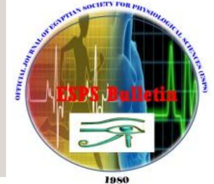
Bull. Egypt. Soc. Physiol. Sci. *Vol. (45), Issue (1)*,123-137

Bull. of Egyp. Soc. Physiol. Sci.

(Official Journal of Egyptian Society for Physiological Sciences)

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



Renoprotective Effect of Maresin-1 in Deoxycorticosterone Acetate-Salt-Induced Hypertension in Rats: Targeting TLR4/NF-kB Pathway

Ahmad M. Gaafar[1], Suzan A. Khodir[1*], Mohamed Shebl Amer[1], Amany Wahb[2], Noha M. Abd El-aziz[3], Amany Tawfik Elfakhrany [4], Marwa Mohamed Safwat[5,6], Heba Gamal Sherif[7], Alaa M. Bahbah[8], Asmaa I. Bayomi[9] , Samar M. Hussein[10]

[1] Medical PhysiologyDepartment, Faculty of Medicine, Menoufia University, Shebin El-Kom, Menoufia, Egypt.
[2] Medical Biochemistry and Molecular Biology Department, Faculty of medicine, Helwan University, Egypt.
[3]Anatomy and Embryology Department, Faculty of Medicine, Menoufia University, Shebin El-Kom, Menoufia, Egypt.
[4]Clinical Pharmacology Department, Faculty of medicine, Menoufia University, Shebin El-Kom, Menoufia, Egypt.
[5]Human Anatomy and Embryology Department, Faculty of Medicine, Ain Shams University, Ain Shams, Cairo, Egypt
[6]Anatomy Department, IbnSina National College for Medical Studies, Jeddah.
[7]Internal Medicine department Faculty of Medicine, Menoufia University, Shebin El-Kom, Menoufia, Egypt.
[8]Pathology department, Faculty of Medicine, Menoufia University, Shebin El-Kom, Menoufia, Egypt.
[9]Zoology Department - Faculty of Science, Menoufia University, Menoufia, Egypt.
[10] Medical Physiology Department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt.

Submit Date : 05 Dec. 2024
Revised Date : 10 Dec. 2024
Accept Date : 13 Dec. 2024

Keywords

- Caspase-3
- DOCA
- Hypertension
- Maresin-1
- NF-Kb
- TLR4

Abstract

Background: Essential hypertension causes the kidney illness known as hypertensive nephropathy, which frequently results in both structural and functional renal impairment..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 demonstrate the underlying mechanisms and renoprotective impact of MaR1 in rats with deoxycorticosterone acetate-salt-induced hypertension. Material &Methods: Thirty Wister albino male rats divided into control, DOCA, DOCA+MaR1 groups. After 8 weeks rats were subjected to ABP, renal blood flow velocity (RBFV) and renal artery resistance (RAR) measurement and serum levels of urea, creatinine in addition to creatinine clearance, urinary albumin, renal MDA, SOD, TNF-α, IL-6, renal genes expression of TLR4 and NF-κB were assessed. Renal tissue was evaluated histopathologically and immunohistochemically. Results: The DOCA group's measured SBP, DBP, MABP, RAR, serum levels of urea, creatinine, renal MDA, renal TNF-α, renal IL-6, and renal genes TLR4 and NF-kB were all dramatically elevated than those of the control group, but their RBFV, renal SOD, and creatinine clearance values were significantly lower. MaR1 significantly enhanced the alterations brought about by DOCA. Conclusion: MaR1 alleviated DOCA induced hypertensive nephropathy by anti-oxidant, anti-inflammatory, anti-apoptotic mechanisms and by down-regulation of TLR4 and NF-κB renal genes expression.

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

Introduction

A persistent rise in blood pressure (BP) is the hallmark of hypertension, a serious global health issue. It is the primary cause of the worldwide burden of disease [1].Uncontrolled hypertension damages organs, particularly the kidneys, which can ultimately result in end-stage renal disease (ESRD) [2].

Essential hypertension causes the renal illness known as hypertensive nephropathy (HN), which frequently results in both structural and functional renal impairment. Nearly 27.5% of new dialysis patients in the USA are due to HN, according to recent reports [3], and 20.78% of hospitalized patients with chronic renal disease in China are due to HN, according to statistical statistics [4].In many nations, after diabetic nephropathy, HN is emerging as the second most frequent cause of ESRD[5].

One of the sensitive markers of early kidney impairment in hypertensive individuals is proteinuria. As one of the features of hypertensive renal impairment and a significant risk factor for the advancement of ESRD, proteinuria is thought to have a significant impact on the prognosis of hypertension [6].

The pathophysiology of hypertension-induced renal injury is a complex process involving several pathways, including hemodynamics, inflammation, oxidative stress, and endothelial dysfunction [7]. According to earlier research, one of the key mechanisms behind this pathophysiology is oxidative stress [8].One of the main organs harmed by high blood pressure is the kidneys. Recent studies have revealed that the kidneys of hypertensive animal models produce excessive amounts of reactive oxygen species (ROS) [9].The generation of ROS causes protein oxidation and disrupts cell signaling, which results in inflammation and fibrosis [10].

The deoxycorticosterone acetate (DOCA)-salt hypertensive rat is one of the most often used experimental models in the research of antihypertensive activity. Treatment with DOCA-salt results in inflammation in autonomic nerve centers and hyperactivity of the sympathetic nervous system. An overactive sympathetic nervous system causes and maintains hypertension. Additionally, rats given DOCA-salt develop sodium retention and excessive salt consumption, which leads to volume-dependent hypertension [11].

Cell membrane-based pattern recognition receptors, such toll-like receptors (TLRs), are able to identify aberrant chemical patterns that pose a threat to cells [12]. TLRs, namely TLR4, use the nuclear transcription factor kappa B (NF-kB) signaling pathway to enhance cytokine production and mediate innate immune responses [13].

Antihypertensive medications, primarily angiotensin converting enzyme inhibitors and angiotensin receptor blockers, are the mainstay of the current therapeutic therapy for hypertensive nephropathy. Antihypertensive medications may work primarily by reducing oxidative stress and correcting chronic hypoxia [14]. In order to prevent and cure hypertensive renal impairment, complementary treatments are suggested as potential approaches [15].

One of the newest groups of mediators supporting inflammatory regression is maresin 1 (MaR1), a lipid mediator that is naturally generated from omega-3 fatty acids and produced and released by macrophages [16].MaR1 is one of the specialized

proresolving lipid mediators (SPMs). According to earlier research, MaR1 protects against a number of chronic inflammatory disorders by preventing neutrophil infiltration, lowering proinflammatory factor production, increasing macrophage phagocytosis, and preventing NF-κB activation [17, 18].

Therefore, the current investigation was conducted for the first time to the best of our knowledge to determine if the possible protective effects of MaR1 in this experimental model of DOCA-salt are linked to the TLR4/NF-kB signaling pathway.

Materials and Methods

Animals

Using statistics and the sample size pro software version 6, a sample size of 30 rats was established. The study's confidence level is 95% and its power is 80%. At the beginning of the trial, thirty male Wister albino rats weighing between 200 and 230 grams each were placed in animal cages at a random temperature of 25 degrees Celsius. Rats were given unrestricted access to standard laboratory diet and water. Every technique described in this work was carried out in strict accordance with the Animal Research Reporting of In Vitro Experiments (ARRIVE) guidelines. In addition, the National Organizations of Health's Public Health Service Policy on Research Laboratory Animals was followed to ensure the animals' appropriate treatment. The Ethical Committee of Menoufia University's College of Medicine in Egypt approved the study with IRB NO:12/2024ANAT17. Each cage included five animals in a specific pathogen-free environment with a temperature of 24 ± 3 °C, a 12-hour light/dark cycle, and unlimited access to food and water.

Group 1 (control group): For eight weeks, rats were given intraperitoneal (i.p.) injections of dimethyl sulfoxide (DMSO) from Fisher Scientific, Loughborough, UK. Rats were given twice-weekly subcutaneous (s.c.) injections of olive oil (1 mL/kg) for the last four weeks.

Group 2 (DOCA): received intraperitoneal (i.p.) dimethyl sulfoxide (DMSO) (Fisher Scientific, Loughborough, UK) DMSO injection for 8 weeks. In the last 4 weeks, rats received s.c. injections of DOCA (Sigma, St Louis, MO, USA) (25 mg/kg dissolved in olive oil) twice weekly and 1% NaCl in their drinking water. They had not been uninephrectomized [11,19, 20]

Group 3 (DOCA-induced hypertension MaR1-treated (DOCA+MaR1) group): rats were given DOCA as in group 2 plus daily intraperitoneal (4 ng/g per BW) of MaR1 (Cayman Chemical, Ann Arbor, MI, USA), with 50 mg of Mar1 dissolved in 1 ml of DMSO For eight weeks [21–23],.In experimental illness models, the MaR1 dose was chosen to be higher than the levels linked to authorized MaR1 bioactivity [21]. Prior to receiving a DOCA injection, rats were given MaR1 once daily for four weeks. For the next four weeks, they received DOCA and MaR1 at three-hour intervals.

Rats were subjected to ABP measurement then given anesthesia, for the measurement of renal artery blood flow velocity (RBFV) and resistance (RAR) after 8 weeks, after which they were placed in metabolic cages for a 24-hour urine collection period.Following that, the blood sample was prepared for further biochemical analysis. Rats were killed by dislocating their cervical spines.After being dissected, the right kidney was preserved for histological and

immunohistochemical analyses. For the biochemical test, the left kidney was kept at a temperature of -80.

Assessment of renal hemodynamics

As previously mentioned, a pulsed Doppler flowmeter (Hadeco, Hayashi Denki Co. Ltd., Japan) was used to measure renal blood flow velocity (RBFV) and renal artery resistance (RAR) [25]. In short, rats were given with xylazine and ketamine at doses of 2 mg/kg and 60 mg/kg, respectively to induce anesthesia. The left renal artery was then exposed by a midline abdominal incision. The probe was positioned above the blood vessel until a stable record could be obtained after the tip had been filled with coupling gel.

Non-invasive blood pressure measurement

The diastolic and systolic blood pressures were measured non-invasively using the MP 36R Ultimate System R (BIOPAC, Aero Camino, USA). Before the experiment, the rat was put in the animal heating chamber's heating section while within the restrainer. The rat's temperature reached the ideal level after half an hour. The restrainer remained within the animal heating chamber, and the sensor was fastened to the rat's tail. Blood pressure measurements were taken after the rat had calmed down. Numerical data was analyzed using acknowledge software [26].

Urine Collection. Urine samples were collected for 24 hours from rats housed in separate metabolic cages. Urine samples were measured for volume, and then centrifuged for 10 minutes at 1000 revolutions per minute (rpm) to measure creatinine and microalbuminuria.

Measurements of Creatinine Clearance. Urinary microalbuminuria was measured using the immunoturbidimetric method (Gcell Company, Beijing, China).Urine creatinine concentration (mg/dL) multiplied by urine volume (mL/min) to plasma creatinine concentration (mg/dL) yielded the creatinine clearance (ml/min), with values reported in mL/min. [27]. Colorimetric kits (Biodiagnostic Co., Giza, Egypt) were used to measure the concentrations of urea and creatinine in plasma and urine.

Biochemical analysis

A retroorbital blood sample was then taken in heparinized capillary tubes and extracted at 500×g centrifuge for 20 min at 4 °C to gain the serum and reserved at a temperature of −80°C till biochemical analysis of serum urea and creatinin using colorimetric kits (Biodiagnostic Co., Giza, Egypt)

Renal homogenate preparation

The tissue was homogenized and centrifugation was done at 4000 r.p.m. for 15 minutes using a tissue homogenizer (MPW120; MPW, Medical Instruments, China), and the supernatant was removed and stored at -80°C for further biochemical assay of renal malondialdehyde (MDA) and super oxide dismutase (SOD) using specific colorimetric kits (Biodiagnostic Co., Giza, Egypt) according to the kit instructions and measurement of renal TNF-α and IL-6 using the corresponding rat ELISA kits (TNF-α: ERT2010-1, Assaypro LLC, Saint Charles, Missouri, USA, cystatin C: ab201281, IL-6: ab100772, Abcam, Cambridge, UK) following the manufacturer's procedure.

Evaluation of TLR4 and NF-kB genes expression levels by real-time polymerase chain reaction (RT-PCR) quantitative analysis

Qiagen RN Easy Plus Universal Kit, manufactured in the United States, was used to isolate the total RNA of renal tissues. The purity and quality of the

RNA were then confirmed. Until it was ready for use, the RNA was stored at -80 °C. First, cDNA was produced using the QuantiTect Reverse Transcription Kit, which is produced by Qiagen in the United States.For one set, they used the Applied Biosystems 2720 heat cycler (Singapore). GAPDH primers were used as an RNA loading control in RT-PCR techniques. The cDNA was amplified in the second step, and it was then utilized in a quantitative real-time PCR using SYBR green to measure the relative quantity (RQ) of BDNF gene expression. Primers made in Midland, Texas, and the SensiFASTTMSYBR Lo-ROX Kit from the USA wereutilized. The forward primer for TLR4 was (TCAGCTTTGGTCAGTTGGCT), and the Reverse was (GTCCTTGACCCACTGCAAGA). For The NF-kB forward primer was (TCGACCTCCACCGGATCTTTC). The reverse primer was (GAGCAGTCATGTCCTTGGGT). Lastly, data scanning was finally finished with Applied Biosystems 7500 software version 2.0.1 [28].With a comparative ΔΔCt method, the RQ of TLR4 and NF-kB gene expression was carried out.This technique compares the gene's mRNA to a control after normalizing it to an endogenous reference gene (GAPDH).

Histopathological method

Kidney tissue slices were fixed at formalin for histological investigations. 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. [29].

Kidney paraffin slices (4 µm) were treated for 10 minutes with 5% hydrogen peroxide for immunohistochemistry investigations. After being cleaned with PBS and blocked for 30 minutes with BCA solution, the samples were probed overnight at 37 °C using 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 specie peroxidase. Lastly, using image J software (Maryland, USA), the intensities of Caspase-3 and NF-kB immune-stained fields were assessed in five randomly chosen distinct microscopic fields for each group at a magnification level of x400.

Statistical analysis

The data's normality is assessed using the Shapiro test. The mean ± standard deviation of the results is shown. Analysis of variance (ANOVA) was used to assess the collected data. The data was examined using SPSS (Version 23) (SPSS Inc., Armonk, NY, USA). The mean±SD was used to express the data. A statistical criterion of $P < 0.05$ was used to establish the significance.

Results

The measured SBP,DBP , MABP, RAR, serum levels of urea, creatinine addition to urinary albumin, renal MDA, renal TNF-α, renal IL-6, renal genes TLR4 and NF-kB of DOCA group were dramatically increased compared to control however RBFV, renal SOD and creatinine clearance values of DOCA group were substantially decreased compared to control. The measured SBP, DBP, MABP, RAR, serum levels of urea, creatinine addition to urinary albumin, renal MDA, renal TNF-α, renal IL-6, renal genes TLR4 and NF-kB of DOCA+MaR1group were substantially decreased compared to DOCA but still dramatically increased compared to control, however RBFV, renal SOD and creatinine

clearance values of DOCA+MaR1were substantially increased compared to DOCA but still substantially decreased compared to control (Fig. 1, Table 1).

Table (1): The measured SBP,DBP, MABP, RBFV, RAR, serum urea, creatinine, creatinine clearance, urinary albumin, renal TNF-α, renal IL-6, renal MDA, SOD, and renal TLR4 and NF-kB genes expressionin all studied groups

	Control group	DOCA group	DOCA+MaR1 group
SBP (mmHg)	105.6±2.11	190.8±1.4*	146.6±0.17 *#
DBP (mmHg)	68±1.9	119±2.8*	93.1±3.1 *#
MABP (mmHg)	85.2±2.3	148.3±2.1 *	117.9±2.3 *#
RBFV (cm/second)	5.3±0.02	3.1±0.11 *	4.33±0.13 *#
RAR (PRU)	0.85±0.04	1.71±0.01 *	1.3±0.03 *#
Serum Urea (mg/dl)	60.8±4.2	160±4.2 *	88±2.6 *#
Serum Creatinine (mg/dl)	0.41±.09	1.9±0.1 *	0.91±0.07 *#
Creatinine clearance (mL/min)	1.41±0.07	0.561±0.09*	0.83±0.08 *#
urinary albumin (mg/day)	21.8±2.15	175±3.8*	91±3.5 *#
Renal MDA (nmol/ gm. Tissue)	7.2 ±0.9	24± 0.9*	15.2± 1.04*#
Renal SOD (U/gm. Tissue)	10.2 ±1.03	3.08±0.31*	6.89±0.07*#
Renal TNF-α (ng/ml)	20.8±0.57	52.3±12.91*	31.1±2.1*#
Renal IL-6 (pg/mL)	120.5±5.42	201±2.99*	142.5±3.1*#
Renal TLR4 gene expression	1	5.1±0.3*	2.9±0.19*#
Renal NF-κB gene expression	1	4.2±0.19*	2.4±0.11*#

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

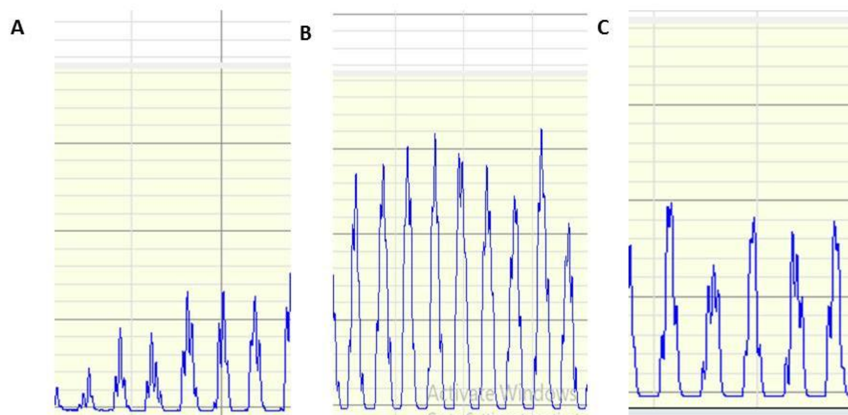


Fig (1): Blood pressure values in all studied groups: A- control, B- DOCA and C- DOCA+MaR1

Hematoxylin and Eosin staining:

Sections from the control group showed normal renal parenchyma without any glomerular and vascular abnormalities (Fig. 2A). The DOCA group showed severe glomerulosclerosis, tubulointerstitial infiltration and renal artery sclerosis (Fig. 2B). The DOCA+MaR1 group showed dramatic improvement of the renal parenchyma with mild glomerular sclerosis (Fig. 2C).

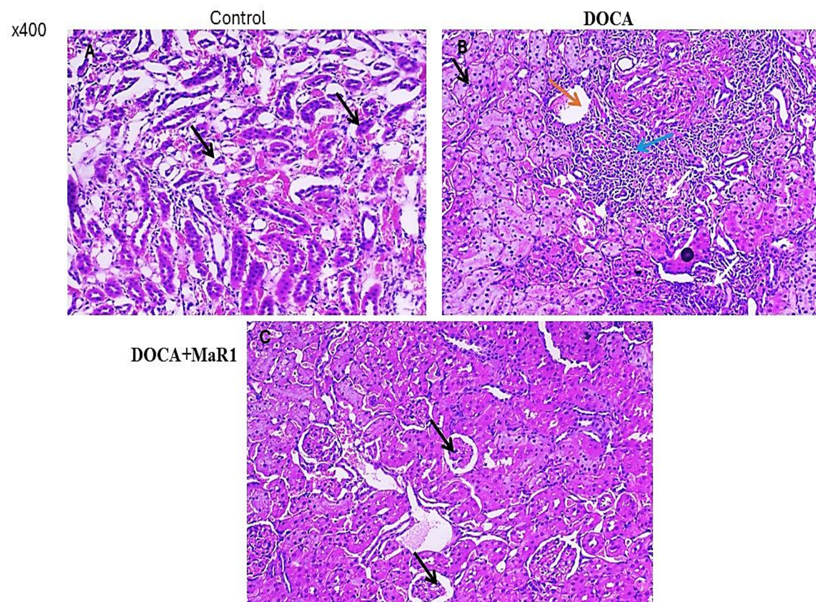


Fig (2):H& E-stained kidney sections in the studied groups (H&E ×400): (A) are photomicrographs of control group showed normal renal parenchyma without any glomerular and vascular abnormalities (black arrow). (B) is a photomicrograph of DOCA group showed severe glomerulosclerosis (white arrow), tubulointerstitial infiltration (blue arrow), proximal convoluted tubule hydropic degeneration (black arrow) and renal artery sclerosis (red arrow). (C) is a photomicrograph of DOCA+MaR1 group showed mild glomerulosclerosis in the renal tissue.

Immunohistochemical results

In Caspase-3-stain, DOCA group showed dramatically increased in the percentage area of caspase-3 when compared to control group (52±0.63 vs 9±0.54., respectively, P<0.05). However, the DOCA+MaR1 showed a substantial decrease in this percentage when compared to DOCA (20±0.03 vs 52±0.63., respectively, P<0.05), but still increased from the control (Fig. 3: A-D).

In NF-kB stain, DOCA showed a substantial increase in the percentage area of NF-kB when compared to control group (60±0.12 vs10±0.04.,P<0.05). However, the DOCA+MaR1 group showed dramatically decrease in this percentage when compared to DOCA (22±0.25 vs 60±0.12, respectively, P<0.0). but still increased from the control (Fig. 3: E-H).

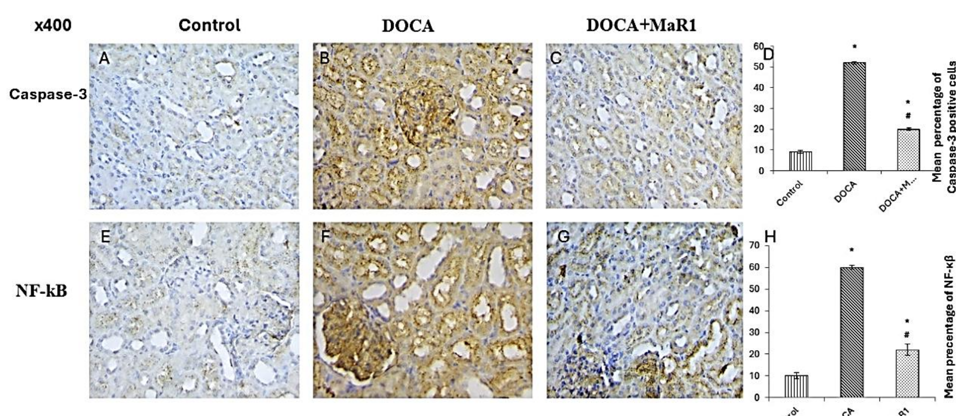


Fig (3): Representative micrographs showing a substantial elevation of the Caspase-3 (A-D) and NF-kB (E-H) immunoreaction in the DOCA group and a significant downregulation in the DOCA+MaR1.

Discussion

Long-term exposure to elevated blood pressure damages target organs, including the kidney [30]. According to Safaeian et al.'s research, giving DOCA-salt for four weeks effectively created hypertension, as shown by the DOCA group's dramatically higher BP than the control group [20].The direct effects of mineralocorticoid receptors on the heart, blood vessels, kidneys, adrenal medulla, and brain areas involved in blood pressure control [31], as well as an increase in sympathetic nerve activity and catecholamine levels [32], are what produce DOCA-induced hypertension.

ABP levels were significantly reduced by DOCA+MaR1. Serum MaR1 levels in hypertension individuals decreased. Additionally, it was shown that the MaR1/LGR6 axis is essential for hypertensive vascular remodeling and that there was a negative connection between MaR1 concentration and systolic blood pressure. Additionally, in the AngII-infused animals, the MaR1 therapy relieved hypertensive vascular remodeling and decreased blood pressure rise[33].

According to earlier research, DOCA significantly hampered renal function, as seen by increased microalbuminurea, decreased creatinine clearance, and raised blood urea and creatinine when compared to control [34].In addition to being one of the target organs harmed by hypertension, the kidney plays a crucial role in controlling blood pressure. According to earlier research, hypertension is a risk factor for ESRD on its own. Microalbuminuria is an indication of renal impairment in the early stages of hypertension. Changes in renal hemodynamics brought on by chronic hypertension, inflammation and podocyte destruction are linked to the pathogenesis of microalbuminuria.[35].A prior study found that SHRs had a considerably higher level of proteinuria than control rats.[14]. Histological changes seen in the renal tissue of DOCA-induced hypertension rats further supported these findings and are consistent with previous study [34].

In our investigation, MaR1 significantly enhanced kidney function, which is consistent with our histopathological findings. The renoprotective effect of MaR1 was reported in previous sudies [36, 37].

MaR1 can significantly lower acute kidney damage and death in sepsis mice. It may do this by lowering neutrophil infiltration and inhibiting NF-kB/STAT3/MAPK activation, which lowers pro-inflammatory cytokine levels and raises anti-inflammatory cytokine levels [38].

Oxidative stress plays a significant role in the pathogenesis of hypertension and the damage that hypertension causes to target organs, according to a number of studies. In hypertensive rats, ROS can worsen renal impairment [39], this is consistent with our findings that DOCA significantly increased oxidative stress. Experimental data suggests that ROS may have a significant pathophysiological function in hypertension [40].Significant oxidative stress was seen in the DOCA-salt group, which was consistent with the findings of the Safaeian et al. research [20].

MaR1 significantly reduced DOCA-induced oxidative damage. The antioxidant impact of MaR1 was demonstrated in previous studies [41, 42]. The protective effect of MaR1 could result from reducing ROS generation and inhibiting mitochondrial damage[41].

One of the main causes of hypertension is inflammation [43].In line with earlier research, the DOCA-salt group exhibited a substantial rise in inflammatory cytokines when compared to the control group [44].

MaR1 significantly reduced DOCA-induced inflammation, which is consistent with previous study [45]. Nrf2 mediates the anti-inflammatory route of MaR1 via blocking the TLR4/NF-κB pathway [46, 47]. By attaching to the receptors on the membrane surface to control the essential inflammatory protein, maresin1 may initiate the downstream pathways, preventing the production of inflammatory components and reducing inflammation [48], in addition to MaR1 ability to increase IL4 and IL10 production [49].

NF-kB activation sets off an inflammatory cascade that generates inflammatory factors and contributes significantly to HTN as glomerular disease progresses [50]. An innate immune receptor called TLR4 promotes the generation of pro-inflammatory cytokines in several tissues and recognizes pathogens that pose a threat to the cell [51]. By activating the transcription factor NF-kB, the TLR4 pathway sets off a downstream signaling cascade that regulates the production of pro-inflammatory cytokines. According to these findings, TLR4 contributes significantly to renal inflammation and injury through its downstream effector protein NF-kB [13].

TLR4/NF-κB is an important inflammatory signal pathway in renal disorders [52]. When comparing the DOCA group to the control, a substantial rise in renal NF-κB gene expression, immunoreactivity, and TLR4 gene expression was noted. According to earlier findings, DOCA-salt mice given an anti-TLR4 antibody had a decrease in blood pressure [53].

A major contributing factor to hypertension is dysfunctional innate immune activation, mainly through Toll-like receptors (TLRs).Research indicates that these receptors serve as entry points for oxidative stress, vascular remodeling, and persistent low-grade inflammation [54]

We evaluated the effect of MaR1 on TLR4/NF-kB pathway activation in DOCA-induced hypertension. In contrast to the DOCA group, the DOCA+MaR1 group in this study showed a notable downregulation of NF-κB gene expression and immunoreactivity. These results imply that MaR1 can exert its renoprotective action against DOCA-induced hypertension via reducing NF-κB activation. In a rat model of chronic liver injury, MaR1 may also decrease NF-κB activity [55]. MaR1 has been shown to protect against renal IRI by blocking the TLR4/MAPK/NF-κB pathways, which mediate anti-inflammatory effect of MaR1 [37].

Vascular remodeling and alterations in renal hemodynamics are the main pathogenic events. The renal arterioles will be impacted by the alteration in renal hemodynamics that happens when hypertension develops. The arteriole will first become more sensitive to vasoconstrictive drugs, which will raise vascular resistance and reduce local blood flow [56]. This was demonstrated by our findings, which showed that DOCA significantly raised RAR and lowered RBFV when compared to control. whereas MaR1 significantly reduced DOCA-induced alterations in renal blood flow. NOD-like receptor protein 3 (NLRP3) transcriptionis aided by NF-κB activation. Cytokines and caspase-3 are activated

in part by the NLRP3 inflammasome [57].When compared to control, DOCA significantly increased renal caspase-3 immunoreactivity. Prior research showed that DOCA caused apoptosis in tissues [11].When compared to the DOCA group, the DOCA+MaR1 group in this research also showed a significant downregulation of caspase-3 immunoreactivity. This was consistent with previous studies. [41, 42].This may be due to the anti-oxidant and anti-inflammatory properties of MaR1

Conclusion

Through anti-oxidant, anti-inflammatory, and anti-apoptotic pathways, as well as the down-regulation of TLR4 and NF κB renal genes expression, MaR1 reduced DOCA-induced hypertensive nephropathy.

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. **Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, Amann M, Anderson HR, Andrews KG, Aryee M, et al**. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380:2224–60.
2. **Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, IzzoJr JL, et al.** The Seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure the JNC 7 report. J Am Med Assoc 2003;289:2560e72.
3. **Saran R, Robinson B, Abbott KC, Bragg- Gresham J, Chen X, Gipson D, et al.** US renal data system 2019 annual data report: epidemiology of kidney disease in the United States. Am J Kidney Dis. 2020;75(1 Suppl 1): A6–7. https://doi.org/10.1053/j.ajkd.2019.09.003
4. **Yang C, Gao B, Zhao X, Su Z, Sun X, Wang HY, et al.** Executive summary for China kidney disease network (CK-NET) 2016 annual data report. Kidney Int. 2020;98(6):1419–23.
5. **Dong Z, Dai H, Feng Z, Liu W, Gao Y, Liu F, et al.** Mechanism of herbal medicine on hypertensive nephropathy (Review). Mol Med Rep. 2021;23(4):234.
6. **Nagase M, Shibata S, Yoshida S, Nagase T, Gotoda T, Fujita T.** Podocyte injury underlies the glomerulopathy of Dahl salt-hypertensive rats and is reversed by aldosterone blocker. Hypertension 2006;47:1084e93.
7. **Cohuet, G, Struijker-Boudier, H.** Mechanisms of target organ damage caused by hypertension: Therapeutic

potential. Pharmacol&Ther. 2006; 111(1): 81–98.

8. **Rubattu, S, Pagliaro B, Pierelli G, Santolamazza C, Di Castro S,** Mennuni S, Volpe M. Pathogenesis of target organ damage in hypertension: Role of mitochondrial oxidative stress. Int J Mol Sci. 2014; 16(1): 823–839.
9. **Kamisah Y, Ang S.-M, Othman F, Nurul-Iman B S, Qodriyah HMS**. Renoprotective effect of virgin coconut oil in heated palm oil diet-induced hypertensive rats.ApplPhysiolNutrMetab. 2016; 41(10): 1033–1038.
10. **Touyz RM, Rios FJ, Alves-Lopes R, Neves KB, Camargo LL,** MontezanoAC.Oxidative Stress: a Unifying Paradigm in Hypertension. Can J Cardiol. 2020;36(5):659–70.doi:10.1016/j. cjca.2020.02.081.13.
11. **Khodir, S. A., Sweed, E., Gadallah, M., &Shabaan, A. (2022).**Astaxanthin attenuates cardiovascular dysfunction associated with deoxycorticosterone acetate-salt-induced hypertension in rats. *Clinical and Experimental Hypertension*, *44*(4), 382-395.
12. **Xiong W, Meng XF, Zhang C.** Nlrp3 inflammasome in metabolic-associated kidney diseases: An update. Front Immunol. (2021) 12:714340. doi: 10.3389/fimmu. 2021.714340
13. **El-Domiaty, H. F., Sweed, E., Kora, M. A., Zaki, N. G., &Khodir, S. A. (2022).** Activation of angiotensin-converting enzyme 2 ameliorates metabolic syndrome-induced renal damage in rats by renal TLR4 and nuclear transcription factor κBdownregulation. *Frontiers in medicine*, *9*, 904756.
14. **Shi, W., Guo, L., Liu, G., Peng, T., Li, H., Xie, T., ...& Yang, X. (2018).** Protective effect of calcitriol on podocytes in spontaneously hypertensive rat.*Journal of the Chinese Medical Association*, *81*(8), 691-698.
15. **Cai, W., Zhang, Z., Huang, Y., Sun, H., &Qiu, L. (2017).** *Vaccarin alleviates hypertension and nephropathy in renovascular hypertensive rats. ExpTher Med. 2018; 15 (1): 924-32.*
16. **S. Tang, M. Wan, W. Huang, R. C. Stanton, and Y. Xu,** “Maresins: specialized proresolving lipid mediators and their potential role in inflammatory-related diseases,” Mediators Inflamm, vol. 2018, article 2380319, pp. 1–8, 2018.
17. **M. Spite,** “Resolving inflammation in nonalcoholic steatohepatitis,” Journal of Clinical Investigation, vol. 130, pp. 1524–1526, 2019.
18. **S. Tang, C. Gao, Y. Long et al., “**Maresin 1 mitigates high glucose-induced mouse glomerular mesangial cell injury by inhibiting inflammation

and fibrosis," Mediators of Inflammation, vol. 2017, Article ID 2438247, 11 pages, 2017.

19. **Titze J, Luft FC, Bauer K, Dietsch P, Lang R, Veelken R, Wagner H, Eckardt K-U, Hilgers KF .** Extrarenal Na+ Balance, Volume, and Blood Pressure Homeostasis in Intact and Ovariectomized Deoxycorticosterone-Acetate Salt Rats.Hypertension. 2006;47 (6):1101–07.doi:10.1161/01.HYP.0000221039.17735.1a.
20. **Safaeian L, Emami R, Hajhashemi V, Haghighatian Z.** Antihypertensive and antioxidant effects of protocatechuic acid in deoxycorticosterone acetate-salt hypertensive rats. Biomed Pharmacother. 2018;100:147–55.doi:10.1016/j.biopha.2018.01.107. 25.
21. **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
22. **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
23. **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
24. **Linsenmeier RA, Beckmann L, Dmitriev AV.** Intravenous ketamine for long term anesthesia in rats. Heliyon. 2020 2020/12/01/;6(12):e05686.
25. **Haywood, J.R., et al.,** *Regional blood flow measurement with pulsed Doppler flowmeter in conscious rat.*Am J Physiol, 1981. 241(2): p. H273-8.
26. **Das K, Kundu A, Karmakar S, Ghosh M (2020)** Novel structured diacylglycerol (DAG) rich oleo formulations activate the Nrf2 pathway and impedes NF-κB translocation to mitigate pre-clinical conditions of hypertension. J Funct Foods 64:103650.
27. **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.

28. **Garcés MF, Sanchez E, Torres-Sierra AL, et al.** Brain-derived neurotrophic factor is expressed in rat and human placenta and its serum levels are similarly regulated throughout pregnancy in both species. ClinEndocrinol (Oxf). 2014 Jul;81(1):141-51.
29. **Bancroft, J.D. and Layton, C. (2013).** 'The hematoxylin and eosin, connective and mesenchymal tissues with their stains. In: Suvarna SK, Layton C, Bancroft JD, editors. Bancroft's Theory and Practice of Histological Techniques', 7th ed. Philadelphia, PA:Churchill Livingstone;, pp. 173-212.
30. **Van Der Veen PH, Geerlings MI, Visseren F LJ, Nathoe HM, Mali WPTM, Van Der Graaf Y.** And Muller M. Hypertensive target organ damage and longitudinal changes in brain structure and function. Hypertension. 2015; 66:1152-1158.
31. **Gomez-Sanchez EP.DOCA/Salt:** much More Than a Model of Hypertension. J CardiovascPharmacol. 2019;74(5):369–71. doi:10.1097/FJC.0000000000000753.
32. **Bouvier M, de Champlain J.**Increased basal and reactive plasma norepinephrine and epinephrine levels in awake DOCA-salt hypertensive rats. J AutonNerv Syst. 1986;15(2):191–95.doi:10.1016/0165-1838(86)90014-7.
33. **Yin, Z., Zhang, J., Zhao, M., Peng, S., Ye, J., Liu, J., ...& Wang, M. (2024).** Maresin-1)) ameliorates hypertensive vascular remodeling through its receptor LGR6. *MedComm*, *5*(3), e491
34. **Garba, M. S., &Bouderbala, S. (2022).** Protective effects of olive cake against heart and kidney injury in dexamethasone-induced hypertensive rats
35. **Ma J, Zhang B, Liu S, Xie S, Yang Y, Ma J, et al.** 1,25-Dihydroxyvitamin D(3) inhibits podocyteuPAR expression and reduces proteinuria. PLoS One 2013;8:e64912.
36. **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
37. **Qiu, Y., Wu, Y., Zhao, H., Sun, H., &Gao, S. (2019).** Maresin 1 mitigates renal ischemia/reperfusion injury in mice via inhibition of the TLR4/MAPK/NF-κB pathways and activation of the Nrf2 pathway. *Drug design, development and therapy*, 739-745
38. **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.

39. **Kamisah Y, Ang S.-M, Othman F, Nurul-Iman B S, Qodriyah HMS**. Renoprotective effect of virgin coconut oil in heated palm oil diet-induced hypertensive rats. ApplPhysiolNutrMetab. 2016; 41(10): 1033–1038.
40. .**Puddu P, Puddu GM, Cravero E, Rosati M, MuscariA**. The molecular sources of reactive oxygen species in hypertension. Blood Press. 2008;17(2):70–77.
41. **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
42. **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.
43. **Visioli F, ArtariaC.** Astaxanthin in cardiovascular health and disease: mechanisms of action, therapeutic merits, and knowledge gaps. Food Funct. 2017;8(1):39–63.
44. **Del Mauro JS, Prince PD, Plantamura YS, Allo MA, Parola L, Machulsky NF, Morettón, MA, Bin, EP, González, GE, Bertera, FM, et al.** Nebivolol is more effective than atenolol for blood pressure variability attenuation and target organ damage prevention in L-NAME hypertensive rats. Hypertension Research. 2021;44(7):791–802.
45. **Cezar, T. L., Martinez, R. M., Rocha, C. D., Melo, C. P., Vale, D. L., Borghi, S. M., ... &Casagrande, R. (2019).** Treatment with maresin 1, a docosahexaenoic acid-derived pro-resolution lipid, protects skin from inflammation and oxidative stress caused by UVB irradiation. *Scientific reports*, *9*(1), 3062.
46. **S. Qiu, P. Li, H. Zhao, X. Li, Maresin 1** alleviates dextran sulfate sodium-induced ulcerative colitis by regulating NRF2 and TLR4/NF-kB signaling pathway, IntImmunopharmacol. 78 (2020), 106018, https://doi.org/10.1016/j. intimp.2019.106018.
47. **H. Bian, G. Wang, J. Huang, L. Liang, Y. Zheng, Y. Wei, H. Wang, L. Xiao, H. Wang,** Dihydrolipoic acid protects against lipopolysaccharide-induced behavioral deficits and neuroinflammation via regulation of Nrf2/HO-1/NLRP3 signaling in rat, J. Neuroinflamm. 17 (1) (2020) 166, https://doi.org/10.1186/s12974-020-01836- y.
48. **Wang, X., Botchway, B. O., Zhang, Y., Huang, M., & Liu, X. (2023).**

Maresin1 can be a potential therapeutic target for nerve injury. *Biomedicine & Pharmacotherapy*, *161*, 114466.

49. **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*.
50. **Zhao Y, Chen X, Lin Y, Li Z, Su X, Fan S, et al.** USP25 inhibits renal fibrosis by regulating TGFb-SMAD signaling pathway in Ang II-induced hypertensive mice. BiochimEtBiophysActaMol Basis Dis. (2023) 1869:166713. doi: 10.1016/j.bbadis.2023.166713
51. **Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS.** Tlr4 links innate immunity and fatty acid–induced insulin resistance. J Clin Invest. (2006) 116:3015– 25.
52. **Rabadi MM, Ghaly T, Goligorksy MS, Ratliff BB (2012)** HMGB1 in renal ischemic injury. Am J Physiol Renal Physiol 303:F873–F885
53. **Nunes, K. P., Bonfim, G. F., Szasz, T., Carvalho, M. H., and Webb, R. C. (2012).**Treatment with anti-TLR4 antibody lowers blood pressure and improves erectile function in DOCA-salt rats
54. **McCarthy, C. G., Goulopoulou, S., Wenceslau, C. F., Spitler, K., Matsumoto, T., and Webb, R. C. (2014).** Toll-like receptors and damage-associated molecular patterns: novel links between inflammation and hypertension. Am. J. Physiol. Circ. Physiol. 306, H184–H196. doi: 10.1152/ajpheart.00328.20.
55. **Rodríguez MJ, Sabaj M, Tolosa G, Herrera Vielma F, Zúñiga MJ, González DR, Zúñiga-Hernández J (2021)** Maresin-1 Prevents Liver Fibrosis by Targeting Nrf2 and NF-κB, Reducing Oxidative Stress and Inflammation. Cells 10
56. **Wang, L., Wang, J., Zhang, Y., & Zhang, H. (2024).** Current perspectives and trends of the research on hypertensive nephropathy: a bibliometric analysis from 2000 to 2023. *Renal Failure*, *46*(1), 2310122
57. **Li Z, Guo J, Bi L (2020)** Role of the NLRP3 inflammasome in autoimmune diseases. Biomedicine & Pharmacotherapy 130:110542