นิพนธ์ต้นฉบับ . Original Article

ผลของไดออสมินต่อโครงสร้างและการทำงานของไตในหนูแรทความดันเลือดสูงเนื่องจากหลอดเลือดแดงที่ไตตีบ

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The Effects of Diosmin on Kidney Morphology and Functions in Renovascular Hypertensive Rats

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บทคัดย่อ

หลักการและวัตถุประสงค์: ภาวะความดันเลือดสูงเนื่องจากหลอดเลือดแดงที่ไตตีบ มีรายงานว่ามีความสัมพันธ์กับการทำงานที่ผิดปกติของหลอดเลือดและไต โดยกลไกที่เกี่ยวข้องอาจเกิดจากภาวะเครียดออกซิเดชัน ไดออสมินเป็นสารกลุ่มฟลาโวนอยด์ที่พบมากในพืชตระกูลส้ม มีคุณสมบัติทางชีวภาพมากมาย เช่น คุณสมบัติต้านอนุมูลอิสระและลดความดันเลือดสูง อย่างไรก็ตามยังไม่มีการศึกษาผลของไดออสมินในความดันเลือดสูงเนื่องจากหลอดเลือดแดงที่ไตตีบ ดังนั้นการศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของไดออสมินต่อโครงสร้างและการทำงานของไตในหนูแรทความดันเลือดสูงเนื่องจากหลอดเลือดแดงที่ไตตีบ

วิธีการศึกษา: การศึกษานี้ใช้หนูแรทเพศผู้สายพันธุ์ Sprague-Dawley น้ำหนัก 150-160 กรัม โดยเหนี่ยวนำให้เกิดความดันเลือดสูงด้วยการผ่าตัดคลิปหลอดเลือดแดงที่ไตให้ตีบ หลังผ่าตัด 3 สัปดาห์ หนูถูกแบ่งออกเป็น 5 กลุ่ม (5ตัว/กลุ่ม) ได้แก่ กลุ่มควบคุม กลุ่มความดันเลือดสูง (2K-1C) กลุ่มความดันเลือดสูงที่ได้รับสารไดออสมิน 50 และ 100 มก./กก./วัน และกลุ่มความดันเลือดสูงที่ได้รับยาลดความดันเทลมิซาร์แทน 5 มล./กก./วัน เป็นเวลา 4 สัปดาห์ ความดันเลือดถูกวัดทุกสัปดาห์ เมื่อสิ้นสุดการทดลองตัวอย่างปัสสาวะและไตของหนูทั้งหมดจะถูกเก็บเพื่อนำมาศึกษาทางชีวเคมีและสัณฐานวิทยา

ผลการศึกษา: การรักษาด้วยสารไดออสมิน 100 มล./กก./วัน มีผลในการลดความดันเลือดสูงอย่างมีนัยสำคัญทางสถิติในหนูความดันเลือดสูงเนื่องจากหลอดเลือดแดงที่ไตตีบ ($p<0.05$) การเพิ่มขึ้นของระดับของมาลอนไดอัลดีไฮด์และซุปเปอร์ออกไซด์ในเนื้อเยื่อลดลงในกลุ่มที่รักษาด้วยสารไดออสมิน 100 มก./กก./วัน ($p<0.05$) นอกจากนี้ยังปรับปรุงการทำงานของไตจากการเพิ่มขึ้นของครีเอตินินและยูเรียไนโตรเจนในปัสสาวะเมื่อเปรียบเทียบกับกลุ่มความดันเลือดสูง ($p<0.05$) อีกทั้งยังปรับปรุงโครงสร้างของไตผ่านการลดลงของพื้นที่หน้าตัดของโกลเมอรูลัส กลุ่มหลอดเลือดโกลเมอรูลัส ส่วนของช่องว่างระหว่างพื้นที่หน้าตัดของโกลเมอรูลัสและกลุ่มหลอดเลือดโกลเมอรูลัส และพื้นที่ปริมาตรของไตข้างที่ไม่ได้คลิป ($p<0.05$) ขณะที่กลุ่มยาลดความดันเทลมิซาร์แทนนั้นให้ผลไม่แตกต่างจากกลุ่มสารไดออสมิน (100 มก./กก./วัน)

สรุป: สารไดออสมินมีฤทธิ์ลดความดันเลือดสูง ปรับปรุงโครงสร้างและการทำงานของไตในหนูแรทความดันเลือดสูงเนื่องจากหลอดเลือดแดงที่ไตตีบ ซึ่งการเปลี่ยนแปลงนี้อาจเกี่ยวข้องกับการเป็นสารต้านอนุมูลอิสระ

คำสำคัญ: ไดออสมิน, ความดันเลือดสูงเนื่องจากหลอดเลือดที่ไตตีบ, โครงสร้างของไต, การทำงานของไต

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Abstract

Background and objectives: Renovascular hypertension associated with vascular and kidney dysfunctions have been reported. The possible mechanisms might be related to oxidative stress. Diosmin is a flavonoid that is abundant in the *Rutaceae* family. It exerts numerous biological activities such as anti-oxidation and antihypertension. However, the study of diosmin in renovascular hypertension is still unexplored. Thus, the purpose of this study was to investigate the effects of diosmin on kidney morphology and function in two-kidney, one-clip (2K-1C) hypertension.

Method: Male Sprague-Dawley rats (150-160g) were used in this study. The left renal artery was clipped with a silver clip to induce hypertension. Three weeks after the operation, the rats were divided into five groups (n=5/each) including sham rats treated with vehicle (1.5 ml/kg/day); 2K-1C rats treated with vehicle (1.5 ml/kg/day); 2K-1C rats treated with diosmin (50 and 100 mg/kg/day); and 2K-1C rats treated with telmisartan (5 mg/kg/day). Systolic blood pressure (SP) was measured weekly using the tail-cuff method. At the end of the experiment, the urine sample and the kidney of all rats were collected for biochemical and morphological studies.

Results: Treatment with diosmin (100 mg/kg/day) significantly reduced high blood pressure in 2K-1C hypertensive rats ($p<0.05$). Increases in kidney tissue malondialdehyde (MDA) and superoxide productions in 2K-1C rats were ameliorated by diosmin (100 mg/kg/day) ($p<0.05$). Diosmin allso improved kidney function supporting by the elevation of creatinine (Cr) and blood urea nitrogen (BUN) in urine compared with untreated 2K-1C group ($p<0.05$). Moreover, diosmin improved kidney morphology as it reduced glomerular cross-sectional area (CAS), glomerular tuft area, Bowman's space area, and glomerular volume in the non-clipped kidney ($p<0.05$). In addition, treatment with telmisartan had a similar effect to diosmin (100 mg/kg/day).

Conclusion: Diosmin exerts an antihypertensive effect in renovascular hypertensive rats. It also improved kidney morphology and functions in 2K-1C rats. This effect might be relevant to its antioxidative effect.

Keywords: Diosmin, renovascular hypertension, kidney morphology, kidney function

Introduction

Hypertension (HT) is a medical condition characterized by chronic arterial blood pressure elevation and is a major contributor to morbidity and mortality worldwide[1]. Particularly, it is a major risk factor for cardiovascular disease and various diseases such as kidney dysfunction[2]. The two-kidney, one-clip (2K-1C) model used in this study is an excellent model to study renin-angiotensin system (RAS)-mediated in renovascular hypertension. Renal artery occlusion stimulates the RAS, leading to angiotensin II (Ang II) formation[3]. Overactivation of RAS results in excessive Ang II production, a strong vasoconstrictor that affects blood pressure[4, 5]. Moreover, Ang II enhances reactive oxygen species (ROS) production via stimulating nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. It is well-accepted that high levels of ROS are involved in many pathological conditions of renovascular hypertension such as kidney and endothelial dysfunctions[6]. Kidney dysfunction in the 2K-1C hypertensive rat model has been reported, because of the reductions of creatinine clearance, BUN and creatinine in urine. While elevations of albuminuria, proteinuria, serum BUN and creatinine[5, 7, 8]. Additionally, the 2K-1C hypertensive rat model demonstrates kidney morphology alteration have been reported. The non-clipped kidney shows compensatory enlargement and hyperfunction supported by elevations in glomerular cross-sectional area (CSA), glomerular tuft area and glomerular volume, while the clipped kidney shows progressive atrophy[5]. Therefore, alleviation of oxidative stress might be the appropriate strategy for improving high blood pressure, morphology alteration and dysfunction of the kidney induced by hypertension.

Presently, there are several ways to treat HT, including using antihypertensive drugs. Telmisartan is one of the antihypertensive drugs classified as angiotensin receptor blockers (ARBs) which has a direct effect on cardiovascular end-organ protection. Diosmin (3,5,7-trihydroxy-4-methoxyflavone-7-rutinoside) is a flavonoid that is abundant in the *Rutaceae* family. It exerts numerous biological activities such as antihypertension, anti-oxidation, anti-inflammation and many other effects[9, 10]. Diosmin exhibits antioxidant activity since it is a flavonoid that acts as scavenging free radicals and then prevention of lipid peroxidation and free radical formation reactions[11]. A previous study found that co-administration of 5 mg/kg detamethrin and 50 mg/kg diosmin, orally for 28 days, could provide protection against deltamethrin-induced toxicity and organ damage in rats[12]. Moreover, in deoxycorticosterone acetate (DOCA)-salt-induced hypertension in male Wistar rats, treatment with diosmin for six weeks can alleviate high BP and the dose of 50 mg/kg body weight showed maximum efficacy[13]. However, the study of diosmin in renovascular hypertension is still unexplored. Thus, the purpose of this study was to investigate the effects of diosmin on kidney morphology and function in 2K-1C hypertension.

Materials and methods

Chemicals

Diosmin was obtained from INDOFINE Chemical Company, Inc. (NJ, USA).

Animals

This study complied with the standard for the care and use of experimental animals that was approved by the Animal Ethics Committee of Khon Kaen University, Khon Kaen, Thailand (IACUC- KKU-38/65). Male Sprague-Dawley rats weighing 110-120 g were purchased from Nomura Siam International Co., Ltd., Bangkok, Thailand. Rats were housed in the HVAC (Heating, Ventilation, and Air-Conditioning) System (23±2 ℃) with a 12-hours dark-light cycle, and free access to food and water at Northeast Laboratory Animal Center.

Two-kidney, one-clip (2K-1C) model of hypertension

After one week of the adaptation period, male Sprague-Dawley rats weighing 150-160 g were anesthetized with an intraperitoneal injection of zoletil (1 mL/kg) and xylazine (0.25 mL/kg). Thereafter, a silver clip (0.20 mm) was clipped on the left renal artery. The sham animals were done the same surgical procedure, with no introduction of the clip. The rats were considered 2K-1C hypertensive rats when systolic blood pressure (SP) was more than 160 mmHg after three weeks of surgery[7].

Experimental designs

After three weeks of the induction period, the animals were randomly assigned into 5 groups (n=5/each) including sham-treated with the vehicle (1.5 ml/kg/day); 2K1C-treated with vehicle (1.5 ml/kg/day); 2K1C-treated with diosmin (50 and 100 mg/kg/day); and 2K1C-treated with telmisartan (5 mg/kg/day), dissolved in 10% dimethyl sulfoxide + 90% propylene glycol for four weeks by oral gavage[7, 14]. At the end of the experimental day, rats were sacrificed, the urine and kidney samples were collected for biochemical and morphological studies.

Blood pressure (BP) measurement

Indirect blood pressure measurements were performed once a week for seven weeks. SP was measured in conscious rats by tail-cuff method (CODA software, non-invasive blood pressure system; Kent Scientific., Torrington, CT, USA). The mean values of ten measurements in each rat were recorded. These data were presented as the mean ± SEM.

Oxidative stress marker assessments

Superoxide ($O_2^{\cdot -}$) production and malondialdehyde (MDA) levels

The $O_2^{\cdot -}$ production in kidney tissue was evaluated by lucigenin-enhanced chemiluminescence as described previously with some modifications[15, 16]. The level of thiobarbituric acid reactive substances (TBARs) expressed in the MDA equivalent was assessed in the non-clipped kidney tissue based on a previously described method[17]. The absorbance of the supernatant was measured at 532 nm by a spectrophotometer. A standard curve was generated at different concentrations 0.3 to 10 µmol/ L of 1,1,3, 3-tetraethoxypropane (TEP).

Kidney function measurement

Before and after treatment, the rats were placed in individual metabolic cages with free access to water for 24 hours to collect urine. The volume of urine was recorded. The urine was then centrifuged at 3500 rotations per minute (rpm) for 30 min. The supernatant was separated and stored at -20°C until the analysis. The concentration of creatinine (Cr) and blood urea nitrogen (BUN) in urine was expressed as mg/dL.

Kidney histological and morphology analysis

After dissection, the non-clipped kidney tissue was placed in 4% formaldehyde for 72 hours. The tissues were paraffin-embedded and sliced into 5 µm thick sections. The kidney morphology changes were evaluated using the hematoxylin and eosin (H&E) techniques. Images were captured using a light microscope (Nikon, Tokyo, Japan) at magnification x 400. The glomerular CSA, glomerular tuft area, and Bowman's space area were calculated using ImageJ morphometric software (National Institutes of Health, Bethesda, MD, USA). Glomerular volume was calculated using the formula below. 1.38 represents the shape coefficient and 1.01 represents the size distribution coefficient[8].

$$\text{Glomerular volume} = \text{area}^{1.5} \times (1.38/1.01)$$

Statistical analysis

All data were recorded and presented as mean ± standard error of the mean (SEM). The statistical analysis was performed using GraphPad prism (version 9.4) and one-way analysis of variance (ANOVA), followed by Tukey tests. A p-value of <0.05 was considered statistically significant.

Results

Effects of diosmin and telmisartan on blood pressure

At the beginning of the experiment, SP was not different among groups. After three weeks of the operation, SP was progressively increased in the 2K-1C-operated groups and was significantly higher than that in the sham-operated group. Oral administration of diosmin (100 mg/kg/day) and telmisartan (5 mg/kg/day) for four weeks reduced SP in hypertensive rats ($p<0.05$), while diosmin (50 mg/kg/day) had not significantly reduced SP in hypertensive rats, as shown in Fig 1.

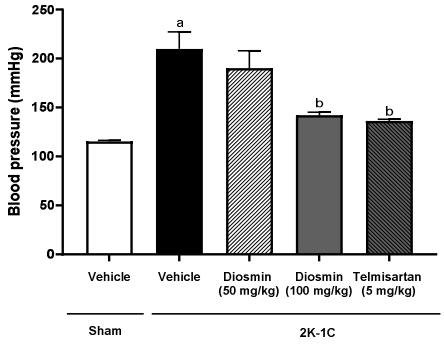

Figure 1 Effects of diosmin and telmisartan on systolic blood pressure at week 7 in 2K-1C hypertensive rats (n=5). The data are expressed as the means ± SEM. [a]p<0.05 vs. sham, [b]p<0.05 vs. 2K-1C. 2K-1C; two-kidney, one-clip.

Effects of diosmin and telmisartan on oxidative stress markers

The $O_2^{\cdot -}$ production and MDA levels elevated in non-clipped kidney tissue were observed in the untreated 2K-1C group when compared with the sham-operated group ($p < 0.05$). After oral administration for four weeks, diosmin (100 mg/kg/day) and telmisartan (5 mg/kg/day) reduced $O_2^{\cdot -}$ production and MDA levels in non-clipped kidney tissue when compared with the untreated 2K-1C group ($p<0.05$), while diosmin (50 mg/kg/day) had not significantly reduced oxidative stress markers, as shown in Fig 2.

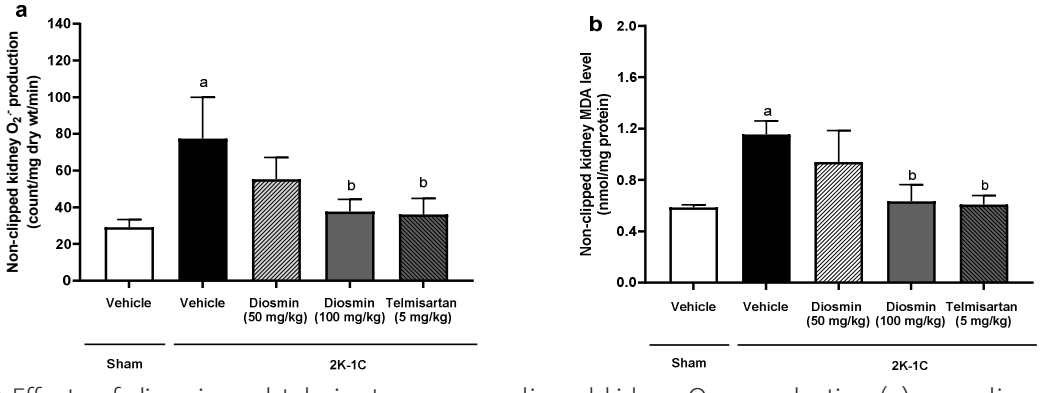

Figure 2 Effects of diosmin and telmisartan on non-clipped kidney $O_2^{\cdot -}$ production (**a**), non-clipped kidney MDA levels (**b**) in 2K-1C hypertensive rats (n=5). The data are expressed as the means ± SEM. [a]p<0.05 vs. sham, [b]p<0.05 vs. 2K-1C. 2K-1C; two-kidney, one-clip.

Effects of diosmin and telmisartan on kidney function

Seven weeks after the 2K-1C operation, kidney dysfunction was observed in the untreated 2K-1C group, as represented by the reductions of Cr and BUN in urine compared with the sham-operated group ($p<0.05$). After treatment with diosmin or telmisartan for four weeks, diosmin (100 mg/kg/day) and telmisartan (5 mg/kg/day) significantly improved Cr and BUN levels in urine when compared with the 2K-1C untreated group ($p<0.05$). While diosmin (50 mg/kg/day) had no effect on kidney function, as shown in Table 1.

Table 1 Effects of diosmin and telmisartan on kidney function in 2K-1C hypertensive rats (n=5). The data are expressed as the means ± SEM. [a]p<0.05 vs. sham, [b]p<0.05 vs. 2K-1C. 2K-1C; two-kidney, one-clip, BUN; blood urine nitrogen, Cr; creatinine.

Group	Pre-treatment		Post-treatment	
	Urine BUN (mg/dL)	Urine Cr (mg/dL)	Urine BUN (mg/dL)	Urine Cr (mg/dL)
Sham + Vehicle	2252.60 ± 282.21	71.30 ± 8.92	2281.00 ± 190.25	69.88 ± 8.14
2K-1C + Vehicle	1153.60 ± 175.88 [a]	35.94 ± 6.47 [a]	728.20 ± 129.86 [a]	19.84 ± 4.07 [a]
2K-1C + Diosmin50 mg/kg	1202.00 ± 275.56 [a]	36.96 ± 8.22 [a]	1081.00 ± 117.93 [a]	36.32 ± 6.96 [a]
2K-1C + Diosmin100 mg/kg	1102.80 ± 171.65 [a]	36.78 ± 5.39 [a]	1683.40 ± 132.77 [b]	50.64 ± 4.70 [b]
2K-1C + Telmisartan 5 mg/kg	1140.40 ± 217.10 [a]	39.48 ± 4.97 [a]	1761.20 ± 117.93 [b]	53.78 ± 3.22 [b]

Effects of diosmin and telmisartan on kidney morphology

The non-clipped kidney of 2K-1C hypertensive rats showed renal hypertrophy which was supported by elevation in glomerular CSA, glomerular tuft area, Bowman's space area and glomerular volume when compared with the sham-operated group ($p<0.05$). After four weeks of treatment, diosmin (100 mg/kg/day) and telmisartan (5 mg/kg/day) significantly improved kidney hypertrophy compared with the 2K-1C untreated group ($p<0.05$). While diosmin (50 mg/kg/day) had no effect on kidney morphology (Table 2). Representative images are shown in Fig 3.

Table 2 Effects of diosmin and telmisartan on non-clipped kidney morphology in 2K-1C hypertensive rats (n=5). The data are expressed as the means ± SEM. [a]$p<0.05$ vs. sham, [b]$p<0.05$ vs. 2K-1C. 2K-1C; two-kidney, one clip.

Group	Sham + Vehicle	2K-1C + Vehicle	2K-1C + Diosmin 50 mg/kg	2K-1C + Diosmin 100 mg/kg	2K-1C + Telmisartan 5 mg/kg
Glomerular CSA (x 10^3 μm^2)	7.98 ± 0.74	12.75 ± 0.97[a]	10.14 ± 0.95	8.20 ± 0.53[b]	8.15 ± 0.40[b]
Glomerular tuft area (x $10^3\mu m^2$)	6.06 ± 0.68	9.57 ± 0.72[a]	7.08 ± 0.65	6.13 ± 0.55[b]	6.09 ± 0.38[b]
Bowman's space area (x 10^3 μm^2)	1.92 ± 0.15	3.18 ± 0.29[a]	3.07 ± 0.30	2.08 ± 0.08[b]	2.04 ± 0.13[b]
Glomerular volume (x 10^6 μm^3)	0.99 ± 0.14	2.00 ± 0.22[a]	1.43 ± 0.20	1.07 ± 0.11[b]	1.00 ± 0.08[b]

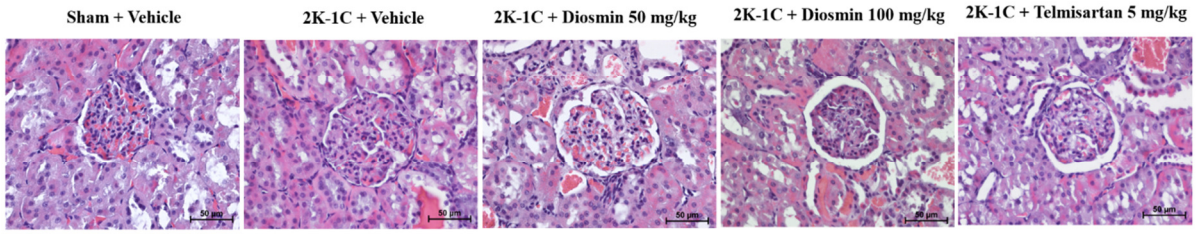

Figure 3 Effects of diosmin and telmisartan on hematoxylin and eosin (H&E) staining for non-clipped kidney tissue in 2K-1C hypertensive rats (n=5). 2K-1C; two-kidney, one clip.

Discussion

The present study demonstrated that clipping the left renal artery causing increased blood pressure, increased oxidative stress status as well as induced kidney damage in the rats. These abnormalities were reduced by treatment with diosmin (100 mg/kg) or telmisartan (5 mg/kg) for four weeks. 2K-1C causing systemic hypertension related to RAS overactivation has been reported. Unilateral occlusion of the renal artery causes reduced renal perfusion pressure that promotes RAS activation[8]. Overactivation of RAS results in excessive Ang II production, a strong vasoconstrictor substance, that affects high BP[4, 5]. Moreover, RAS overactivation enhances ROS production via NADPH oxidase stimulation, leading to elevated oxidative stress. It activates eNOS uncoupling and affects nitric oxide (NO) production, which is a key component of the endothelium-dependent vasodilators that regulate blood pressure. This information was supported by the evidence that increased oxidative stress is the important mechanism contributing to the hypertension induced by 2K-1C method[18]. Our result in this study found that diosmin and telmisartan reduced the blood pressure in 2K-1C hypertensive rats. This result is consistent with previous study found that diosmin acts as an antihypertensive agent against deoxycorticosterone acetate (DOCA)-salt induced hypertension[13]. The possible mechanism of diosmin alleviated BP might be relevant to the scavenging of superoxide anions by diosmin is attributed to its antioxidant effect.

Kidney damage observed in 2K-1C hypertensive rats has been reported in several studies associated with excessive ROS production and renal hemodynamic change[5, 8]. The higher ROS activated fibrosis, apoptosis and inflammation in the kidney develop into

morphology alteration and dysfunction of kidney[19]. In the 2K-1C model, the non-stenotic kidney also undergoes structural changes, probable as a result of the hypertension that developed or as a result of systemic or local humoral changes that were switched as a compensatory response. However, the mechanisms underlying glomerular injury in hypertension remain unclear. In addition, two potential mechanisms have been proposed: (i) hemodynamics-dependent mechanisms and (ii) hemodynamics-independent mechanisms, which include renal oxidative stress and inflammation[20]. The kidney damage that occurred in 2K-1C hypertensive rats in this study was supported by the non-clipped kidney, which showed increases in glomerular CSA, glomerular tuft area, Bowman's space area and glomerular volume. These results corresponded with those of Poasakate and colleagues, who found that non-clipped kidneys had glomerular hypertrophy and atrophy of the clipped kidney[8]. In 2K-1C rats, induced hypertension caused changes in pressure and volume load on the kidney leading to kidney damage and dysfunction[5]. The kidney dysfunction that occurred in 2K-1C hypertensive rats in this study was supported by reduced Cr and BUN in urine, which was consistent with previous study[7]. This study found that diosmin and telmisartan improved kidney morphology and function in 2K-1C rats. These results are consistent with previous studies that co-administration of deltamethrin and diosmin could provide protection against deltamethrin-induced toxicity and organ damage in rats through improved oxidative stress by increasing antioxidant enzyme[12]. The results of this study might be related to two possible mechanisms that could support the effect of diosmin on improved kidney morphology and function. First, diosmin reduced blood pressure leading to decreased pressure load on the kidney. Second, diosmin reduced oxidative stress via it reduced superoxide production and MDA level of the non-clipped kidney in 2K-1C hypertensive rats.

Conclusion

In conclusion, diosmin reduced BP and improved kidney morphology and function in 2K-1C hypertensive rats. These results might be related to its antioxidative effect.

Acknowledgments

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