Bull. Egypt. Soc. Physiol. Sci. Vol. (45), Issue (2),246-257

Bull. of Egyp. Soc. Physiol. Sci.

(Official Journal of Egyptian Society for Physiological Sciences)

(pISSN: 1110-0842; eISSN: 2356-9514)

Maresin-1 mitigates streptozotocin induced diabetic nephropathy in rats via up-regulation of Nrf2/HO-1 pathway

Suzan A. Khodir[1*], Dina Elzeiny[2,3], Noha M. Abd El-aziz[4], Ahmed Abdel-monem ELmetwally[5], Sarah Mohamed Mowafy[6] , Sara Fahmy Abo EL-Soud[7] , Ahmed Mohamed Kamal[8], Shorouk E. M. Elmorshdy[9],Rania Salah Mansour[10]

[1] Medical Physiology Department, Faculty of Medicine, Menoufia University, Shebin El-Kom, Menoufia, Egypt.
[2] Medical Biochemistry and Molecular Biology Department, Faculty of medicine, Mansoura University, Egypt.
[3] Medical Biochemistry and Molecular Biology Department, Faculty of medicine, New Mansoura University, Egypt.
[4]Anatomy and Embryology Department, Faculty of Medicine, Menoufia University, Shebin El-Kom, Menoufia, Egypt.
[5] Clinical Pharmacology Department, Faculty of medicine, Mansoura University, Egypt.
[6]Anatomy and Embryology Department, Faculty of Medicine, Portsaid University, Portsaid, Egypt
[7] Pathology Department, Faculty of Medicine, Menoufia University Shebin El-Kom, Menoufia, Egypt.
[8] Internal Medicine and Nephrology department, Faculty of Medicine, Menoufia University Shebin El-Kom, Menoufia, Egypt.
[9] Medical Physiology Department, Faculty of Medicine, Mansoura, Egypt.
[10]Medical Physiology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Submit Date : 23 Dec.2024
Revised Date : 07 Jan. 2025
Accept Date : 09 Jan. 2025

Keywords

- Diabetic Nephropathy
- HO-1
- Maresin-1
- NF-kB
- Nrf2

Abstract

Introduction: One serious health issue is diabetic nephropathy (DN). A lipid mediator that is naturally produced from omega-3 fatty acids, maresin-1 (MaR1), may be able to considerably lessen the symptoms of a number of inflammatory illnesses. ***Objective***: to assess the potential underlying processes and the protective impact of Maresin-1 in DN caused by STZ**.** ***Materials and Methods*:** Thirty adult male albino rats were divided into: control, DN group, and DN+MaR1 group (10/group). Levels of urine albumin, renal SOD, creatinine clearance, renal MDA, renal TNF-α, renal IL-6, and renal genes expression of nuclear factor E2-related factor 2 (Nrf2) and heme-oxygenase1 (HO-1) in addition to serum levels of glucose, glycated HbA1c, urea, and creatinine were assessed. Furthermore, renal histopathology and immunohistochemical analyses were performed. ***Results*** Along with urinary albumin, renal MDA, renal TNF-α, and renal IL-6, DN group significantly increased serum levels of glucose, glycated HbA1c, urea, and creatinine when compared to the control group. Additionally, it dramatically reduced the expression of the renal genes Nrf2 and HO-1, renal SOD, and creatinine clearance levels. In addition, the DN group's NF-kB immunoreaction and Caspase-3 were both upregulated in comparison to the control. The DN+MaR1 group showed a considerable improvement in STZ-induced DN changes. ***Conclusion***: Through anti-oxidant and anti-inflammatory mechanisms, apoptosis suppression, and the up-regulation of Nrf2 and HO-1 renal gene expressions, MaR1 provides significant protective benefits against STZ-induced DN.

Corresponding author: Suzan A. Khodir, Email: suzan.abdalhameed.12@med.menofia.edu.eg, ORCID iD: 0000-0002-2535-9445.Medical PhysiologyDepartment, Faculty of Medicine, Menoufia University, Shebin El-Kom, Menoufia, Egypt.

Introduction

Diabetic nephropathy (DN) is a leading cause of end-stage renal disease worldwide and one of the main microvascular consequences of diabetes. Within 20 to 25 years after the commencement of their diabetes, 25 to 40 percent of diabetic people have DN [1]. End-stage renal disease is the result of glomerular degeneration, proteinuria, and tubule-interstitial lesions [2]. The high rates of death and disability in diabetes patients are explained by DN [1].It has been demonstrated that DN is linked to elevated levels of inflammatory cytokine production and advanced renal glycation end products (AGEs) [3].

The prevalence of DN is still rising, and many DN patients develop end-stage renal disease as a result of a steady reduction in kidney function [4]. Therefore, in order to find novel treatment targets and enhance clinical care, it is imperative to have a deeper understanding of the pathophysiology of DN.

Numerous molecular mechanisms, including oxidative stress induction, proinflammatory cytokine release, fibroblast activation, the renin-angiotensin system, and apoptosis are implicated in the renal damage caused by hyperglycemia in DN according to an expanding body of research [5].

Through the antioxidant response element (ARE), nuclear factor E2-related factor 2 (Nrf2), the primary regulator of the antioxidant response, regulates the expression of many genes that code for antioxidant proteins [6]. The oxidant-responsive transcription factor Nrf2 facilitates cytoprotection against oxidative stress by regulating the expression of heme-oxygenase1 (HO-1) [7].

Bioactive specialized pro-resolving lipid mediators (SPMs) such as maresins, which are derived from omega-3 polyunsaturated fatty acids, have been found as potential pharmacological treatments for both acute and chronic inflammatory illnesses and actively aid in the resolution of inflammation [8]. Along with their anti-inflammatory qualities, SPMs also control glucose homeostasis, insulin signaling, and other metabolic regulators in many tissues [9].

Renal inflammation can be brought on by metabolic disorders and hemodynamic alterations prompted by chronic hyperglycemia [10]. A strong and endogenous inflammatory cascade reaction may be directly or indirectly triggered by reactive oxygen species (ROS) produced by persistent hyperglycemia in renal tissues, leading to the onset and progression of DN [11]. Macrophages produce and release maresin-1 (MaR1), a lipid mediator that is naturally produced from omega-3 fatty acids. Studies on diseases linked to inflammation have demonstrated that MaR1 protects the body by reducing the generation of proinflammatory cytokines, improving macrophage phagocytosis, and restricting neutrophil infiltration [12]. It is unknown, therefore, how MaR1 and the pathophysiology of DN are related. MaR1 was found to have a protective impact on experimental models of acute kidney injury [13,].

The aim of this study was to illustrate the renoprotective impact of MaR1 in STZ induced diabetic nephropathy and the underlying processes involved with referral to Nrf2/HO-1 signaling pathway

Methods

Animals

This study was carried out in accordance with the rules established by the Animal Experimentation Ethics Committee of Menoufia University's Faculty of Medicine with IRB NO: 1/2025PATH17-1.The weight of the thirty mature male Wister rats ranged from 150 to 180 g. The rats were housed in a housing that had a 12-hour light and 12-hour dark cycle and ranged in temperature from 20 to 24 degrees Celsius. Regular rat food and tap water were also freely available to them. The rats were allowed to adapt for 10 days before the tests started.

DM induction and grouping: Type 1 diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ, 65 mg/kg) in 0.2 ml of citrate buffer (Sigma-Aldrich Chemical Co., USA) [14].

It was confirmed that 48 hours following the STZ injection, diabetes had developed. Animals having a greater fasting blood glucose level than 200 mg/dl were diagnosed with diabetes and participated in the experiment.

Thirty rats were divided into three groups (n = 10). In the control group, rats were given a single intraperitoneal (i.p.) injection of 10 mmol/L citrate buffer and for eight weeks i.p. injections of dimethyl sulfoxide (DMSO) from Fisher Scientific, Loughborough, UK.

In the diabetic Nephropathy (DN) group: diabetes was induced as previously mentioned, and then rats received i.p. DMSO injection for 8 weeks

In the maresin-1-treated DN group (DN+MaR1), rats were given 4 ng/g per BW of MaR1 i.p. every day for eight weeks after diabetes was induced. Fifty milligrams of MaR1 were dissolved in 1 ml of DMSO [15–17]. In experimental illness models, the MaR1 dose was chosen to be higher than the levels linked to authorized MaR1 bioactivity [15].

Rats kept in different metabolic cages had urine samples taken daily for a whole day. After the volume of the urine samples was determined, they were centrifuged for ten minutes at 1000 revolutions per minute (rpm). In order to assess creatinine and microalbuminuria, the supernatant was then removed and stored at -20°C.

To determine the quantity of albumin in the urine, the Microalbuminuria ELISA kit (Exocell Inc., Philadelphia, USA) was utilized. The creatinine clearance, with values expressed in mL/min, was calculated by multiplying the urine creatinine concentration (mg/dL) by the urine volume (mL/min) divided by the serum creatinine concentration (mg/dL) [18].

Following a 12-hour overnight fast, blood samples were extracted from the retroorbital venous plexus under light anesthesia and divided equally between two tubes. For 10 minutes, the first tube was centrifuged at 4,000 revolutions per minute (rpm). Until it was needed for additional analysis, the collected serum was kept at -20 degrees. The other fraction was used to estimate the HbA1c when the blood was taken into the second tube, an EDTA tube. Rats were then sacrificed by cervical dislocation, and the left kidney was utilized for a biochemical test, while the right kidney was removed for histological analysis.

Fasting serum glucose (Diamond Diagnostic, Egypt) and HbA1c (Stanbio Glycohemoglobin, Egypt) were measured by colorimetric kits. Colorimetric kits (Spectrum Diagnostics, Egypt) were used to measure the concentrations of creatinine in serum, urine, and urea.

Biochemical tests of oxidative stress and inflammatory markers

Making a Tissue Homogenate, a tissue homogenizer (MPW120, MPW Medical Instruments, China) was used to homogenize each of the weighted kidney specimens separately. Before being kept at -80°C for testing, the crude tissue homogenate was spun for 15 minutes at 10,000 rpm in an ice-cold centrifuge. According to the manufacturer's instructions, renal MDA and superoxide dismutase (SOD) were measured by spectrophotometric method using available commercial kits (Biodiagnostic CO., Dokki, Giza, Egypt). ELISA kits for renal TNF-α (Cat.: MBS2507393, MyBioSource, Sandiego, CA, USA), and renal IL-6 (Cat.: MBS269892, MyBioSource, Sandiego, CA, USA) according to the manufacturer's instructions were used.

Gene expression quantification using real-time reverse transcription polymerase chain reaction (qRT-PCR)

RT-PCR was used to analyze the relative mRNA levels of the Nrf2 and HO-1 genes in the renal tissue. The TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA from tissues in accordance with the manufacturer's instructions. The extracted RNA was stored at -80 C until it was needed. The quantity and purity of RNA were determined by measuring the absorbance at 260 nm (A260) with Nanodrop 2000 (Thermo Scientific, USA). In the first PCR step, complementary DNA (cDNA) synthesis (reverse transcription phase) was carried out using ThermoScript RT reagent kits (Invitrogen). cDNA was then amplified using SYBR Green Mix kits (Stratagene-USA) and applied Biosystems™ 7500 real-time PCR instrument (Applied Biosystems-USA). Each amplification curve's cycle threshold, or Ct, values were determined. The reference gene was glyceraldehyde-3-phosphate dehydrogenase (GAPDH). GAPDH primers sequence was:
(1) Forward primer:
5′-GTCTCCTCTGACTTCAACAGCG-3′
(2) Reverse Primer:
5′-ACCACCCTGTTGCTGTAGCCAA-3′.
Using the comparative ΔΔCt method, Nrf2 and HO-1 relative quantification and gene expression were carried out.
The following primers were used for the Nrf2 gene:
(1) Forward primer:
5′-GGTTGCCCACATTCCCAAATC-3′.
(2) Reverse primer:
5′-CAAGTGACTGAAACGTAGCCG-3′.
The following primers were used for the HO-1 gene:
(1) Forward primer:
5′-AGGTGCACATCCGTGCAGAG-3′.
(2) Reverse primer:
5′-CTTCCAGGGCCGTATAGATATGGTA-3′.

Histopathological evaluation

Kidney tissue slices were fixed at 10% neutral buffered formalin. They were then dried in ethyl alcohol, washed in xylol, and lastly placed with paraffin. Haematoxylin and Eosin was used to stain the 4 μm-thick sections.
Kidney paraffin slices (4 μm) were treated for 10 minutes at room temperature with 3% hydrogen peroxide for immunohistochemistry investigations. Blocking with BCA solution for 30 minutes was followed by overnight at 37 °C probing with primary antibodies against caspase-3 (1:100 dilution, Elabscience Corp., Wuhan, China) and NF-kB (monoclonal, dilution 1:200, Abcam).

Finally, sections were treated for 30 minutes at room temperature with a secondary antibody conjugated with specific peroxidase. Lastly, using image J software (Maryland, USA), the intensities of Caspase-3 and NF-kB immune-stained fields were assessed at a magnification level of x400.

Statistical Analysis

The data was tabulated and analyzed using the Statistical Package for the Social Sciences (SPSS) software, version 23. The Shapiro-Wilk test was used to determine if the data sets were normally distributed. The quantitative data expression was the mean, plus or minus the standard deviation. After doing a one-way analysis of variance (ANOVA), we employed a post hoc Tukey test to determine if the group differences were statistically significant.

Results

Biochemical and molecular results

While the DN group's serum levels of glucose, glycated HbA1c, urea, creatinine, urinary albumin, renal MDA, renal TNF-α, and renal IL-6 were dramatically higher than those of the control group, the group's renal SOD, creatinine clearance values, and the expression of the Nrf2 and HO-1 genes were dramatically lower. While renal SOD, creatinine clearance values, and renal gene expression of Nrf2 and HO-1 in DN+MaR1group were dramatically higher than those of DN but still dramatically lower than those of control, serum levels of glucose, glycated HbA1c, urea, creatinine, urinary albumin, renal MDA, renal TNF-α, and renal IL-6 of the DN+MaR1 group were dramatically lower than those of DN but still significantly higher than those of the control group.(Table 1).

Table (1): The measured glycated HbA1c, serum glucose, urea, creatinine, creatinine clearance, urinary albumin, renal TNF-α, renal IL-6, MDA, SOD, TNF-α, IL-6, renal Nrf2 gene expression , renal HO-1 gene expression in all studied groups.

	Control group	DN group	DN+ MaR1 group
Serum Glucose (mg/dl)	84.2±2.3	315±4.2 *	214±4.8 *#
Glycated HbA1c (% of normal Hb)	3.9±0.19	11.8±0.35 *	8.1±0.47 *#
Serum Urea (mg/dl)	55.8±6.3	141.9±7.2 *	86.8±2.4 *#
Serum Creatinine (mg/dl)	0.39±.07	1.77±0.1 *	0.9±0.3 *#
Creatinine clearance (mL/min)	1.29±0.09	0.61±0.18*	0.93±0.08 *#
urinary albumin (mg/day)	60.5±3.18	177.5±6.8*	104.2±6.2 *#
Renal MDA (nmol/ gm. Tissue)	7.9 ±0.9	33.5± 1.3*	19.3± 1.1*#
Renal SOD (U/gm. Tissue)	10.2 ±0.9	3.6±0.08*	6.9±0.21*#
Renal TNF-α (pg/ml)	105.2±3.1	295.5±3.91*	180.5±4.2*#
Renal IL-6 (pg/ml)	160.2±6.3	399.2±2.98*	275.8±6.1*#
Renal Nrf2 gene expression	1	0.38±0.09*	0.72±0.08*#
Renal HO-1 gene expression	1	0.46±0.29*	0.81±0.1*#

* Significant compared with control, # Significant compared with DN.

Histopathological results

The control group's sections revealed normal renal parenchyma devoid of tubular and glomerular anomalies (Fig. 1A).The DN group displayed tubular atrophy with thicker tubular basement membrane and glomerular mesangial enlargement with thickened glomerular basement membrane (Fig.1 B).The renal tubules and glomerulus improved in the DN+MaR1 group (Fig. 1C)

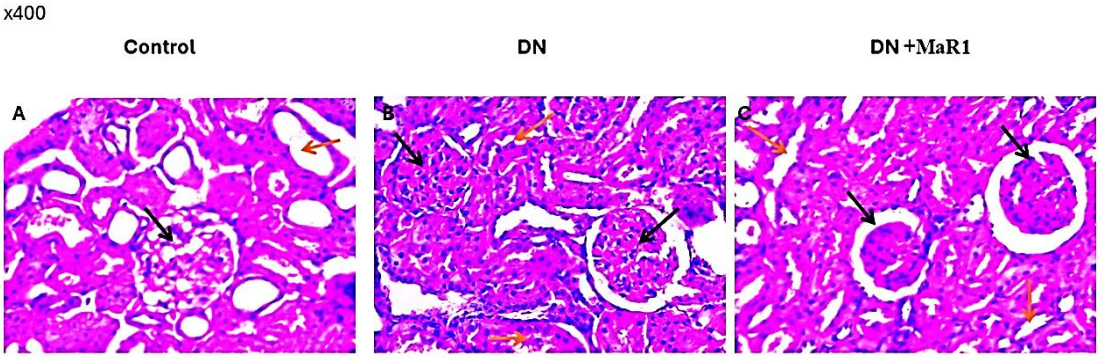

Fig 1: Kidney slices stained with H&E in the groups under study (H&E ×400):The control group's photomicrographs (A) revealed normal renal parenchyma devoid of any tubular (orange arrow) or glomerular (black arrow) anomalies. The DN group photomicrograph (B) displays tubular atrophy with thickened tubular basement membrane (orange arrows) and glomerular mesangial enlargement with thicker glomerular basement membrane (black arrows).A photomicrograph of the DN+MaR1 group in (C) revealed a marked improvement in the renal tubules (orange arrows) and glomerulus (black arrows).

Immunohistochemical results

The percentage area of caspase-3 was dramatically higher in the DN group than in the control group (47±0.33 vs. 7±0.45, P<0.05).This proportion was higher than that of the control group, but it was dramatically lower in the DN+MaR1 group than in the DN group (18±0.35 vs. 47±0.33, P<0.05).(Fig. 2: A–D).

In NF-kB stain, DN group showed a dramatic increase in the percentage area of NF-kB when compared to control group (54±0.11 vs 8±0.03, P<0.05). However, the DN+MaR1 group showed a dramatic decrease in this percentage when compared to DN group (16±0.05 vs 54±0.11, P<0.05), but still increased from the control (Fig. 2: E-H).

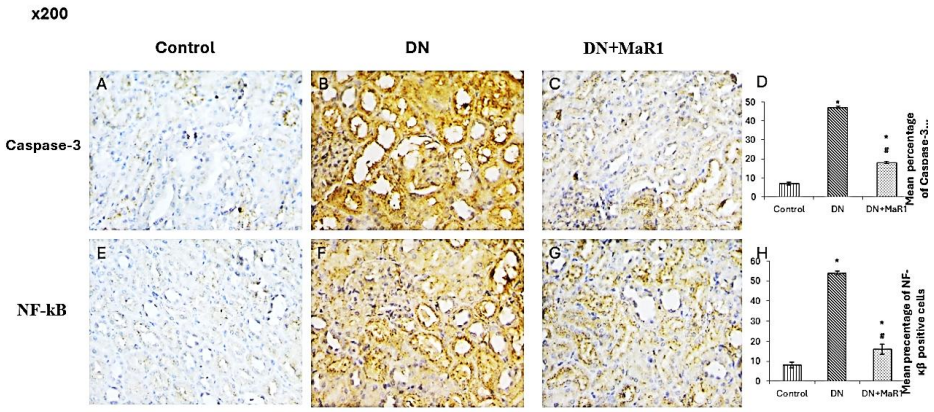

Fig (2): Representative micrographs of the different experimental groups showing a substantial elevation of the Caspase-3 (A-D) and NF-kB (E-H) immunoreaction in the DN group and a significant downregulation in the DN+MaR1 group.

Discussion

In a DN model produced by STZ, our study provides evidence that MaR1 can stop kidney damage. We showed that MaR1's renoprotective action against STZ-induced DN in rats is mediated through the cell signaling pathway Nrf2/HO-1.

The results from our DN group indicated a changed glycemic state, which was in line with findings from previous research.[19].

MaR1 significantly reduced STZ-induced hyperglycemia. There is growing evidence that pro-resolving mediators can reduce adipose inflammation and renal dysfunction, two

conditions linked to diabetes. Macrophages' production of MaR1 has strong pro-resolving and tissue homeostatic effects [20].

Additionally, Hong et al. discovered that maresin-like mediators contributed to wound healing by giving diabetic macrophages (Mfs) their reparative functions back; furthermore, they may reduce Mfs inflammatory activation and potentially reduce chronic inflammation in diabetic wounds brought on by Mfs activation [21]. This suggests that MaR1 may protect against diabetic nephropathy by reducing inflammation and early fibrosis.

According to Asleh et al., podocytes were mainly implicated in DN with proteinuria because of alterations in podocyte shape, and they were extremely vulnerable to the negative effects of hyperglycemia. These results were in line with the DN group's findings of decreased renal function in comparison to the control group [22].

MaR1 dramatically ameliorated renal impairment caused by STZ. The renoprotective impact of MaR1 was documented in a previous study [13]. According to Morita et al., participants with stage 3–4 diabetic nephropathy had lower urine MaR1 than those in the control group and those with stage 1 or 2 diabetic nephropathy [23]. MaR1 protects mouse glomerular mesangial cells (GMCs) against glucotoxicity by reducing inflammation and reactive oxygen species (ROS) [20].

A different study shows a correlation between the onset of DKD and lower serum MaR1 levels.MaR1 may reduce inflammation brought on by glucotoxicity and DKD. HbA1c and the duration of diabetes were substantially inversely correlated with serum MaR1 levels [24]. Previous studies have shown that MaR1 can alleviate DKD by inhibiting ROS generation [20].

For instance, by reducing inflammation and fibrosis, MaR1 helps mice's renal mesangial cells recover from the damage caused by hyperglycemia [20]. In diabetic kidney disease (DKD), maresin-1 reduces oxidative stress and inflammation [24]. Thus, our present findings identify that MaR1 has a therapeutic potential for DN.

The pathophysiology of DN is significantly influenced by oxidative stress. Our results are in line with previously published data, which demonstrate that oxidative stress is caused by STZ-induced diabetes in rats. This is demonstrated by a large rise in renal MDA levels and a decrease in SOD in the DN group [25]. Hyperglycemia can worsen oxidative stress and lipid peroxidation by increasing free radicals and decreasing cellular antioxidants. DN symptoms result from structural damage to proteins, lipids, DNA, and RNA caused by oxidative stress [26].

MaR1 dramatically improved oxidative stress caused by STZ this goes in line with a previous study [27]. Through a mechanism that may include the liver's enhanced antioxidant glutathione peroxidase enzyme activity, MaR1 reduces oxidative damage caused by ischemia/ reperfusion (I/R) injury [28].MaR1 antioxidant impact may be due to the inhibition of mitochondrial impairment and decreased production of ROS [29]

Nephropathy is influenced by renal inflammation [30]. Proinflammatory indicators significantly increased in the DN group as in previously reported results [25]. Albuminuria, inflammatory chemicals produced in response to albuminuria, and severe hyperglycemia can all contribute to

increased inflammation by promoting the synthesis of chemokines [31].

MaR1 significantly reduced the inflammatory response that STZ induced. Previous studies have shown that MaR1 prevents neutrophil infiltration, reduces the generation of proinflammatory factors, increases macrophage phagocytosis, and inhibits NF-κB activation to protect against a variety of chronic inflammatory illnesses [20].

Furthermore, the administration of MaR1 increased the synthesis of IL10 while decreasing the production of IL17. By lowering proinflammatory circumstances and modifying T-cell responses to support an anti-inflammatory state, our findings highlight the anti-inflammatory role of MaR1 [32].

NF-κB is a family of transcription factors that regulate genes linked to cell survival, inflammation, and immunology [33]. Proinflammatory cytokines are released by the NF-κB signaling pathway. Our findings indicate that, in comparison to control, DN shows a similar up-regulation in NF-kB immunoreaction. These results align with previous studies [34].

When compared to the DN group, MaR1 significantly reduced the kidney's NF-kB immunoreaction. By reducing neutrophil infiltration and blocking NF-κB/STAT3/MAPK activation, which decreases pro-inflammatory cytokine levels and boosts anti-inflammatory cytokine levels, MaR1 can considerably reduce acute kidney injury, according to a prior research [35].Also MaR1 dramatically downregulated NF-kB in Ovalbumin (OVA)-induced asthma inflammation [36].

By preventing oxidative damage, controlling apoptosis, and reducing inflammation, the Nrf2-dependent gene program and its target protein, HO-1, the inducible form of hemeoxygenase, had positive impacts on both pathogenic and developmental situations [37].

Diabetic complications have been studied in relation to the Nrf2/HO-1 pathway [38,39]. By activating the Nrf2/HO-1 pathway, high mobility group box 1 (HMGB1) down-regulation shields mesangial cells in diabetic kidneys from glucose-induced ferroptosis [40]. In the DN group, STZ downregulated Nrf2/HO-1 genes expression. When compared to the control group, MaR1 significantly up-regulated Nrf2/HO-1.This is consistent with earlier research where MaR1 up-regulated the Nrf2/HO-1 pathway [41].

In the pathophysiology of renal injury, complex pathway interaction accelerated renal tubular apoptosis as well as increased oxidative stress and inflammation [42].Caspases-3 levels dramatically increased in the DN group, which is in line with previous studies [43].

In this study, the DN+MaR1 group also showed a substantial downregulation of caspase-3 immunoreactivity in comparison to the DN group. This is in line with previous findings [27, 44]. This might be attributed to MaR1 anti-inflammatory and anti-oxidant qualities.

Conclusion

MaR1 reduced the effects of STZ-induced diabetic nephropathy by upregulating the expression of the renal genes Nrf2 and HO-1, as well as by the antioxidant, anti-inflammatory, and anti-apoptotic pathways.

Acknowledgment: The authors would like to thank the Faculty of Medicine, Menoufia University, for providing the necessary facilities.

Funding: This paper was not funded.

Competing Interests: The authors have no relevant financial or non-financial interests to disclose

References

1- **X. Deng, J. Cheng, and M.** Shen, "Vitamin D improves diabetic nephropathy in rats by inhibiting renin and relieving oxidative stress," *Journal of Endocrinological Investigation*, vol. 39, no. 6, pp. 657–666, 2016.

2- **Y. Zhao, W. Zhang, Q. Jia et al.,** "High dose vitamin E attenuates diabetic nephropathy via alleviation of autophagic stress," *Frontiers in Physiology*, vol. 9, p. 1939, 2019.

3- **Y. Yuan, H. Sun, and Z. Sun,** "Advanced glycation end products (AGEs) increase renal lipid accumulation: a pathogenic factor of diabetic nephropathy (DN)," *Lipids HealthDisease*, vol. 16, no. 1, p. 126, 2017.

4- **P. Ruggenenti, P. Cravedi, and G. Remuzzi,** "*e RAAS in the pathogenesis and treatment of diabetic nephropathy," *NatureReviews Nephrology*, vol. 6, no. 6, pp. 319–330, 2010

5- **Ma LIANAN, Liu SHOU PENG, Zhen XIAO EN et al**. Astaxanthin delayed the pathogenesis of diabetic nephropathy in type 1 diabetic rats. Biocell, 2022; 46(8), 1911-1916.

6- **Khodir, S., Alafify, A., Omar, E., & Al-Gholam, M. (2021).** Protective potential of ginseng and/or coenzyme Q10 on doxorubicin-induced testicular and hepatic toxicity in rats. *Open Access Macedonian Journal of Medical Sciences*, *9*(A), 993-1005.

7- **Luo Y, Shang P, Li D. Luteolin:** a flavonoid that has multiple cardio-protective effects and its molecular mechanisms. Front Pharmacol 2017;8:692

8- **Serhan CN.** Pro-resolving lipid mediators are leads for resolution physiology. Nature 2014; 510: 92–101.

9- **Hellmann J, Tang Y, Kosuri M, Bhatnagar A, Spite M.** Resolvin D1 decreases adipose tissue macrophage accumulation and improves insulin sensitivity in obese diabetic mice. FASEB J 2011; 25: 2399–2407.

10- **P. M. Garc´ıa-Garc´ıa, M. A.** Getino-Meli´an, V. Dom´ınguez-Pimentel, and J. F.Navarro-Gonz´alez, "Inflammation in diabetic kidney disease," *World Journal of Diabetes*, vol. 5, no. 4, pp. 431– 443, 2014.

11- **C. Wang, Y. Pan, Q.-Y. Zhang, F.-M. Wang, and L.-D. Kong,** "Quercetin and allopurinol ameliorate kidney injury in STZ treated rats with regulation of renal NLRP3 inflammasome activation and lipid accumulation," *PLoS ONE*, vol. 7, no. 6, Article ID e38285, 2012

12- **J. Dalli, M. Zhu, N. A. Vlasenko et al.,** "The novel 13S,14Sepoxy-maresin is converted by humanmacrophagestomaresin 1 (MaR1), inhibits leukotriene A4 hydrolase (LTA4H), and shifts macrophage phenotype," *FASEB Journal*, vol. 27, no. 7, pp. 2573–2583, 2013.

13- **Li J, Zhang Z, Wang L, Jiang L, Qin Z, Zhao Y, Su B (2021)** Maresin 1 Attenuates Lipopolysaccharide-Induced Acute Kidney Injury via Inhibiting NOX4/ROS/NF-κB Pathway. Frontiers in pharmacology 12:782660

14- **Al-Awar A, Kupai K, Veszelka M et al.** Experimental diabetes mellitus in different animal models. Journal of Diabetes Research 2016; 1–12. DOI 10.1155/2016/9051426.

15- **Francos-Quijorna I, Santos-Nogueira E, Gronert K, Sullivan AB, Kopp MA, Brommer B, David S, Schwab JM, López-Vales R (2017)** Maresin 1 Promotes Inflammatory Resolution, Neuroprotection, and Functional Neurological Recovery After Spinal Cord Injury. J Neurosci 37:11731-11743

16- **Jung TW, Kim H-C, Abd El-Aty AM, Jeong JH (2018)** Maresin 1 attenuates NAFLD by suppression of endoplasmic reticulum stress via AMPK–SERCA2b pathway. Journal of Biological Chemistry 293:3981-3988

17- **Soto G, Rodríguez MJ, Fuentealba R, Treuer AV, Castillo I, González DR, Zúñiga-Hernández J (2020)** Maresin 1, a Proresolving Lipid Mediator, Ameliorates Liver Ischemia-Reperfusion Injury and Stimulates Hepatocyte Proliferation in Sprague-Dawley Rats. Int J Mol Sci 21

18- **T. Bazzano, T. I. Restel, L. C. Porfirio, A. S. D. Souza, and I. S. Silva,** "Renal biomarkers of male and female Wistar rats (*Rattusnorvegicus*) undergoing renal ischemia and reperfusion," *ActaCirurgicaBrasileira*, vol. 30, no. 4, pp. 277–288, 2015.

19- **Khodir, S. A., Samaka, R. M., &Ameen, O. (2020).** Autophagy and mTOR pathways mediate the potential renoprotective effects of vitamin D on diabetic nephropathy. International Journal of Nephrology, 2020(1), 7941861

20- **Tang, S., Gao, C., Long, Y., Huang, W., Chen, J., Fan, F., ...& Xu, Y. (2017).** Maresin 1 mitigates high glucose-induced mouse glomerular mesangial cell injury by inhibiting inflammation and fibrosis. *Mediators of Inflammation*, *2017*(1), 2438247.

21- **S. Hong, Y. Lu, H. Tian et al.,** "Maresin-like lipid mediators are produced by leukocytes and platelets and rescue reparative function of diabetes-impaired macrophages," *Chemistry andBiology*, vol. 21, no. 10, pp. 1318–1329, 2014.

22- **Asleh R, Nakhoul FM, Miller-Lotan R et al.** Poor lysosomal membrane integrity in proximal tubule cells of haptoglobin 2-2 genotype mice with diabetes mellitus," Free Radical Biology and Medicine, 2012; 53(4): 779–786, 2012.

23- **Y. Morita, M. Kurano, E. Sakai, M. Sawabe, J. Aoki, and Y.** Yatomi, "Simultaneous analyses of urinary eicosanoids and related mediators identified tetranor-prostaglandin E metabolite as a novel biomarker of diabetic nephropathy," Journal of Lipid Research, vol. 62, article 100120, 2021.

24- **Li, X., Xu, B., Wu, J., Pu, Y., Wan, S., Zeng, Y., ...& Xu, Y. (2022).** Maresin 1 alleviates diabetic kidney disease via LGR6-mediated cAMP-SOD2-ROS pathway. *Oxidative medicine and cellular longevity*, *2022*(1), 7177889.

25- **Mahmoud AM, Ashour MB, Abdel-Moneim A et al.** Hesperidin and naringin attenuate hyperglycemia- mediated oxidative stress and proinflammatory cytokine production in high fat fed/streptozotocin-induced type 2 diabetic rats, Journal of Diabetes and its Complications, 2012; 26(6):483–490.

26- **Manda G, Checherita AI, Comanescu MV et al.** Redox signaling in diabetic nephropathy: hypertrophy versus death choices in mesangial cells and podocytes, Mediators of Inflammation, 2015; vol. 2015, Article ID 604208, 13 pages, 2015.

27- **Khodir, S. A., Sweed, E. M., Faried, M. A., Abo Elkhair, D. M., Khalil, M. M., Afifi, K. H., & El Agamy, D. F. (2025).** Neuroprotective Effect of Maresin-1 in Rotenone-Induced Parkinson's Disease in Rats: The Putative Role of the JAK/STAT Pathway. *Neurochemical Research*, *50*(1), 30

28- **Tang, D., Fu, G., Li, W., Sun, P., Loughran, P. A., Deng, M., ...&Billiar, T. R. (2021).** Maresin 1 protects the liver against ischemia/reperfusion injury via the ALXR/Akt signaling pathway. *Molecular Medicine*, *27*, 1-15.

29- **Gu J, Luo L, Wang Q, Yan S, Lin J, Li D, Cao B, Mei H, Ying B, Bin L, Smith FG, Jin SW (2018)** Maresin 1 attenuates mitochondrial dysfunction through the ALX/cAMP/ROS pathway in the cecal ligation and puncture mouse model and sepsis patients. Laboratory investigation; a journal of technical methods and pathology 98:715-733

30- **Landon R, Gueguen V, Petite H et al.** Impact of astaxanthin on diabetes pathogenesis and chronic complications. Marine drugs 2022; 18(7), 357.

31- **Navarro-Gonz´alez F, Mora-Fern´andez C.** role of inflammatory cytokines in diabetic nephropathy," Journal of the American Society of Nephrology 2008; 19(3): 433–442.

32- **Zahoor, I., Nematullah, M., Ahmed, M. E., Fatma, M., Mir, S., Ayasolla, K., ...&Giri, S. (2023).** Maresin-1 promotes neuroprotection and prevents disease progression in experimental models of multiple sclerosis through metabolic reprogramming and shaping innate and adaptive disease-associated cell types. *bioRxiv*.

33- **Yuan YH, Sun JD, Wu MM, Hu JF, Peng SY, Chen NH (2013)** Rotenone could activate microglia through NFκB associated pathway. Neurochemical research 38:1553-1560

34- **Awad AM, Elshaer SL, Gangaraju R et al.** Ameliorative effect of montelukast against STZ induced diabetic nephropathy: targeting HMGB1, TLR4, NF-κB, NLRP3 inflammasome, and autophagy pathways. Inflammopharmacology, 2024; 32(1), 495-508.

35- **Sun, S., Wang, J., Wang, J., Wang, F., Yao, S., & Xia, H. (2019).** Maresin 1 mitigates sepsis-associated acute kidney injury in mice via inhibition of the NF-κB/STAT3/MAPK pathways. *Frontiers in pharmacology*, *10*, 1323.

36- **Ou, G., Liu, Q., Yu, C., Chen, X., Zhang, W., Chen, Y., ...&Zeng, M. (2021).** The Protective Effects of Maresin 1 in the OVA-Induced Asthma Mouse Model. *Mediators of Inflammation*, *2021*(1), 4131420.

37- **Fang, P., Wang, Y., Sun, F., Lin, H., & Zhang, X. (2023).** Effects of albiflorin on oxidative stress and inflammatory responses in rats with acute spinal cord injury. *Immunity, Inflammation and Disease*, *11*(9), e1015.

38- **Aleksunes LM, Reisman SA, Yeager RL, Goedken MJ, Klaassen CD**. Nuclear factor erythroid 2-related factor 2 deletion impairs glucose tolerance and exacerbates hyperglycemia in type 1 diabetic mice. J PharmacolExpTher. 2010;333(1):140–51.

39- **Bitar MS, Al-Mulla F. A** defect in Nrf2 signaling constitutes a mechanism for cellular stress hypersensitivity in a genetic rat model of type 2 diabetes. Am J PhysiolEndocrinolMetab. 2011;301(6):E1119–29

40- **Wu Y, Zhao Y, Yang HZ, Wang YJ, Chen Y.** HMGB1 regulates ferroptosis through Nrf2 pathway in mesangial cells in response to high glucose. Biosci Rep. 2021;41(2).

41- **Yang W, Wang Y, Zhang C, Huang Y, Yu J, Shi L, et al**. Maresin1 protect against Ferroptosis-Induced Liver Injury through ROS Inhibition and Nrf2/HO-1/GPX4 activation. Front Pharmacol. 2022;13:865689

42- **Martınez-Castelao A, Hueso M, Sanz V et al.** Treatment of hypertension after renal transplantation: long-term efficacy of verapamil, enalapril, and doxazosin. Kidney Int. 1998; 68: S130–S134

43- **Ahmed OM, Ali TM, Abdel Gaid MA et al.** Effects of enalapril and paricalcitol treatment on diabetic nephropathy and renal expressions of - -TNF-α, p53, caspase-3 and Bcl-2 in STZ-induced diabetic rats. PloS one, 2019;14(9), e0214349.

44- **Tang, D., Fu, G., Li, W., Sun, P., Loughran, P. A., Deng, M., ...&Billiar, T. R. (2021).** Maresin 1 protects the liver against ischemia/reperfusion injury via the ALXR/Akt signaling pathway. *Molecular Medicine*, *27*, 1-15.