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Resveratrol improved kidney function and structure in malignantly hypertensive rats by restoration of antioxidant capacity and nitric oxide bioavailability

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OBJECTIVE

Background: The main cause of death among patients with malignant hypertension is a kidney failure. The promising field in essential and malignant hypertension therapy could be centered on the amelioration of oxidative stress using antioxidant molecules like resveratrol. Resveratrol is a potent antioxidative agent naturally occurred in many plants that possess health-promoting properties.
Methods: In the present study, we investigated the therapeutic potential of resveratrol, a polyphenol with antioxidative activity, in N^G-L-Arginine Methyl Ester (L-NAME) treated spontaneously hypertensive rats (SHR) - malignantly hypertensive rats (MHR).
Results: Resveratrol significantly improves oxidative damages by modulation of antioxidant enzymes and suppression of prooxidant factors in the kidney tissue of MHR. Enhanced antioxidant defense in the kidney improves renal function and ameliorates the morphological changes in this target organ. Besides, protective properties of resveratrol are followed by the restoration of the nitrogen oxide (NO) pathway. 4) Conclusion: Antioxidant therapy with resveratrol could represent promising therapeutical approach in hypertension, especially malignant, against kidney damage.

1. Introduction

According to the World Health Organization the most common causes of death all over the world are non-communicable diseases (NCDs), accounting for almost two-thirds of all global deaths [1]. Cardiovascular disease (CVD) and chronic kidney disease (CKD) are the major NCDs. Hypertension is one of the most important cardiovascular risk factors. Other cardiovascular risk factors include impaired endothelial function, reduced production/release, or increased inactivation of endothelium-derived vasodilators, as well as interactions of NO with

Abbreviations: NCDs, non-communicable diseases; CVD, Cardiovascular disease; CKD, chronic kidney disease; ROS, reactive oxygen species; RNS, reactive nitrogen species; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; SHR, spontaneously hypertensive rats; L-NAME, N^G-L-Arginine Methyl Ester; IMR, Institute for Medical Research; SHR+R, spontaneously hypertensive rats treated with resveratrol; MHR, malignantly hypertensive rats; MHR+R, malignantly hypertensive rats treated with resveratrol; Up, urine protein; Cr, creatinine; MDA, malondialdehyde; AOPP, advanced oxidation protein products; $O_2^{\cdot-}$, superoxide anion radical; SOD, superoxide dismutase; PON1, paraoxonase 1; PAB, prooxidant-antioxidant balance; TOS, total oxidant status; TAC, total antioxidant capacity; OSI, oxidative stress index; PAS, periodic acid Schiff; RDS, Renal Damage Score.

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angiotensin 2, reactive oxygen species (ROS), reactive nitrogen species (RNS) as well as oxidized lipoproteins. Unfortunately, the precise cause of blood pressure elevation often cannot be determined. In the general population, 30–45 % of death is caused by hypertension, with a tendency to increase incidence from the age of 50. Hypertension and kidney diseases are closely interconnected. Hypertension induced a cluster of disorders of the heart and blood vessels which causes the damage of the one organ that leads to dysfunction of the other, eventually failing both organs [2]. In the past decades, although numerous studies have been performed aiming to develop better strategies for treating high blood pressure, the therapeutic outcome has been still unsatisfactory. Untreated hypertension in the human population (about 10%) results in a developed malignant or accelerated form, defined by extremely high systolic and diastolic arterial pressure (SAP > 200; DAP > 130). Unfortunately, the prognosis of patients with malignant hypertension is highly unfavorable because almost eight out of ten patients die within 2 years [3,4]. The main cause of death among patients with malignant hypertension is a kidney failure [4]. This situation requires an urgent need for better developing appropriate therapeutic strategies.

Animal models have been useful for unravelling the pathogenesis of hypertension and for testing novel therapeutic strategies. Spontaneously hypertensive rats (SHR) are widely used as a rat model of primary or essential hypertension because they have similar vascular anatomy and structure as humans [5,6]. Malignant hypertension can develop from essential through serious long-term nitric oxide (NO) inhibition by L-NAME resulting in a significant increase in blood pressure, profound vasoconstriction, oxidative stress, and structural alterations of the conduit and large arteries, as well as cardiac hypertrophy [7,8].

In addition, it has been demonstrated that the administration of antioxidant agents, such as vitamins C and E, improved arterial stiffness and endothelial function in essential hypertensive patients [9]. Resveratrol (RSV; 3,5,4 -trihydroxy stilbene, $C_{14}H_{12}O_3$) is one of the most highly investigated and well-known antioxidant molecules [10–12]. Numerous studies have demonstrated beneficial effects of resveratrol on human health, including CVD [7,8,10,12,13] and kidney disease [10, 13]. Resveratrol is one of the natural polyphenol compounds found in pomegranates, berries, peanuts, and red wine. Furthermore, it is found with an elevated level in grape juice. Over the years, many investigators have shown a beneficial effect of resveratrol on endothelial function, carcinogenesis, neuroprotection, and inflammation [14]. Data obtained using different animal models such as ischemia-reperfusion injury [15], drug-induced kidney injury [16], kidney toxicity [17], diabetic nephropathy [18], and sepsis-induced kidney injury [19] have shown the well-proven utility of resveratrol. Although resveratrol was shown to alleviate kidney injury in a several experimental models, the possible protective effect of resveratrol on malignantly hypertension induced target organ injury has not yet been completely investigated.

New antihypertensive strategies that target not only blood pressure, but also exhibit beneficial organ-protective effects, candidate resveratrol as effective tool in protection and preservation of the kidney in hypertensive condition. Thus, the aim of the present study was to examine the effects of resveratrol treatment on kidney function and structure, oxidative stress, and antioxidant defense in malignantly hypertensive rats (MHR).

2. Materials and methods

2.1. Reagents

All reagents and chemicals were provided with the highest analytical grade from Sigma-Aldrich Merck and Acros Organic. The chemicals obtained from Sigma-Aldrich: resveratrol, L-NAME, lithium-heparin, nitroblue tetrazolium, nicotinamide, adenine dinucleotide, phenazine methosulfate, dithiobis (2-nitrobenzoic acid, phenylacetate, tetramethylbenzidine, azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), periodic acid schiff. The chemicals obtained from Acros Organic: trichloroacetic acid, thiobarbituric acid, and Trolox. The chemicals obtained from Centrohem: citric acid, potassium iodide, alcohol, formalin, and hydrogen peroxide. The plasma/urine creatinine and urine protein were evaluated using commercial kits from Hoffmann-La Roche, Leitch Diagnostic, Germany. Anti-3-Nitrotzrosine antibody obtained from Abacam.

2.2. Ethics Statement

The experimental protocol was in accordance with the National Law of Animal Welfare (the Official Gazette of the Republic of Serbia, No.: 41/2009 and 39/2010) and the Directive on the protection of animals used for scientific purposes (2010/63/EU). The study protocol was approved by the Ethics Committee of the Institute for Medical Research (IMR), University of Belgrade, Serbia and Veterinary Directorate, Ministry of Agriculture and Environmental Protection, Republic of Serbia (No. 0316–1/11).

2.3. Animals

The experiments were performed on 6-month-old female SHR rats (200–250 g) bred at the IMR. They were descendants of breeders originally obtained through Taconic Farms, Germantown, NY, USA. The experimental animals were housed in plastic cages (Macrolon® cage type 4, Bioscape, Germany) with sawdust bedding (Versele-Laga, Belgium) certificated as having contaminant levels below toxic concentrations. The environmental conditions were controlled and monitored by a central computer-assisted system with a temperature of 22 ± 2^0 C, relative humidity of 55 ± 15%, 15 – 20 airchanges/h, and artificial lighting of approximately 220 V (12 hrs light/dark cycle). The experimental animals had free access to food, commercial pellets for rats (Zavod Subotica, Serbia) and tap water from municipal mains, filtered through a 1.0 μm filter (Skala Green, Serbia).

2.4. Experimental design

All animals were examined for control values of systolic blood pressure by an indirect method using a tail-cuff, pneumatic pulse detector and direct recorder (Physiograph Four, Narco Bio-System, Houston, TX, USA) [7]. After assuring that all animals had been hypertensive, they were divided randomly into four groups according to the 4 weeks drug treatment: (1) SHR (n = 10), SHR received tap water 0.5 ml by gavage; (2) SHR+R group (n = 10), SHR treated with resveratrol (Resveratrol, $C_{14}H_2O_3$, Mr 228.2, Sigma-Aldrich, USA) in a dose of 10 mg/kg/day, by gavage; (3) MHR group, SHR rats (n = 10) treated with L-NAME (N^G-L-Arginine Methyl Ester, Sigma), dissolved in tap water in a dose of 10 mg/kg/day, and (4) MHR+R group, SHR rats (n = 10) treated with the combination of L-NAME and resveratrol in the same dosages (Fig. 1).

2.5. Blood pressure measurements

At the end of treatment, all animals were anaesthetized (by 35 mg/kg *i.p.* sodium pentobarbital) and then direct blood pressure measurement was performed [7]. Systolic (SAP) and diastolic (DAP) arterial pressure and heart rate (HR) were measured directly through a femoral artery catheter (PE–50, Clay-Adams Parsippany, NJ, USA) using a low volume displacement transducer P23 Db (Statham, Oxnard, CA, USA), and recorded on a direct writing recorder.

2.6. Biochemical measurements

Twenty-four-hour urine samples were collected in graduated cylinders with an accuracy of 0.1 ml. These urine samples were used for creatinine protein concentration measuring. After blood pressure measurement, blood samples were obtained by abdominal aorta puncture,

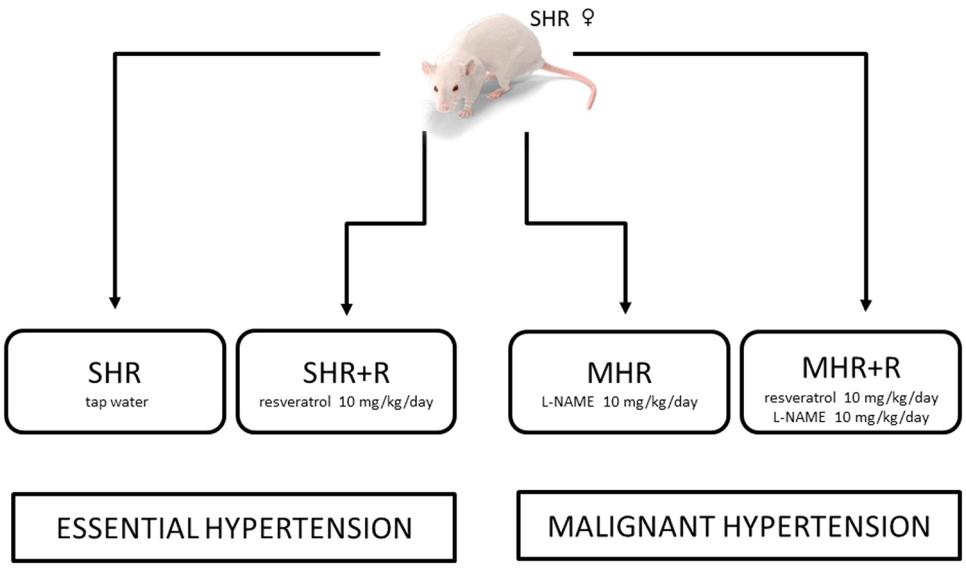

Fig. 1. Scheme of the experimental design.

using an anticoagulant lithium-heparin (Li-heparin, Sigma-Aldrich, St. Louis, MO, USA), and centrifuged at 4000 rpm, 4 °C for 20 min. Plasma was collected and stored at −20 °C until assaying. Plasma creatinine, a marker of glomerular filtration, as well as urine protein (Up) and creatinine (Cr) levels in 24 h urine, were measured by an automatic COBAS INTEGRA 400 plus analyzer (Hoffmann-La Roche, Leitch Diagnostic, Germany). Urine protein and urine-to-creatinine ratio (Up/cr) were used for the assessment of proteinuria.

2.7. Redox state of kidney

Kidneys were removed, rinsed in ice-cold saline, and weighed. The tissue was immediately frozen in liquid nitrogen and stored at − 70 °C for later analysis. Malondialdehyde (MDA), as a marker of lipid peroxidation, was measured by using phosphoric and thiobarbituric acid solution according to the method of Mihara and Uchiyama [20]. Advanced oxidation protein products (AOPP) was measured in an acidic condition in the presence of potassium iodide [21]. The concentration of superoxide anion radical ($O_2^{\cdot-}$) in kidney tissue was measured at 530 nm, after the reaction of nitro blue tetrazolium in TRIS buffer [22]. The activity of antioxidant enzyme superoxide dismutase (SOD) was measured in kidney homogenates by the spectrophotometric method, as previously described [23]. Quantification of free protein thiol groups was determined by derivatization with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), by the Ellman method [24]. Paraoxonase 1 (PON1) in the kidney was evaluated by phenylacetate [25]. Determination of prooxidant-antioxidant balance (PAB) in the kidney tissue was performed using 3,3′, 5,5′-tetramethylbenzidine as a chromogen [26]. Total oxidant status (TOS) of tissue is based on the oxidation of ferrous iron to ferric iron by the various types of peroxides contained in the samples, in the presence of xylenol orange which produces a colored ferric-xylenol orange complex whose absorbance can be measured spectrophotometrically [27]. Total antioxidant capacity (TAC) method is based on the bleaching of characteristic color of a more stable ABTS [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] radical cation by antioxidant sample constituents [28]. Ratio of TOS to TAC level was accepted as oxidative stress index (OSI) and expressed in arbitrary units. All spectrophotometric analyses were performed in duplicate with an Ultrospec 3300 pro UV/Visible spectrophotometer (Amersham Biosciences Corp., USA).

2.8. Histopathological and semiquantitative examination

The left kidney was excised, dissected longitudinally and tissue slices were fixed in 10% buffered formalin solution. Renal tissue samples were dehydrated in alcohol, blocked in paraffin wax, cut into 5 μm slices and stained by periodic acid Schiff (PAS) reaction for light microscopy observation. The whole visual field from each renal slice was analyzed and photographed at magnification 200x, by using a light microscope connected with a digital camera (Olympus-2, Tokyo, Japan) for histopathological examination, as per our method previously published in the literature [29]. The severity of renal impairment expressed as Renal Damage Score (RDS) is shown in Table 1.

2.9. Immunohistochemistry examination

The standard protocol was followed for the immunohistochemistry staining on 5 μm deparaffinized and rehydrated tissue sections. The sections were treated by microwave for 20 min at 400 W in citric acid buffer (pH 6.0) to reduced nonspecific background staining. Slides were incubated in 3% hydrogen peroxide for 10 min. Primary antibody for 3-nitrotyrosine (Abacam, Anti-3-Nitrotzrosine antibody [39B6] (ab61392), dilution 1:1000) was applied according to the manufacturer's recommended protocol. The slides were washed thoroughly with phosphate buffer saline, pH 7.4, between the steps. 3,30-Diaminobenzidine (DAB) (TL-015-HDJ, Thermo Scientific Lab Vision UltraVision ONE Detection System) was used as chromogen, to develop the antigen-antibody complex, and all slides were then counterstained with

Table 1
Tissue scoring scale for renal alterations—Renal Damage Score (RDS).

Degree	Description
0	Normal finding.
1	Mild damage: Single glomerular cells slightly enlarged. Mild dilatation of small blood vessels. A few foci of inflammatory cell infiltrates.
2	Moderate damage: < 50% glomerular cells with proliferation. Severe vasodilation is associated with hyperemia, oedema and prominent inflammatory cell infiltrates.
3	Severe and focal damage: > 50% glomerular cells with proliferation. Transmural rupture of the blood vessels (up to 50 %) and accumulation of inflammatory cells.
4	Severe and diffuse damage: Complete loss of the normal glomerular architecture and endothelial cells of the blood vessels (> 50 %). High-intensity haemorrhages and diffuse accumulation of inflammatory cells.

hematoxylin, dehydrated, and mounted. Appropriate positive and negative controls were processed in parallel. The slides were analyzed with a microscope (Olympus-2, Tokyo, Japan) at 200x magnification. For these sections, the average number of immune-positive cells (tubular cells with intensive optical density expression of nitrotyrosine across twenty non-successive fields were calculated by two independent pathologists in a blinded manner [23], using ImageJ software 1.50 (National Institute of Health, Bethesda, Rockville, MD, United States). Quantitative measurement of the immuno-positive tubular cells was expressed according to this formula:

Percentage of 3-NT positively stained cells = the number of positively stained tubular cells x 100 / total number of tubular cells

2.10. Statistical analysis

Results are expressed as mean ± S.D. One way analysis of variance (ANOVA) was applied as appropriate. Fisher LSD test was performed as a post hoc multiple comparison test STATISTICA 12, StatSoft Inc, (Tulsa, OK, USA). The differences between examined groups were significant if $p < 0.05$.

3. Theory/calculation

Considering previous, resveratrol seems to serve as an effective dietary supplement for the protection and preservation of the kidney injury in a several experimental models, but the question is its efficacy in malignant hypertension. In this study, we have addressed this interesting issue to examination of the resveratrol effects on kidney structure and function and correlated it with oxidative stress and inflammation status of kidney in the spontaneously and malignant hypertensive rats.

4. Results

4.1. Blood pressure

Blood pressure parameters are shown in Table 1. SAP and DAP arterial pressures were 172.04 ± 9.68 mmHg and 133.33 ± 12.65 mmHg in the SHR group, respectively. After 4 weeks of blocked NO synthesis in MHR, SAP raised to 201.11 ± 6.00 mmHg and DAP to 156.12 ± 2.48 mmHg. The application of resveratrol significantly reduced this blood pressure in both SHR and MHR groups ($p < 0.001$). The HR remained unchanged in all experimental groups. (Table 2).

4.2. Biochemical parameters

The data of biochemical parameters showed in Fig. 2. After 4 weeks of blocked NO synthesis in the MHR group plasma creatinine level and urine protein were significantly elevated compared to SHR rat. The same significance between these two groups exists when followed Up/Cr ($p < 0.001$). Chronic application of resveratrol, in both SHR and MHR groups, significantly reduced this increase of plasma creatinine

Table 2
Systolic and diastolic arterial pressure (SAP and DAP) and heart rate (HR).

Treatments	SAP	DAP	HR
SHR	172.04 ± 9.68	133.33 ± 12.65	307 ± 4.83
SHR+R	138.21 ± 16.05 ***	110.85 ± 14.07 ***	305 ± 5.27
MHR	201.11 ± 6.00 ***	156.12 ± 4.83 ***	307 ± 15.67
MHR+R	162.77 ± 19.07 ###	145.46 ± 23.43 #	299 ± 14.49

SHR – spontaneously hypertensive rat; SHR+R – spontaneously hypertensive rat treated with resveratrol; MHR – malignant hypertensive rat; MHR+R – malignant hypertensive rat treated with resveratrol; Values are means ± S.D; *** indicate $p < 0.001$ vs. SHR; ###, # indicate $p < 0.001$, 0.05 vs. MHR.

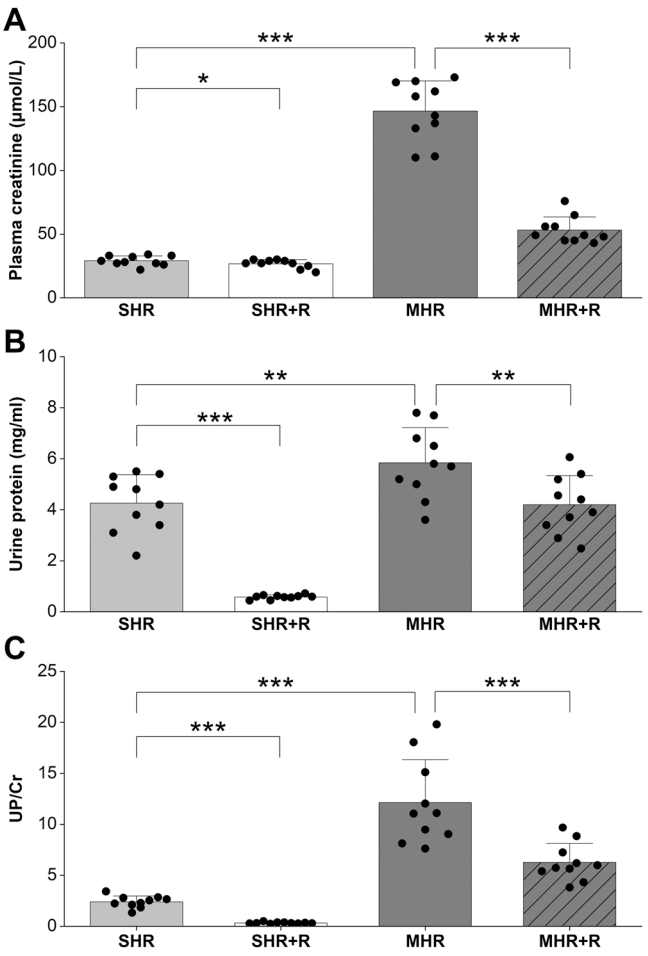

Fig. 2. Plasma creatinine (A), urine protein (B), urine protein/creatinine ratio (Up/Cr) (C) in experimental groups. SHR- spontaneously hypertensive rat, SHR+R – spontaneously hypertensive rat treated with resveratrol; MHR – malignant hypertensive rat; MHR+R – malignant hypertensive rat treated with resveratrol. Values are means ± S.D. *** , **, * indicate $p < 0.001$, 0.01, 0.05.

($p < 0.05$, $p < 0.001$, respectively), urine protein ($p < 0.001$, $p < 0.01$, respectively), and Up/Cr ($p < 0.001$, respectively).

4.3. Redox state

In the kidney tissue of MHR level of $O_2^{\cdot-}$ significantly increased ($p < 0.001$), followed by significantly higher MDA ($p < 0.01$) and AOPP ($p < 0.01$) compared to the SHR group (Fig. 3). Interestingly, the application of resveratrol declines all prooxidant parameters in both SHR+R and MHR+R compared to their respective controls (Fig. 3).

The blockade of NO syntheses decreased all antioxidant parameters ($p < 0.001$) in MHR compared to the control group (Fig. 4). As expected, these parameters were significantly increased after treatment with resveratrol in SHR+R ($p < 0.001$), and in MHR + R ($p < 0.001$), almost to the value of the control group (Fig. 4).

Oxidative stress parameters were significantly higher in MHR compared to SHR (Fig. 5). However, chronic resveratrol treatments significantly reduced TOS and PAB ($p < 0.001$, respectively) and significantly increased TAC level ($p < 0.001$) in the SHR+R group compared to SHR. A similar trend was maintained in the MHR+R group, the TOS ($p < 0.05$) and PAB ($p < 0.01$) were significantly lower, but the level of TAC ($p < 0.01$) was higher compared to MHR.

OSI showed similar trend in MHR so that it became significantly increased ($p < 0.01$), and the application of resveratrol significantly reduced these parameters in both treated groups ($p < 0.001$,

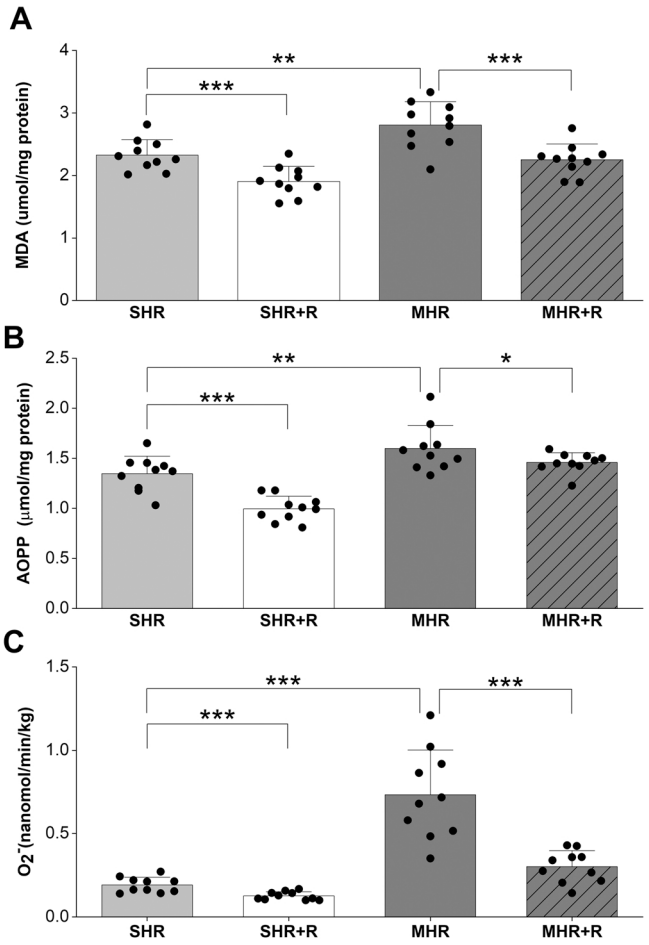

Fig. 3. Prooxidant parameters: MDA- Malondialdehyde (A), AOPP-advanced oxidation protein products (B), $O_2^{\cdot-}$ – superoxide anion radical (C) in experimental groups. SHR – spontaneously hypertensive rat, SHR+R- spontaneously hypertensive rat treated with resveratrol; MHR – malignant hypertensive rat; MHR+R – malignant hypertensive rat treated with resveratrol. Values are means ± S.D. *** , **, * indicate $p < 0.001$, 0.01, 0.05.

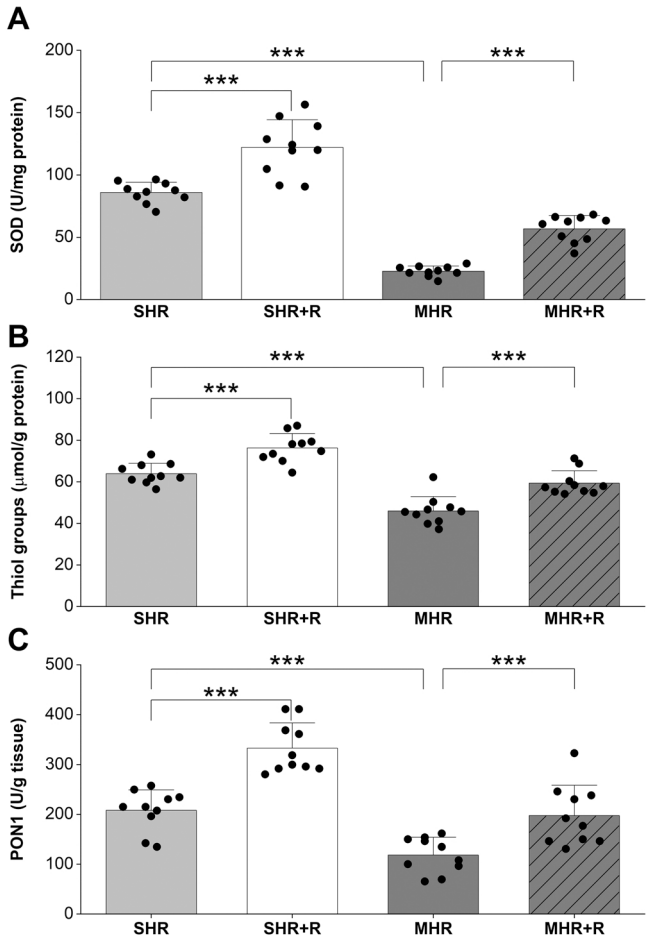

Fig. 4. Antioxidant parameters: SOD – superoxide dismutase (A), Thiol groups (B), PON1-paraoxonase 1 (C) in experimental groups. SHR – spontaneously hypertensive rat, SHR+R – spontaneously hypertensive rat treated with resveratrol; MHR – malignant hypertensive rat; MHR+R- malignant hypertensive rat treated with resveratrol. Values are means ± S.D. *** , **, * indicate $p < 0.001$, 0.01, 0.05.

respectively), compared to their respective controls (Fig. 6).

4.4. Histopathological parameters

Morphological changes in the kidney tissue are shown in Fig. 7. The high blood pressure in SHR leads to minimal change which is characterized by mild oedema and hyperemia. Only individual glomeruli showed hypercellularity, with increased numbers of both resident cells and infiltrating leukocytes, a mean RDS was 2.56 ± 0.13 (Figs. 7A, B and Fig. 6B). Histological changes are more intensive in MHR, including hypercellular glomerulus and poorly defined capillary loops. Almost completely atrophy of tubules with PAS intensive positive casts, proximal and distal convoluted tubules showed diffuse epithelial cell swelling, loss of brush border, vascular degeneration, and focal necrosis. Increased numbers of epithelial cells and pericapillary infiltration by polymorphonuclear leukocytes and erythrocytes were in the glomerular lesion. These changes are correlated with the RDS (Fig. 6B) of 3.69 ± 0.12 confirming that the L-NAME in MHR induced severe renal damage (Fig. 7E and Fig. 7F). However, the application of resveratrol significantly ameliorates the morphological changes caused by hypertension. The normal glomerulus is stained with PAS to highlight basement membranes of glomerular capillary loops and tubular epithelium. Podocytes are present and form the visceral epithelial surface. Bowman's space is seen along with parietal epithelial cells (Fig. 7C and D). Renal specimens taken from the SHR+R rats (Fig. 6B) expert minimal changes 1.81 ± 0.17 in comparison to the SHR ($p < 0.01$). Renal histopathological examination in the MHR+R showed a decreased intensity of tissue damage with the distinct renal tubular epithelial cell swelling and degeneration (Fig. 7G and H). Treatment with resveratrol in this group is indicative of the significantly lower RDS (Fig. 6B) of 2.63 ± 0.13 ($p < 0.01$ vs. MHR group).

Immunohistochemistry of the kidney tissue of SHR showed that 3-nitrotyrosine was granularly present in the cytoplasm (Fig. 8A and B). In the MHR group intense 3-nitrotyrosine staining was observed in the region of proximal and distal convoluted tubules, initial collecting tubules, interlobular vascular endothelium, and glomeruli (Fig. 8E and F). Treatment with resveratrol induced a total absence of 3-nitrotyrosine particular in the SHR+R group (Fig. 8C and D). The MHR+R group treatment with resveratrol showed much less renal staining for 3-nitrotyrosine compared with MHR, and the general distribution of 3-nitrotyrosine staining was in proximal and convoluted tubules, as well as in glomeruli (Fig. 8G and H).

5. Discussion

The major novel findings in the present study are that the chronic treatment of malignantly hypertensive rats with natural polyphenol resveratrol results in reduced proteinuria, better glomerular filtration, and improved renal histology, mainly due to antiinflammatory and antioxidative actions of resveratrol. These effects were similar or slightly

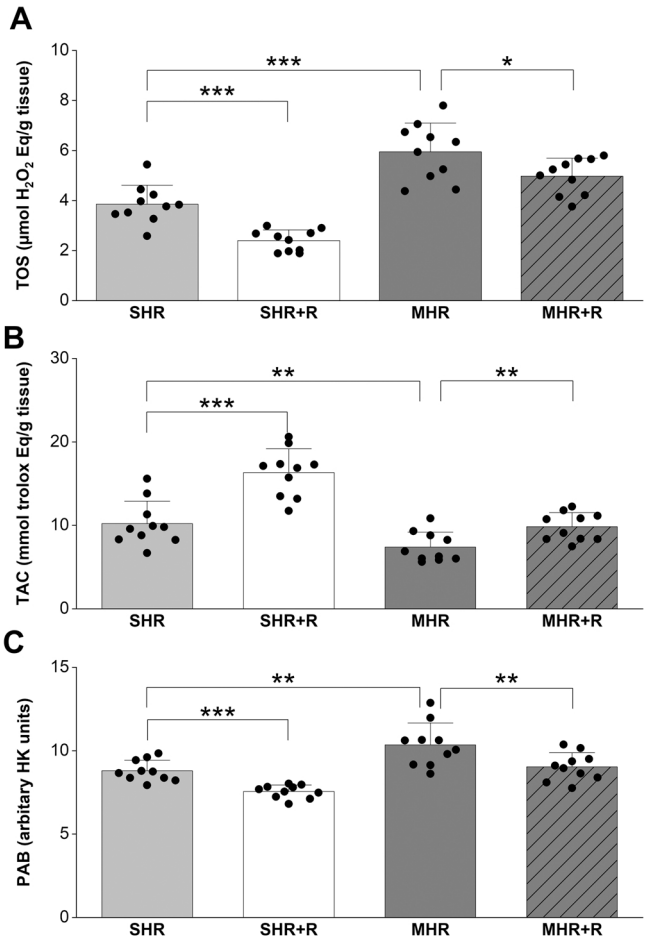

Fig. 5. Oxidative stress parameters: TAC – total antioxidant capacity (A), TOS – total oxidant status (B), PAB – prooxidative balance (C) in experimental groups. SHR – spontaneously hypertensive rat, SHR+R – spontaneously hypertensive rat treated with resveratrol; MHR – malignant hypertensive rat; MHR+R – malignant hypertensive rat treated with resveratrol. Values are means ± S.D. ***, **, * indicate $p < 0.001$, 0.01, 0.05.

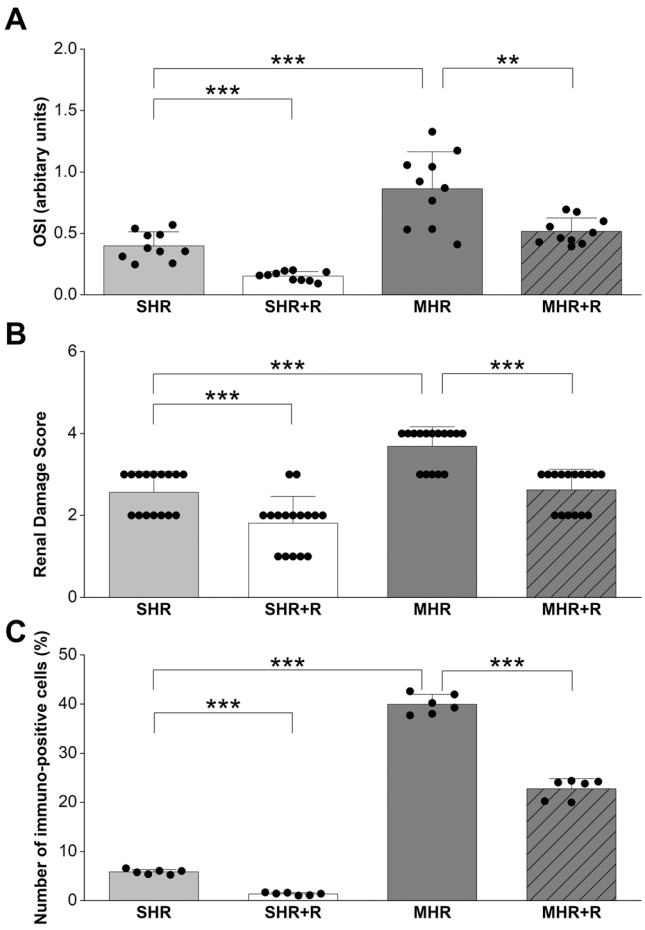

Fig. 6. Risk scores: OSI – oxidative stress index (A), RDS – renal damage score (B), number of immuno-positive cells (C) in experimental groups. SHR – spontaneously hypertensive rat, SHR+R – spontaneously hypertensive rat treated with resveratrol; MHR – malignant hypertensive rat; MHR+R – malignant hypertensive rat treated with resveratrol. Values are means ± S.D. ***, **, * indicate $p < 0.001$, 0.01, 0.05.

blunted than in SHR. These results give a promise that this polyphenol, resveratrol, has a potential to reduction a high blood pressure induced kidney injury in different stadium of hypertension. Actually, this has for the first time showed benefits of resveratrol on the kidney in malignant hypertension.

Our results indicate, similarly to the study of Montoro-Molina [30], that, in a genetic model of hypertension, renal hypertension changes develop to overt proteinuria. There is limited literature data about malignant hypertension and its pathophysiological influence on renal changes. It is well known that the kidney is essential for the long-term control of arterial pressure and that abnormalities in renal function play a key role in the pathogenesis of hypertension. In this study, long term blockade of NO syntheses with L-NAME in MHR rats can contribute to the expected increase in wall tension and the hypertrophy of glomerular capillaries. It also compromises baseline function leading to elevated plasma creatinine and proteinuria (> 147 μmol/L and 6 mg/ml, respectively). This is similar to the two large retrospective analyses of patients with malignant hypertension, the renal outcome was associated with serum creatinine elevation (> 200 μmol/L and 175 μmol/L) and proteinuria (≤ 1 g/24 hrs) [31,32]. Some studies reported that the treatment with the resveratrol reduced serum creatinine in diabetic nephropathy [18], sepsis-induced kidney injury [19], kidney toxicity [17], as well as in acute kidney injury [33]. The results of our investigation revealed that plasma creatinine and urine protein levels decreased after treatment with resveratrol in both models of hypertension, SHR and MHR. Although both hypertensive groups had markedly proteinuria (UP/Cr) the resveratrol treatment induced a statistically significant reduction of this parameter in both hypertensive rats, SHR and MHR ($p < 0.001$, respectively). These results indicate a significant improvement of glomerular filtration and tubular function after resveratrol treatment.

Essential hypertension is among other pathophysiological conditions characterized by the increased presence (bioavailability) of reactive oxygen/nitrogen species known as oxidative stress [2]. Daenen et al. have showed that oxidative stress led to metabolic dysregulation and/or lipid and protein end products oxidation, as well as oxidative damage of cells, tissues, and organs [34]. This is in the line with the results obtained in our study that in the MHR, long term blockade of NO synthesis, induced excessive release of oxygen radicals. Radical storms cannot be buffered because all antioxidant parameters are reduced, resulting in a significant increase in protein and lipid peroxidation.

The present study also demonstrates that superoxide anion production is ameliorated by long-term resveratrol treatment, in both models of hypertension MHR and SHR. Moreover, it has been proposed that resveratrol had decreased all prooxidative changes by scavenging $O_2^{\cdot-}$ and preventing oxidative stress-induced kidney damage. The harmful kidney $O_2^{\cdot-}$ production is likely buffered with preserved kidney antioxidant defense, since superoxide dismutase activity (SOD), paraoxonase 1 activity (PON1) as well as TAC were increased in both resveratrol treated groups. Our results that resveratrol promotes redox benefits even

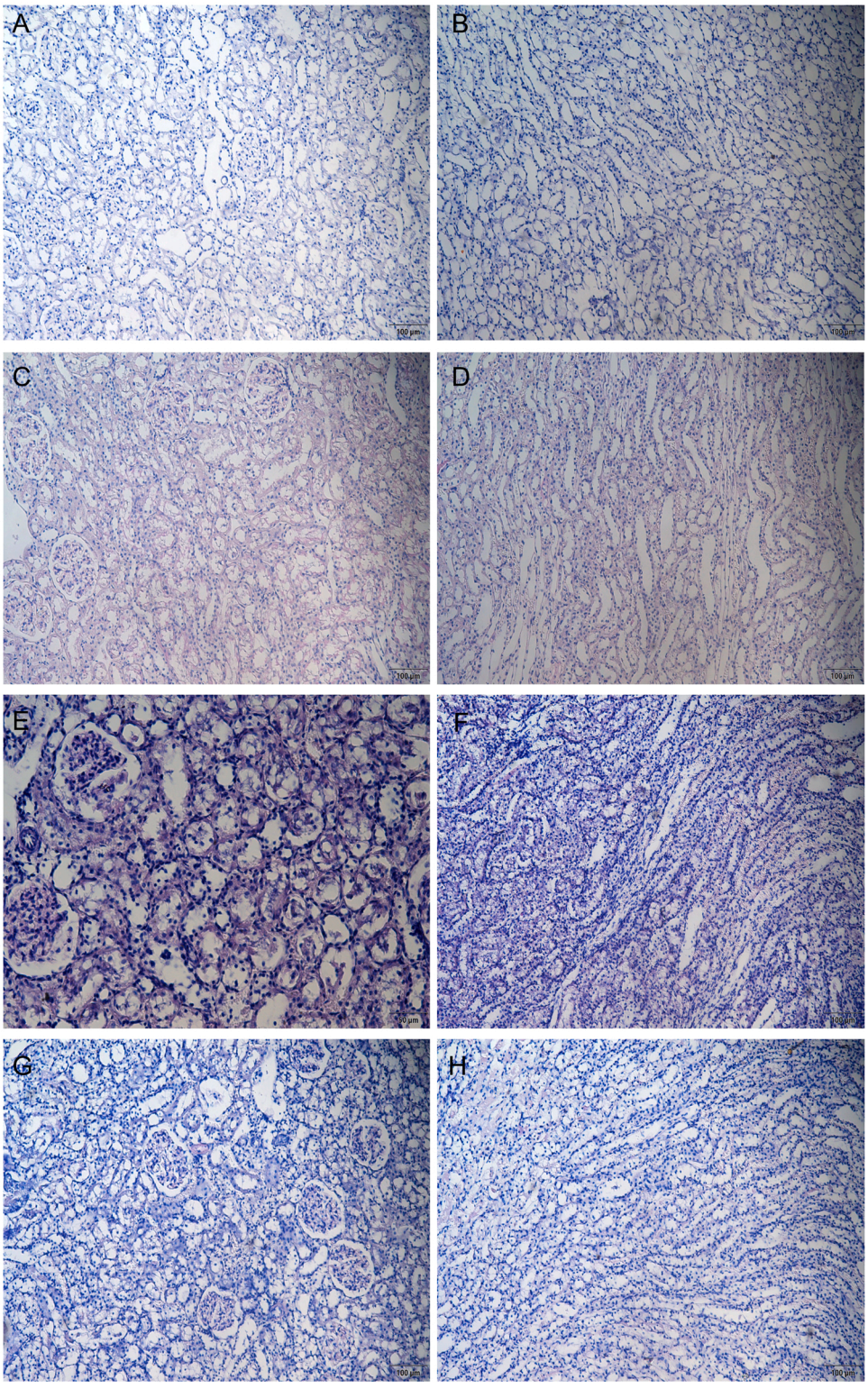

Fig. 7. Protective effects of resveratrol treatment in the kidney tissue of the essential and malignant hypertensive rats, periodic acid Schiff (PAS) method, 200x magnified images, bar = 100 μm. (A-B) Mild oedema of glomeruli and slightly prominent tubular dilatation with infrequent PAS-positive tubular casts in the SHR group. (C-D) The normal shape of glomeruli and tubulointerstitium in the SHR+R group. (E-F) Glomerulus with capsular adhesions and tubular atrophy and dilatation with PAS-positive tubular casts in the MHR group. (G-H) Moderate capsular adhesion and incipient sclerosis; well-preserved tubules and interstitium in MHR+R group.

when the nitric oxide is decreased by the inhibition of nitric oxide synthase are in accordance with the results of Dillenburg et al. [35]. Thus, it is not surprising that great benefits are seen in MHR after resveratrol treatment, especially keeping in mind that L-NAME significantly diminished but did not eliminate resveratrol-induced antioxidative activity.

Evidence obtained from different animal models [36–38] and patients with essential hypertension [37] support the hypothesis that the development of hypertension and hypertension-induced damage in target organs are associated with oxidative stress and inflammation [39]. Our results confirmed elevated blood pressure and typical histopathological changes of the kidney tissue in the SHR group, including arteriole alterations, individual glomerular damage, and moderate oedema of tubulointerstitium, also seen in the studies of other authors [40,41]. These histological changes are more intensive in MHR, followed by a remarkable increase in SAP and DAP. Long term exposure to

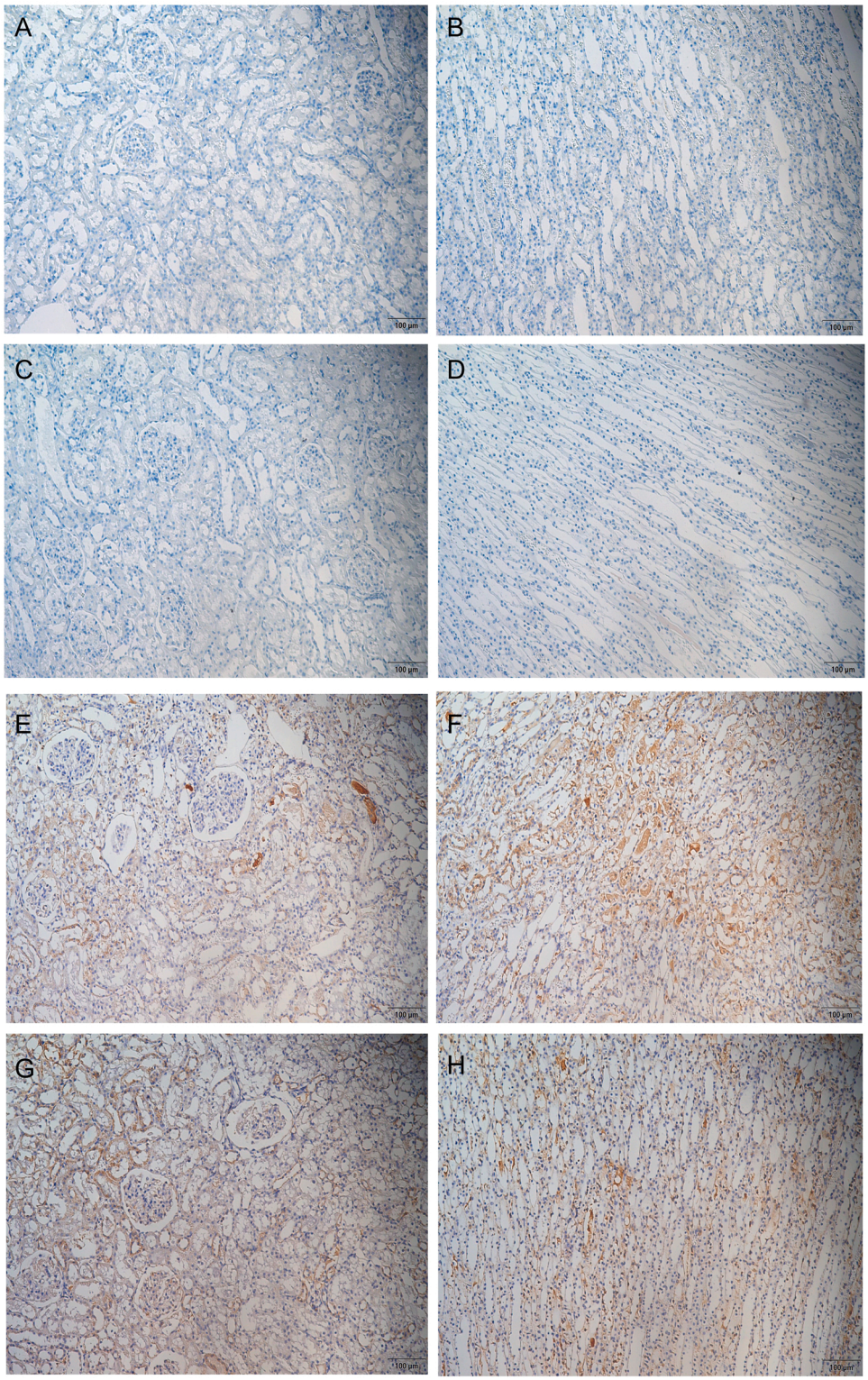

Fig. 8. Immunohistochemical staining of 3-nitrotyrosine in the kidney tissue of the essential and malignant hypertensive rats, magnification 200x. (A-B) Granular appearance of 3-nitrotyrosine particles in SHR. (C-D) negative immunostaining in the SHR+R group. (E-F) intense cytoplasmic staining in the kidney tissue of MHR (dark brown). (G-H) resveratrol induced much less 3-nitrotyrosine staining in the MHR+R group.

very high blood pressure in MHR overcame the wall tension force of these epithelial cells that limit their ability to maintain physical integrity and their ability to withstand mechanical stress load leading to atrophy of tubules, vascular damage, and focal necrosis completely [42].

The central findings of this study are that the treatment with this natural compound resveratrol ameliorates hypertension as well as the renal infiltration of lymphocytes, macrophages, and preserves tubulointerstitial areas of the kidney subjected to the extremely high blood pressure. The precise mechanisms of resveratrol induced blood pressure lowering in SHR we showed in the our previous studies [7,8]. Similar results were obtained in the study of Javkhedkar et al. after 8 weeks of treatment with a much higher dose of resveratrol (50 mg/kg) [43]. Also,

in a model of acute kidney injury, pretreatment of animals with resveratrol (5 and 10 mg/kg) markedly attenuated renal dysfunction, improved morphologic alterations, reduced elevated oxidative stress, and restored the depleted renal antioxidant enzymes [33]. The present study demonstrates, for the first time, interstitial immune cell infiltration, as well as vascular degeneration and atrophy of tubules in the renal tissue of MHR, reduced after treatment with antioxidant resveratrol.

The kidney is one of the most susceptible target organ for hypertension-induced tissue damage [42]. In different models, it has been well described that the downregulation of NO played a decisive role in development of hypertension and kidney disease [44–46]. Reduced NO bioavailability promotes an oxidative imbalance by simultaneously enhancing ROS and RNS production and down-regulating key antioxidant enzymes [47]. Increased levels of ROS indicate excessive production of superoxide, which reacts with NO to form peroxynitrite. Peroxynitrite can cause nitration of tyrosine residues on proteins to form nitrotyrosine [47] and has been identified as an indicator or marker of cell damage and inflammation [48–50]. The elevated level of nitrotyrosine reflects the degree of tissue damage [49], while the presence of nitrotyrosine in the kidney detected with immunohistochemistry could be associated with progressive deterioration of the kidney [51].

In accordance with the earlier study [43], our SHR also exhibited hypercellularity in individual glomeruli of the renal tissue followed with mild oedema and hyperemia. This was associated with marked elevation of TOS and PAB in the kidney tissue, suggesting oxidative stress of these cells. In hypertension, especially malignant, disturbed endothelial cells increase NADPH-oxidase activity that directly reduces the bioavailability of endothelial NO [52]. Also, high intrarenal pressure-induced glomerular injury and advanced production of ROS/RNS [53]. Agents such as L-NAME that reduce local, intra-renal bioavailability of NO or increase renal vasoconstriction may directly increase ROS/RNS production within the kidney and play a role in the aggravation of the histopathological damage of the kidney [54,55]. Its rationale to assume that malignant hypertensive rats of our study have excessive production of different reactive oxygen/nitrogen species including superoxide anion and 3-nitrotyrosine, as well as infiltration of inflammatory cells that lead to direct oxidation of protein within the kidney, induced renal injury with mild glomerular damaged vascular degeneration, and focal necrosis.

The suppression of renal inflammation obtained with resveratrol has been previously shown in different experimental models [18,56]. We measured total antioxidant and total oxidant contents due to the diversity of antioxidant and oxidant agents' interactions. Accordingly, in the present study, the improvements of blood pressure, as well as kidney structure and function in SHR treated with resveratrol, were accompanied by replenished antioxidant defense and lower 3-nitrotyrosine levels, verifying the antioxidant and NO-mediated renoprotective effects of resveratrol.

Our data shows for the first time, that resveratrol treatment in MHR not only decreases blood pressure and oxidative stress but also restores the antioxidant capacity of these animals. Actually, oral administration of resveratrol in MHR increased all antioxidant parameters and decreased levels of kidney non-enzymatic prooxidant, as well as reduced the formation of 3-nitrotyrosine to normal range in SHR rat. In the present study, we also show that resveratrol's protection against hypertension may be related to the restoration of the NO pathway. As far as we know, this is the first study showing that resveratrol decreases overproduction of 3-nitrotyrosine in kidney tissue, therefore it is conceivable that resveratrol targets both antioxidative and inflammatory pathways to protect renal tubules against L-NAME induced hypertension. In line with this, Hong et al. found protective action of resveratrol through upregulation of NO in the kidney cells [57]. Moreover, it has been proposed that resveratrol exerts a renoprotective action as a result of the upregulation of nitric oxide and antioxidant activity.

6. Conclusions

In conclusion, resveratrol significantly attenuates kidney damage in malignantly hypertensive rats at the same way, but a little more subtle than in SHR. Resveratrol decreased proteinuria and inflammation in the kidney tissue and its renoprotective effect is closely related to the inhibition of reactive oxygen species and reactive nitrogen species production as well as restoration of endogenous antioxidants and nitric oxide enzyme activity followed by blood pressure lowering. Our study suggests that resveratrol may be a promising therapeutical approach for different hypertension-induced renal damage, especially in combination with conservative agents.

CRediT authorship contribution statement

Jelica Grujić-Milanović: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Supervision. **Vesna Jaćević:** Methodology, Formal analysis, Writing – review & editing, Visualization. **Zoran Miloradović:** Methodology, Validation, Investigation, Resources, Writing – review & editing, Project administration. **Sladjan D. Milanović:** Methodology, Formal analysis, Investigation, Data curation, Writing – review & editing, Supervision. **Djurdjica Jovović:** Funding acquisition. **Milan Ivanov:** Investigation. **Danijela Karanović:** Investigation. **Una-Jovana Vajić:** Investigation. **Nevena Mihailović-Stanojević:** Validation, Writing – review & editing, Visualization, Supervision. All authors have read and agreed to the published version of the manuscript.

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Institutional review board statement

The study was conducted according to the guidelines of the Directive on the protection of animals used for scientific purposes (2010/63/EU) and the National Law of Animal Welfare (the Official Gazette of the Republic of Serbia, No.: 41/2009 and 39/2010). The study protocol was approved by the Ethics Committee of the Institute for Medical Research (IMR), University of Belgrade, Serbia and Veterinary Directorate, Ministry of Agriculture and Environmental Protection, Republic of Serbia (No. 0316–1/11).

Conflict of interest statement

None.

Data Availability

The data presented in this study are available in article.

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References

[1] World Health Organisation ⟨Https://Www.Who.Int/News-Room/Fact-Sheets/Detail/Noncommunicable-Diseases⟩.
[2] R.M. Touyz, F.J. Rios, R. Alves-Lopes, K.B. Neves, L.L. Camargo, A.C. Montezano, Oxidative Stress: A Unifying Paradigm in Hypertension, Can. J. Cardiol. 36 (2020) 659–670.
[3] B.J.H. Van Den Born, G.A. Van Montfrans, Malignant hypertension, Manag. Acute Kidney Probl. (2021) 305–316, https://doi.org/10.1007/978-3-540-69441-0_32.

[4] M. Domek, J. Gumprecht, G.Y.H. Lip, A. Shantsila, Malignant hypertension: does this still exist? J. Hum. Hypertens. 2019 341 34 (2019) 1–4, https://doi.org/10.1038/S41371-019-0267-Y.

[5] L.O. Lerman, T.W. Kurtz, R.M. Touyz, D.H. Ellison, A.R. Chade, S.D. Crowley, D. L. Mattson, J.J. Mullins, J. Osborn, A. Eirin, et al., Animal models of hypertension: a scientific statement from the american heart association, Hypertensiom 73 (2019) e87–e120.

[6] S.A. Doggrell, L. Brown, Rat models of hypertension, cardiac hypertrophy and failure, Cardiovasc. Res 39 (1998) 89–105.

[7] J. Grujic-Milanovic, Z. Miloradovic, D. Jovovic, V. Jacevic, I. Milosavljevic, S. D. Milanovic, N. Mihailovic-Stanojevic, The red wine polyphenol, resveratrol improves hemodynamics, oxidative defence and aortal structure in essential and malignant hypertension, J. Funct. Foods 34 (2017) 266–276, https://doi.org/10.1016/j.jff.2017.04.035.

[8] J. Grujić-Milanović, V. Jaćević, Z. Miloradović, D. Jovović, I. Milosavljević, S. D. Milanović, N. Mihailović-Stanojević, Resveratrol protects cardiac tissue in experimental malignant hypertension due to antioxidant, anti-inflammatory, and anti-apoptotic properties, Int. J. Mol. Sci. (2021) *22*, https://doi.org/10.3390/ijms22095006.

[9] C.G. Fraga, K.D. Croft, D.O. Kennedy, F.A. Tomás-Barberán, The effects of polyphenols and other bioactives on human health, Food Funct. 10 (2019) 514–528, https://doi.org/10.1039/c8fo01997e.

[10] C.N. Hsu, C.Y. Hou, Y.L. Tain, Preventive aspects of early resveratrol supplementation in cardiovascular and kidney disease of developmental origins, Int. J. Mol. Sci. (2021) *22*, https://doi.org/10.3390/IJMS22084210.

[11] F. Mehri, A. Ranjbar, N. Shirafkan, S.S. Asl, M. Esfahani, The protective effect of resveratrol on diazinon-induced oxidative stress and glucose hemostasis disorder in rats' liver, J. Biochem. Mol. Toxicol. 36 (2022), e23063, https://doi.org/10.1002/JBT.23063.

[12] C.K. Cheng, J.Y. Luo, C.W. Lau, Z.Y. Chen, X.Y. Tian, Y. Huang, Pharmacological basis and new insights of resveratrol action in the cardiovascular system, Br. J. Pharmacol. 177 (2020) 1258–1277, https://doi.org/10.1111/BPH.14801.

[13] N. Xia, A. Daiber, U. Förstermann, H. Li, Antioxidant effects of resveratrol in the cardiovascular system, Br. J. Pharmacol. 174 (2017) 1633–1646, https://doi.org/10.1111/BPH.13492.

[14] B. Salehi, A.P. Mishra, M. Nigam, B. Sener, M. Kilic, M. Sharifi-Rad, P.V.T. Fokou, N. Martins, J. Sharifi-Rad, Resveratrol: a double-edged sword in health benefits, Biomedicines (2018) *6*, https://doi.org/10.3390/BIOMEDICINES6030091.

[15] Z. Xiao, C. Chen, T. Meng, W. Zhang, Q. Zhou, Resveratrol attenuates renal injury and fibrosis by inhibiting transforming growth factor-β pathway on matrix Metalloproteinase 7, Exp. Biol. Med. 241 (2016) 140–146, https://doi.org/10.1177/1535370215598401.

[16] D.H. Kim, Y.J. Jung, J.E. Lee, A.S. Lee, K.P. Kang, S. Lee, S.K. Park, M.K. Han, S. Y. Lee, K.M. Ramkumar, et al., SIRT1 activation by resveratrol ameliorates cisplatin-induced renal injury through deacetylation of P53, Am. J. Physiol. Ren. Physiol. (2011) *301*, https://doi.org/10.1152/AJPRENAL.00258.2010.

[17] Esfahani, M.; Rahbar, A.H.; Soleimani Asl, S.; Mehri, F. Resveratrol: A Panacea Compound for Diazinon-Induced Renal Toxicity. https://doi.org/10.1080/15569543.2021.2008452 2021, doi:10.1080/15569543.2021.2008452.

[18] F. Xu, Y. Wang, W. Cui, H. Yuan, J. Sun, M. Wu, Q. Guo, L. Kong, H. Wu, L. Miao, Resveratrol prevention of diabetic nephropathy is associated with the suppression of renal inflammation and mesangial cell proliferation: possible roles of Akt/NF-KB pathway, Int. J. Endocrinol. 2014 (2014), https://doi.org/10.1155/2014/289327.

[19] N. Wang, L. Mao, L. Yang, J. Zou, K. Liu, M. Liu, H. Zhang, X. Xiao, K. Wang, Resveratrol protects against early polymicrobial sepsis-induced acute kidney injury through inhibiting endoplasmic reticulum stress-activated NF-KB pathway, Oncotarget 8 (2017) 36449–36461, https://doi.org/10.18632/ONCOTARGET.16860.

[20] M. Uchiyama, M. Mihara, Determination of malonaldehyde precursor in tissues by thiobarbituric acid test, Anal. Biochem. 86 (1978) 271–278, https://doi.org/10.1016/0003-2697(78)90342-1.

[21] L. Selmeci, L. Seres, M. Antal, J. Lukács, A. Regöly-Mérei, G. Acsády, Advanced oxidation protein products (AOPP) for monitoring oxidative stress in critically Ill patients: a simple, fast and inexpensive automated technique, Clin. Chem. Lab. Med. 43 (2005) 294–297, https://doi.org/10.1515/CCLM.2005.050.

[22] E. Pick, Y. Keisari, A simple colorimetric method for the measurement of hydrogen peroxide produced by cells in culture, J. Immunol. Methods (1980), https://doi.org/10.1016/0022-1759(80)90340-3.

[23] J.M. McCord, I. Fridovich, The reduction of cytochrome c by milk xanthine oxidase, J. Biol. Chem. 243 (1968) 5753–5760, doi:4972775.

[24] G.L. Ellman, Tissue sulfhydryl groups, Arch. Biochem. Biophys. (1959), https://doi.org/10.1016/0003-9861(59)90090-6.

[25] K.N. Gan, A. Smolen, H.W. Eckerson, B.N. La Du, Purification of human serum paraoxonase/arylesterase. evidence for one esterase catalyzing both activities, Drug Metab. Dispos. (1991).

[26] D.H. Alamdari, M. Ghayour-Mobarhan, S. Tavallaie, M.R. Parizadeh, M. Moohebati, F. Ghafoori, S.M.R. Kazemi-Bajestani, K. Paletas, T. Pegiou, G. Koliakos, Prooxidant-antioxidant balance as a new risk factor in patients with angiographically defined coronary artery disease, Clin. Biochem. 41 (2008) 375–380, https://doi.org/10.1016/J.CLINBIOCHEM.2007.12.008.

[27] O. Erel, A new automated colorimetric method for measuring total oxidant status, Clin. Biochem. 38 (2005) 1103–11111, https://doi.org/10.1016/j.clinbiochem.2005.08.008.

[28] O. Erel, A novel automated method to measure total antioxidant response against potent free radical reactions, Clin. Biochem. 37 (2004) 112–119, https://doi.org/10.1016/j.clinbiochem.2003.10.014.

[29] L. Nežić, R. Škrbić, L. Amidžić, R. Gajanin, Z. Milovanović, E. Nepovimova, K. Kuča, V. Jaćević, Protective effects of simvastatin on endotoxin-induced acute kidney injury through activation of tubular epithelial cells' survival and hindering cytochrome C-mediated apoptosis, Int. J. Mol. Sci. 21 (2020) 1–18, https://doi.org/10.3390/IJMS21197236.

[30] S. Montoro-Molina, A. Quesada, F. O'Valle, N.M. Morales, M.D.C. De Gracia, I. Rodríguez-Gómez, A. Osuna, R. Wangensteen, F. Vargas, The long-term study of urinary biomarkers of renal injury in spontaneously hypertensive rats, Kidney Blood Press. Res. 46 (2021) 502–513, https://doi.org/10.1159/000516843.

[31] F. Amraoui, S. Bos, L. Vogt, B.J. Van Den Born, Long-term renal outcome in patients with malignant hypertension: a retrospective Cohort study, BMC Nephrol. (2012) *13*, https://doi.org/10.1186/1471-2369-13-71.

[32] G.Y.H. Lip, M. Beevers, D. Gareth Beevers, Complications and survival of 315 patients with malignant-phase hypertension, J. Hypertens. 13 (1995) 915–924, https://doi.org/10.1097/00004872-199508000-00013.

[33] V. Chander, K. Chopra, Protective effect of resveratrol, a polyphenolic phytoalexin on glycerol-induced acute renal failure in rat kidney, Ren. Fail. 28 (2006) 161–169, https://doi.org/10.1080/08860220500531112.

[34] K. Daenen, A. Andries, D. Mekahli, A. Van Schepdael, F. Jouret, B. Bammens, Oxidative stress in chronic kidney disease, Pediatr. Nephrol. 34 (2019) 975–991, https://doi.org/10.1007/S00467-018-4005-4.

[35] D.R. Dillenburg, C. Mostarda, I.C. Moraes-Silva, D. Ferreira, D. Bós, S.G. da, A.A. M. Duarte, M.C. Irigoyen, K. Rigatto, Resveratrol and grape juice differentially ameliorate cardiovascular autonomic modulation in L-NAME-treated rats, Auton. Neurosci. Basic Clin. 179 (2013) 9–13, https://doi.org/10.1016/j.autneu.2013.06.002.

[36] N.D. Vaziri, B. Rodríguez-Iturbe, Mechanisms of disease: oxidative stress and inflammation in the pathogenesis of hypertension, Nat. Clin. Pract. Nephrol. 2 (2006) 582–593, https://doi.org/10.1038/ncpneph0283.

[37] J. González, N. Valls, R. Brito, R. Rodrigo, Essential hypertension and oxidative stress: new insights, World J. Cardiol. 6 (2014) 353–366, https://doi.org/10.4330/wjc.v6.i6.353.

[38] D. Konukoglu, H. Uzun, Endothelial dysfunction and hypertension, Adv. Exp. Med. Biol. 956 (2016) 511–540, https://doi.org/10.1007/5584_2016_90.

[39] E.L. Schiffrin, Mechanisms of remodelling of small arteries, antihypertensive therapy and the immune system in hypertension, Clin. Investig. Med. 38 (2015) E394–E402, https://doi.org/10.25011/cim.v38i6.26202.

[40] M.C. Braun, S.M. Herring, N. Gokul, M. Monita, R. Bell, M.J. Hicks, S.E. Wenderfer, P.A. Doris, Hypertensive renal disease: susceptibility and resistance in inbred hypertensive rat lines, J. Hypertens. 31 (2013) 2050–2059, https://doi.org/10.1097/HJH.0B013E328362F9A5.

[41] Z. Wang, S. Wang, J. Zhao, C. Yu, Y. Hu, Y. Tu, Z. Yang, J. Zheng, Y. Wang, Y. Gao, Naringenin ameliorates renovascular hypertensive renal damage by normalizing the balance of renin-angiotensin system components in rats, Int. J. Med. Sci. 16 (2019) 644–653, https://doi.org/10.7150/IJMS.31075.

[42] S. Mennuni, S. Rubattu, G. Pierelli, G. Tocci, C. Fofi, M. Volpe, Hypertension and kidneys: unraveling complex molecular mechanisms underlying hypertensive renal damage, J. Hum. Hypertens. 28 (2014) 74–79, https://doi.org/10.1038/JHH.2013.55.

[43] A.A. Javkhedkar, A.A. Banday, Antioxidant resveratrol restores renal sodium transport regulation in SHR, Physiol. Rep. (2015) *3*, https://doi.org/10.14814/PHY2.12618.

[44] C.N. Hsu, Y.L. Tain, Regulation of nitric oxide production in the developmental programming of hypertension and kidney disease, Int. J. Mol. Sci. (2019) *20*, https://doi.org/10.3390/IJMS20030681.

[45] C. Baylis, Nitric oxide synthase derangements and hypertension in kidney disease, Curr. Opin. Nephrol. Hypertens. 21 (2012) 1–6, https://doi.org/10.1097/MNH.0b013e32834d54ca.

[46] C.S. Wilcox, Oxidative stress and nitric oxide deficiency in the kidney: a critical link to hypertension, Am. J. Physiol. Regul. Integr. Comp. Physiol. (2005) *289*, https://doi.org/10.1152/AJPREGU.00250.2005.

[47] G. Ferrer-Sueta, N. Campolo, M. Trujillo, S. Bartesaghi, S. Carballal, N. Romero, B. Alvarez, R. Radi, Biochemistry of peroxynitrite and protein tyrosine nitration, Chem. Rev. 118 (2018) 1338–1408, https://doi.org/10.1021/acs.chemrev.7b00568.

[48] N. Abello, H.A.M. Kerstjens, D.S. Postma, R. Bischoff, Protein tyrosine nitration: selectivity, physicochemical and biological consequences, denitration, and proteomics methods for the identification of tyrosine-nitrated proteins, J. Proteome Res. 8 (2009) 3222–3238, https://doi.org/10.1021/PR900039C.

[49] J.M. Souza, G. Peluffo, R. Radi, Protein tyrosine nitration-functional alteration or just a biomarker? Free Radic. Biol. Med. 45 (2008) 357–366, https://doi.org/10.1016/j.freeradbiomed.2008.04.010.

[50] H. Ischiropoulos, Protein tyrosine nitration–an update, Arch. Biochem. Biophys. 484 (2009) 117–121, https://doi.org/10.1016/J.ABB.2008.10.034.

[51] R.C. Thuraisingham, C.A. Nott, S.M. Dodd, M.M. Yaqoob, Increased nitrotyrosine staining in kidneys from patients with diabetic nephropathy, Kidney Int 57 (2000) 1968–1972, https://doi.org/10.1046/j.1523-1755.2000.00046.x.

[52] M.W. Cunningham, J.M. Sasser, C.A. West, C.J. Milani, C. Baylis, K.D. Mitchell, Renal nitric oxide synthase and antioxidant preservation in Cyp1a1-Ren-2 transgenic rats with inducible malignant hypertension, Am. J. Hypertens. 26 (2013) 1242–1249, https://doi.org/10.1093/AJH/HPT096.

[53] C.C. Lu, G.H. Wang, J. Lu, P.P. Chen, Y. Zhang, Z.B. Hu, K.L. Ma, Role of podocyte injury in glomerulosclerosis, Adv. Exp. Med. Biol. 1165 (2019) 195–232, https://doi.org/10.1007/978-981-13-8871-2_10.

[54] R. Corremans, P.C. D'haese, B.A. Vervaet, A. Verhulst, L-NAME administration enhances diabetic kidney disease development in an STZ/NAD rat model, Int. J. Mol. Sci. 22 (2021), https://doi.org/10.3390/IJMS222312767.
[55] A.J. Polichnowski, L. Lu, A.W. Cowley, Renal injury in angiotensin II+L-NAME-induced hypertensive rats is independent of elevated blood pressure, Am. J. Physiol. Ren. Physiol. 300 (2011) 1008–1016, https://doi.org/10.1152/AJPRENAL.00354.2010.
[56] L. Chen, S. Yang, E.E. Zumbrun, H. Guan, P.S. Nagarkatti, M. Nagarkatti, Resveratrol attenuates lipopolysaccharide-induced acute kidney injury by suppressing inflammation driven by macrophages, Mol. Nutr. Food Res. 59 (2015) 853–864, https://doi.org/10.1002/MNFR.201400819.
[57] S.H. Hong, H.J. Lee, E.J. Sohn, H.S. Ko, B.S. Shim, K.S. Ahn, S.H. Kim, Anti-nephrolithic potential of resveratrol via inhibition of ROS, MCP-1, hyaluronan and osteopontin in vitro and in vivo, Pharmacol. Rep. 65 (2013) 970–979, https://doi.org/10.1016/S1734-1140(13)71078-8.