411-452. https://doi.org/10.1146/annurev.iy.10.040192.002211

Vergara, A., Jacobs-Cachá, C., Soler, M.J. Sodium-glucose cotransporter inhibitors: beyond glycaemic control. Clinical kidney journal, 2019; 12(3): 322-325. https://doi.org/10.1093/ckj/sfz019

Viau, C., Bernard, A., Ouled, A., Lauwerys, R. Determination of rat beta 2-microglobulin in urine and in serum. II. Application of its urinary measurement to selected nephrotoxicity models. Journal of applied toxicology, 1986; 6(3): 191-195. https://doi.org/10.1002/jat.2550060310

Wang, W., Li, X., Bao, X., Gao, L., Tao, Y. Extraction of polysaccharides from black mulberry fruit and their effect on enhancing antioxidant activity. International journal of biological macromolecules, 2018; 120: Pt B, 1420-1429. https://doi.org/10.1016/j.ijbiomac.2018.09.132

Wautier, M.P., Chappey, O., Corda, S., Stern, D.M., Schmidt, A.M., Wautier, J.L. Activation of NADPH oxidase by AGE links oxidant stress to altered gene expression via RAGE. American journal of physiology-endocrinology and metabolism, 2001; 280(5): E685-E694. https://doi.org/10.1152/ajpendo.2001.280.5.E685

Wendt, T.M., Tanji, N., Guo, J., Kislinger, T.R., Qu, W., Lu, Y., Bucciarelli, L.G., Rong, L.L., Moser, B., Markowitz, G.S., Stein, G., Bierhaus, A., Liliensiek, B., Arnold, B., Nawroth, P.P., Stern, D.M., D'Agati, V.D., Schmidt, A.M. RAGE drives the development of glomerulosclerosis and implicates podocyte activation in the pathogenesis of diabetic nephropathy. Am J Pathol, 2003;162(4): 1123-1137. https://doi.org/10.1016/S0002-9440(10)63909-0

Wiener, Z., Band, A.M., Kallio, P., Alitalo, K. Oncogenic mutations in intestinal adenomas regulate Bim-mediated apoptosis induced by TGF-β. Proceedings of the National Academy of Sciences, 2014; 111(21): E2229-E2236. https://doi.org/10.1073/pnas.1406444111

Wilson, R.D., Islam, M.S. Fructose-fed streptozotocin-injected rat: an alternative model for type 2 diabetes. Pharmacological reports, 2012; PR 64(1): 129-139. https://doi.org/10.1016/S1734-1140(12)70739-9

Xu, L., Yu, M., Niu, L., Huang, C., Wang, Y., Wu, C., Yang, P., Hu, X. Phenolic compounds isolated from Morus nigra and their α-glucosidase inhibitory activities. Natural Product Research, 2020; 34(5): 605-612. https://doi.org/10.1080/14786419.2018.1491041

Yamamoto, T., Nakamura, T., Noble, N.A., Ruoslahti, E., Border, W.A. Expression of transforming growth factor beta is elevated in human and experimental diabetic nephropathy. Proceedings of the National Academy of Sciences, 1993; 90(5): 1814-1818. https://doi.org/10.1073/pnas.90.5.1814

Yang, H., Mei, Z., Chen, W., Pan, Y., Liu, L., Zhao, R., Ni, W., Wang, Y., Fei, C. Therapeutic efficacy of dapagliflozin on diabetic kidney disease in rats. International immunopharmacology, 2022; 113(Pt A): 109272. https://doi.org/10.1016/j.intimp.2022.109272

Yang, Q., Lang, Y., Yang, W., Yang, F., Yang, J., Wu, Y., Xiao, X., Qin, C., Zou, Y., Kang, D., Liu, F. Efficacy and safety of drugs for people with type 2 diabetes mellitus and chronic kidney disease on kidney and cardiovascular outcomes: A systematic review and network meta-analysis of randomized controlled trials. Diabetes research and clinical practice, 2023; 198: 110592. https://doi.org/10.1016/j.diabres.2023.110592

Yang, S., Zhao, L., Mi, Y., He, W. Effects of sodium-glucose cotransporter-2 inhibitors and aldosterone antagonists, in addition to renin-angiotensin system antagonists, on major adverse kidney outcomes in patients with type 2 diabetes and chronic kidney disease: A systematic review and network meta-analysis. Diabetes, Obesity and Metabolism, 2022; 24(11): 2159-2168. https://doi.org/10.1111/dom.14801

Yao, D., Wang, S., Wang, M., Lu, W. Renoprotection of dapagliflozin in human renal proximal tubular cells via the inhibition of the high mobility group box 1-receptor for advanced glycation end products-nuclear factor-κB signaling pathway. Molecular medicine reports, 2018; 18(4): 3625-3630. https://doi.org/10.3892/mmr.2018.9393

Yonekura, H., Yamamoto, Y., Sakurai, S., Watanabe, T., Yamamoto, H. Roles of the receptor for advanced glycation endproducts in diabetes-induced vascular injury. Journal of pharmacological sciences, 2005; 97(3): 305-311. https://doi.org/10.1254/jphs.CPJ04005X

Yuan, Y., Sun, M., Jin, Z., Zheng, C., Ye, H., Weng, H. Dapagliflozin ameliorates diabetic renal injury through suppressing the self-perpetuating cycle of inflammation mediated by HMGB1 feedback signaling in the kidney. European journal of pharmacology, 2023; 175560. https://doi.org/10.1016/j.ejphar.2023.175560

Zhang, H., Ma, Z. F. Phytochemical and Pharmacological Properties of Capparis spinosa as a Medicinal Plant. Nutrients, 2018; 10(2): 116. https://doi.org/10.3390/nu10020116

Zhang, L., Su, S., Zhu, Y., Guo, J., Guo, S., Qian, D., Ouyang, Z., Duan, J. Mulberry leaf active components alleviate type 2 diabetes and its liver and kidney injury in db/db mice through insulin receptor and TGF-β/Smads signaling pathway. Biomedicine & Pharmacotherapy, 2019; 112: 108675. https://doi.org/10.1016/j.biopha.2019.108675