doi: 10.4149/gpb_2019034

Effects of nicorandil on vascular and renal dysfunctions in adenine-induced nephropathy: Possible underlying mechanisms

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Abstract. Vascular dysfunctions in chronic kidney disease (CKD) include endothelial dysfunctions and vascular calcification (VC). In the present study, we examined the possible protective effect of nicorandil (potassium channel opener) on renal and vascular dysfunctions in a rat model of adenine-induced nephropathy and its underlying mechanisms. Thirty-four male Sprague-Dawley rats were randomly allocated into 3 groups: Control group, Adenine group (animals received high-adenine diet for 4 weeks), and Nicorandil group (animals received adenine for 4 weeks and nicorandil 1 mg/kg *per* oral for 4 weeks). The results showed significant reduction in the body weight, heart rate (HR), hemoglobin contents, serum Ca^{2+} and reduction in the expression of mRNA of endothelial nitric oxide synthase (eNOS) and nuclear factor erythroid related factor 2 (nrf2) genes in aortic tissues with significant increase in arterial blood pressure (ABP), serum creatinine, blood urea nitrogen (BUN), plasma renin activity (PRA), K^+ and phosphate (PO_4^{3-}), urinary albumin excretion (UAE) and aortic VC in Adenine group compared to normal group ($p < 0.05$). On the other hand, coadminsitration of nicorandil caused significant improvement in the studied parameters compared to Adenine group ($p < 0.05$). We concluded that nicorandil has a potential protective effect against the vascular and renal impairment induced by adenine, which might be due to attenuation of vascular calcifications, activation of *Nrf2* and *eNOS* genes in aortic tissues.

Key words: Nicorandil — Adenine — Vascular calcifications — Kidney — eNOS — Nrf2

Abbreviations: ABP, arterial blood pressure; CKD, chronic kidney disease; CV, cardiovascular; eNOS, endothelial nitric oxide synthase; ESRD, end stage renal disease; Hb, hemoglobin; HR, heart rate; NO, nitric oxide; nrf2, nuclear factor erythroid related factor 2; PRA, plasma renin activity; RIA, radioimmune assay; UAE, urinary albumin excretion; VC, vascular calcifications.

Introduction

Chronic kidney disease (CKD) is a worldwide health problem that is increasing in both developed and developing countries (Levey et al. 2007). The disease is insidious over many years, and may result in end-stage renal disease (ESRD) that needs enhancement of kidney function by dialysis or transplantation, with poor patient outcomes (Chang et al. 2015), but till now the determinants of progression of CKD to ESRD are poorly understood (Diwan et al. 2018). One of the serious complications of CKD patients is the development of cardiovascular disease, which is considered the main cause of morbidity and mortality in these patients (López-Novoa 2012; Nguy et

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al. 2013). Cardiovascular mortality is 10–30 times higher in patients with CKD compared with age matched controls with normal kidney function (Sarnak et al. 2003) and accounts for approximately 50% of all deaths in patients on dialysis and in recipients of renal transplants (Nguy et al. 2012). Moreover, the majority of CKD patients die from cardiovascular (CV) events before developing end-stage renal disease (Sarnak et al. 2003). Also, the prevalence of hypertension is 87–90% patients with CKD patients (Levin 2003), but the details of interactions between the kidneys and the cardiovascular system remain unclear.

Adenine-induced nephropathy produces progressive CKD with cardiovascular changes, mimicking more closely the pathophysiology of human CKD (Lavi-Moshayoff et al. 2010). The mechanisms underlying renal dysfunctions in adenine-induced nephropathy include oxidative stress, inflammatory reaction, apoptosis and fibrosis (Askari et al. 2016; Diwan et al. 2018). The vascular dysfunctions in adenine-induced nephropathy that leads to development of hypertension might include endothelial dysfunctions and vascular calcifications (VC). VC are positively correlated with cardiovascular morbidity or mortality in patients with CKD and ESRD (Chang et al. 2017) and reported in early stages of CKD (Porter et al. 2007; Sigrest et al. 2007) and in young dialysis patients (Oh et al. 2002) and is considered a real challenge for management of ERSD patients (Franczyk-Skóra et al. 2013). The mechanisms underlying VC are complex in which oxidative stress play a major role (Yamada et al. 2012; Chang et al. 2017). Also, endothelial dysfunction in CKD leads to significant depletion of nitric oxide (NO) produced by endothelial NO synthase (eNOS) with elevation in vasoconstrictors such as endothelin (Peng et al. 2013; Ali et al. 2015). Nuclear factor-erythroid-2-related factor 2 (Nrf2) is a transcription factor that regulates the expression of more than 200 antioxidant and cytoprotective genes (Vomund et al. 2017). Several studies demonstrated decrease in Nrf2 activity and the expression of its targets antioxidant genes in animal models of CKD (Aminzadeh et al. 2013; Soetikno et al. 2013; Trujillo et al. 2013). Therefore, we hypothesized that activation of *nrf2* genes could improve the renal and vascular dysfunctions in adenine-induced nephropathy.

Nicronadil, 2-[(pyridin-3-ylcarbonyl) amino-ethyl nitrate, is a clinically proven anti-anginal agent that causes vasodilatation by opening ATP-dependent K^+ channels and also releasing NO (Barbato 2005). Apart from the proven efficacy in treating CKD, nicorandil is also beneficial in patients with ESRD undergoing hemodialysis, by reducing oxidative damage (Ishii et al. 2007). In addition to these protective effects, nicorandil has been recently reported to have benefit in experimental renal disease including in the renal injury induced by ischemia-reperfusion (Shimizu et al. 2011) and glomerulonephritis (Sudo et al. 2009). Nicorandil also significantly alleviated chronic renal injury of remnant kidney model of chronic renal injury by targeting podocytes and macrophages (Tamura et al. 2012), which indicates the potential protective effects of nicorandil on chronic renal failure and associated cardiovascular complication specially VC. Recently, Ravindran et al. (2017a) demonstrated that the cardioprotective effect of nicorandil against ischemia/reperfusion injury was abolished by VC, and Ravindran et al. (2017b) found that nicorandil ameliorates the oxidative stress and mitochondrial dysfunctions in brain tissues of murine model of VC. Therefore, we hypothesized that nicorandil could attenuate the renal and vascular dysfunctions by attacking VC and vascular antioxidant genes such as *nrf2* and *eNOS*. So, the present study was conducted to investigate the possible protective effect of nicorandil on the vascular and renal dysfunctions in a rat model of adenine-induced CKD and its possible underlying mechanisms.

Materials and Methods

Experimental animals

Thirty-four male Sprague-Dawley rats weighing 180–200 g (8–10 weeks old) were individually housed in standard cages at Department of Pharmacology, Mansoura Faculty of Medicine, maintained on a 12-h light-dark cycle and fed on standard diet and water *ad libitum*. All experimental procedures were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals by National Academy of Sciences (USA) and approved by our local committee of institutional review board (IRB).

Study design

Animals were randomly allocated into 3 main groups:

1. Control group (n = 10): rats received normal standard chow with 0.5 ml saline i.p. daily for 4 weeks
2. Adenine group (n = 12): rats received high-adenine diet and 0.5 ml saline i.p. daily for 4 weeks
3. Nicorandil group (n = 12): rats received high adenine diet and nicorandil 1 mg/kg dissolved in 0.5 ml saline for 4 weeks (Sudo et al. 2008).

Induction of tubulointerstitial nephropathy by high adenine diet rat model

One week before the start of the experiment, rats from all studied groups fed on normal standard chow. Then, rats of Control group continued on the same normal chow and rats of Adenine and Nicorandil groups fed on high adenine diet

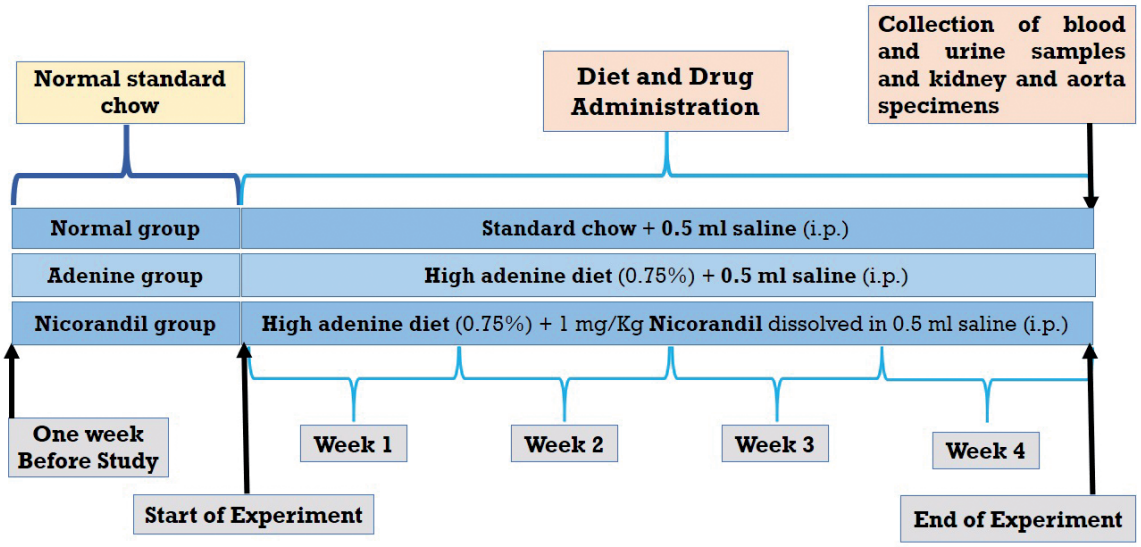


Figure 1. Scheme of experimental study design.

for 4 weeks (see Fig. 1). Table 1 shows the composition of the normal standard chow and high adenine diet.

Measurement of body mass, heart rate and mean arterial blood pressure

By the end of the experiment, the body mass of rats in grams was measured. Heart rate and mean arterial blood pressure were measured by non-invasive indirect rat tail pressure meter (Panlab Harvard apparatus, LE5001, Spain LE 5001) at Department of Medical Physiology, Mansoura Faculty of Medicine. The rats were allowed to adapt the method 3 days before initiation of the measurement. Unanaethetized rat was placed in a restrainer of appropriate size and its tail was placed inside a tail cuff and was allowed to equilibrate for few minutes till its heart rate is settled. Three consecutive reading was obtained for each rat and the mean of these readings was calculated.

Collection of blood and urine samples and harvesting kidney and aorta specimens

The rats were placed in metabolic cages for collection of 24 h and blood samples were obtained from the ophthalmic venous plexus using a fine-walled Pasteur pipette under halothane anesthesia (rats were anesthetized for one minute by placing it in a glass container with piece of cotton soaked with halothane). The blood sample was then centrifuged and the serum was stored at –20°C until biochemical analysis.

By the end of the experiment, rats were sacrificed using high dose of sodium thiopental (120 mg/kg i.p.), then the thorax and abdomen were rapidly opened and kidneys were harvested, bisected and fixed into formalin 10% for histopathological study and the thoracic and abdominal aorta was dissected and divided into 2 parts; one part was fixed in 10% neutral phosphate-buffered saline (PBS) with 4% paraformaldehyde over-night for histological study and the second one was rapidly frozen and stored at liquid nitrogen –170°C for molecular study.

Assay of serum and urinary biomarkers

Serum creatinine and electrolytes (Na^+, K^+, PO_4^-, Ca^{2+}) and hemoglobin contents, were measured by specific kits according to manufacturer instructions. Kits were purchased from Diamond Diagnostics, Egypt (serum creatinine), Stanbio Lab., Texas, USA (urea and uric acid), and Biomed Diagnostics-EGY-Chem., Egypt (Na^+, PO_4^-, Ca^{2+}). Assay of urinary albumin excretion was achieved using specific kits (Fortress Diagnostics Limited Unit 2C Antrim Technology Park, Antrim

Table 1. Composition of standard chow and high adenine diet (for each 100 g pellet)

Standard Chow	High adenine diet
0.92% phosphorus	0.92% phosphorus
1.06% calcium	1.06% calcium
2.5% protein (casein)	2.5% protein (casein)
4.7% fats	4.7% fats
5% cellulose	5% cellulose
75.3% sugars	74.6% sugars
	0.75% adenine

BT41 1QS, United Kingdom). Plasma renin activity (PRA) was determined by RIA kit and expressed as ng AngI/ml/h.

Histopathological examination of kidney

Kidney specimens were dehydrated in alcohol series, cleared in toluene and embedded in paraffin. The paraffin-embedded kidney sections stained with hematoxylin and eosin (H&E) and examined under a photomicroscope (Nikon Eclipse, Japan). H&E stained sections were examined for glomerular lesions (glomerulosclerosis, segmental collapse, proliferation, and mesangial expansion) and tubulointerstitial lesions (leukocyte infiltration, fibrosis, tubular dilatation, or atrophy). The degree of kidney damage was scored as follows: 0, normal; grade 1, <10%; grade 2, 10–24%; grade 3, 25–49%; grade 4, 50–74%; and grade 5, 75–100% (Nicholas et al. 2012).

Histological study of aorta

About 2–3 mm rings of the aorta were embedded upright in paraffin blocks, so that every aortic section comprises on average eight to nine cross-sections (minimum six) at different sites along the vessel. Sections (3–4 μm) some slides were stained with H&E stain. Other slides were stained for calcification with Von Kossa's method. In brief, the sections were first deparaffinized and then re-hydrated to distilled water. Then they were placed in 1% silver nitrated and exposed to ultraviolet light (20 min). Afterwards they were placed in 5% sodium thiosulfate (2 min) and finally counterstained with nuclear Fast Red (5 min). Areas of calcification appeared as dark brown regions in the medial wall of the aortas.

Measurement of endothelial nitric oxide synthase (eNOS) and Nrf2 in aortic tissues RNA extraction and cDNA synthesis for aortic tissues

Total RNA from aortic tissues was isolated by the disruption of 50–100 mg tissues in 1 ml of Trizol, according to the manufacturer's instructions (Invitrogen Corporation, Grand Island, New York, USA). RNA was quantified spectrophotometrically, and its quality was determined by agarose gel electrophoresis and ethidium bromide staining. Only samples that were not degraded and showed clear 18 S and 28 S bands under ultraviolet light were used for real time RT-PCR. Reverse transcription was done using 1 μg total RNA and a cDNA kit (high-capacity cDNA archive kit, Applied Biosystems, USA).

Primer and probe sequences of tested genes

The primer sequences for the tested gene, *eNOS* were as follows: forward, 5= GGACCCAAGTTTCCTCGAGTAA-3=; reverse, 5=GGATCCCAAGCAGCGTCTT-3=; probe, 5=FAM-CAGCAT CACCTACGATACCCTCAGTGCA-TAMRA-3, nrf2: forward, 5'-GCTATTTTCCATTCCCGAGTTAC-3'; reverse, 5'-ATTGCTGTCCATCTCTGTCAG-3'; and GAPDH: forward, 5'-TATCGGACGCCTGGTTAC-3'; reverse, 5'- CTGTGCCGTTGAACTTGC-3-' (Hussein et al. 2016).

Real-time PCR reaction

The reaction was performed in a total volume of 50 μl, which comprised 25 μl of a mixture of 1_ TaqMan® Universal PCR with 25 μl of 20_ TaqMan® Gene Expression Assay Mix, plus 22.5 μl of cDNA diluted in RNase-free water. The cycling parameters were as follows: initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation 95°C for 15 s, annealing at 60°C for 1 min, and extension at 72°C for 1 min. Data analysis was carried out using ABI prism 7000 by the equation $2^{d}CT$ (Livak and Schmittgen 2001).

Statistical analysis

SPSS (Statistical package for social science) program version 17.0 was used for doing statistical analysis. Data were presented in the form of mean ± standard deviation (SD). One-way ANOVA (analysis of variance) test with Tukey *post hoc* test were used to find the statistical significance between studied groups. $p \leq 0.05$ is considered significant.

Results

Animal survival

No animals died in Control group, while in Adenine group, 4 animals were died and in Nicorandil group 2 animals were died.

Effects of nicorandil on final body weight, heart rate, mean ABP, Hb in adenine-induced nephropathy

Adenine feeding (0.75%, w/w) for 4 weeks caused a significant reduction in the body weight, Hb and HR with significant increase in mean ABP compared to Control group ($p < 0.001$). On the other hand, co-administration of nicorandil with adenine attenuated these effects as it significantly increased body weight, HR and Hb with significantly decreased the mean ABP when compared to Adenine group ($p < 0.001$) (Table 2).

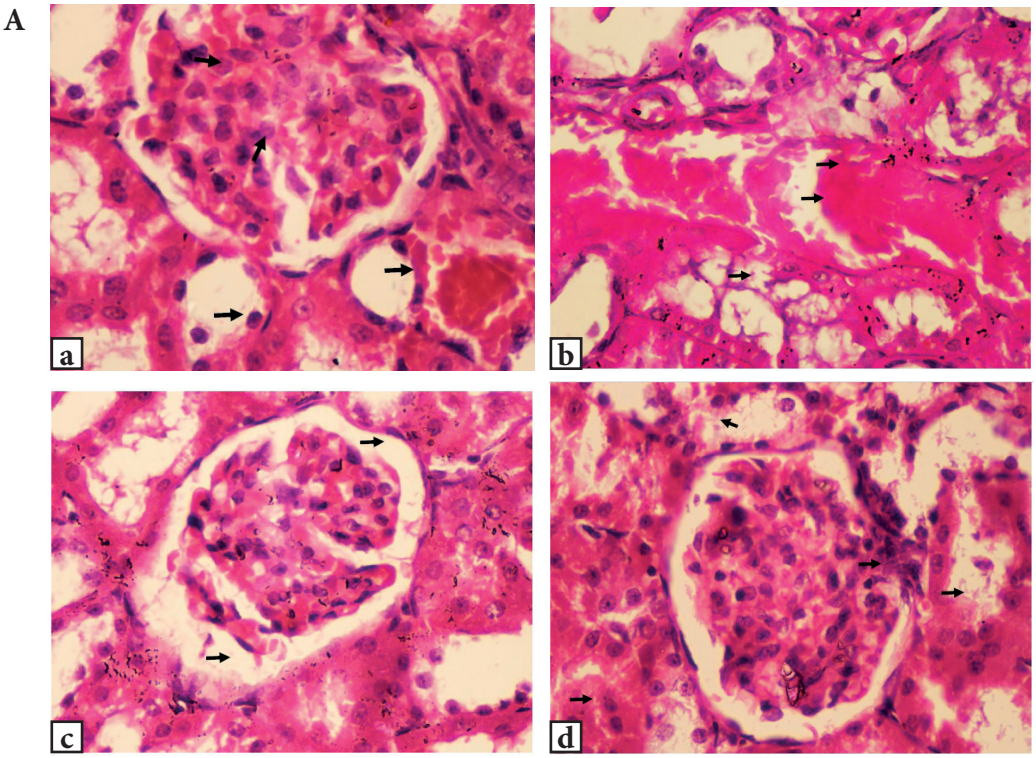
Effects of nicorandil on serum creatinine, serum electrolytes (Na^+, K^+, PO_4^-, Ca^{2+}), urinary protein excretion and PRA in adenine-induced nephropathy

Adenine feeding for 4 weeks caused significant elevation in serum urea, creatinine, Na^+, K^+, PO_4^-, PRA and urinary

Table 2. Effects of treatment with nicorandil on final body weight, heart rate, mean arterial blood pressure (ABP) and hemoglobin level (Hb)

	Control group (n = 10)	Adenine group (n = 8)	Nicorandil group (n = 10)
Final body weight (g)	269.69 ± 10.73	218.4 ± 8.07[a]	231.54 ± 3.26[a,b]
Heart rate (beats/min)	197.30 ± 4.78	166.0 ± 16.20[a]	191.0 ± 7.81[b]
Mean ABP (mmHg)	92.61 ± 3.88	143.2 ± 6.56[a]	126.18 ± 12.99[a,b]
Hb (g/dl)	12.05 ± 0.34	7.33 ± 0.65[a]	8.02 ± 0.46[a,b]

Values are expressed as mean ± SD. $p < 0.05$ was considered significant. [a] $p < 0.0001$ *vs.* Control group, [b] $p < 0.0001$ *vs.* Adenine group (One-way ANOVA followed by Tukey's *post hoc* test for multiple comparison).



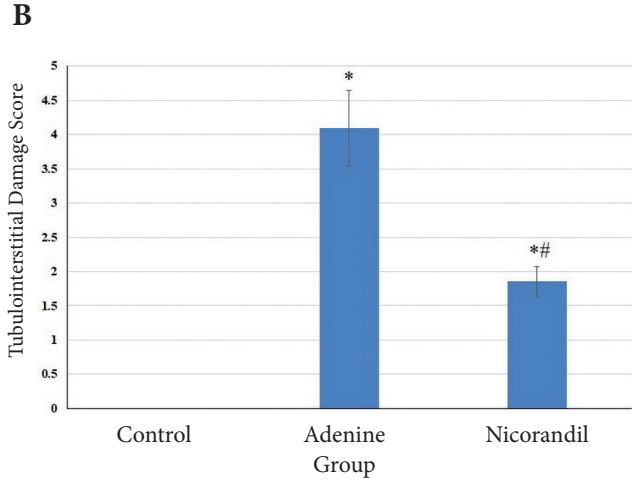


Figure 2. A. Photomicrographs of kidney specimens (H&E, ×400). **a.** Control group. Normal glomerular structure and intact proximal tubular epithelium with normal brush border. **b.** and **c.** Adenine group. Arrow shows interstitial haemorrhage (b) and collapse of renal glomeruli and glomerulosclerosis (c). **d.** Nicorandil group. Hydropic degeneration in proximal tubules. **B.** Effect nicorandil on tubulointerstitial damage score. One-way ANOVA followed by Tukey's *post hoc* test for multiple comparison.* significant *vs.* Control group, # significant *vs.* Adenine group.

protein excretion with significant decrease in serum Ca^{2+} in the Adenine group when compared to Control group ($p < 0.05$). Concomitant administration of nicorandil ameliorated theses effect by causing a significant decrease in serum creatinine, serum Na^+, K^+ and PO_4^-, PRA and urinary protein excretion and a significant increase in serum Ca^{2+} compared to Adenine group ($p < 0.001$) (Table 3).

Effects of nicorandil on kidney morphology

Kidney specimens from Adenine group showed significant increase in the glomerular and tubulointerstitial damage score compared to Control group ($p < 0.001$). Moreover, the degree of renal damage was significantly improved in Nicorandil group compared to Adenine group ($p < 0.001$) (Fig. 2B). Kidneys obtained from Adenine group showed irregular dilated renal tubules with dilated peritubular capillaries and glomerular expansion, and collapse and rarefaction (Fig. 2Aa–Ac), while kidneys obtained from Nicorandil group showing mild tubular injury in the form of few apoptotic cells and regeneration in the form of irregular dilated tubules and solid tubules and mesangial proliferation (Fig. 2Ad).

Effects of nicorandil on structure of aorta and vascular calcification in aortic tissues

H&E staining showed normal structure of aorta in normal rats, while adenine-treated rats showed disorganized aortic endothelium and loss of elastic tissues. Treatment with nicorandil restored the intact endothelium and maintained the wavy collagen fibers (Fig. 3a–f). In Adenine group showed marked increase in VC in aorta by Von Kossa compared to Control group. Concomitant administration of nicorandil significantly decreased these calcifications compared to Adenine group (Fig. 4a–f).

Effects of nicorandil on expression of eNOS and nrf2 genes in aortic tissues

Adenine group showed significant decrease in expression of *eNOS* and *nrf2* genes in aortic tissues by real time PCR compared to Control group ($p < 0.001$). Concomitant administration of nicorandil with adenine significantly increased the expression of both *eNOS* and *nrf2* genes compared to Adenine group ($p < 0.001$) (Fig. 5).

Discussion

In the present study, we found that administration of high adenine diet for 4 weeks caused significant deteriorations of the kidney functions and morphology, which was associated with elevated blood pressure, PRA and aortic VC with reduction in expression of *nrf2* and *eNOS* genes in aortic tissues. On the other hand, administration of nicorandil (vasodilator and K_{ATP} channel opener) resulted in significant improvement in the vascular and renal disturbances induced by adenine.

In the present study, we used a well-established animal model of chronic nephropathy. The mechanisms underlying the pathophysiology of adenine-induced nephropathy include precipitation of dihydroxyadenine in renal tubules causing tubular cell injury and apoptosis, interstitial inflammatory cell infiltration and tubulointerstitial fibrosis resulting in progressive deterioration of kidney functions and CKD (Tamura et al. 2009; Tanaka et al. 2009). In agreement with previous studies, we found that high adenine intake (0.75% w/w) for 4 weeks caused significant reduction in the body mass and Hb contents of rats (Nguy et al. 2012; Ali et al. 2014, 2015; Inami et al. 2014; Hussein et al. 2016) suggesting impairment of RBCs formation due to impairment of kidney functions and deficiency of erythropoietin secretion. In line

Table 3. Effects of treatment with nicorandil on serum creatinine, serum electrolytes (PO_4, K^+, Na^+, Ca^{2+}), urinary protein excretion and plasma renin activity

	Control group ($n = 10$)	Adenine group ($n = 8$)	Nicorandil group ($n = 10$)
Serum creatinine (mg/dl)	0.79 ± 0.03	1.87 ± 0.27[a]	1.05 ± 0.08[a,b]
Serum Ca^{2+} (mg/dl)	7.68 ± 0.44	5.93 ± 0.50[a]	7.25 ± 0.33[b]
Serum PO_4 (mg/dl)	5.14 ± 0.14	18.16 ± 0.75[a]	9.39 ± 0.57[a,b]
Serum Na^+ (mEq/l)	136.53 ± 5.33	169.8 ± 5.20[a]	148.36 ± 5.64[a,b]
Serum K^+ (mEq/l)	4.22 ± 0.59	6.26 ± 0.58[a]	5.03 ± 0.18[a,b]
Urinary protein excretion (g/24 h urine)	0.13 ± 0.02	1.41± 0.26[a]	0.21 ± 0.02[b]
Plasma renin activity (AngI/ml/h)	0.22 ± 0.03	5.5 ± 0.48[a]	4.12 ± 0.32[a,b]

Values are expressed as mean ± SD. $p < 0.05$ was considered significant. [a] $p < 0.0001$*vs.* Control group, [b] $p < 0.0001$ *vs.* Adenine group (One-way ANOVA followed by Tukey's *post hoc* test for multiple comparison).

with this hypothesis, Rahman et al. (2018) demonstrated that administration of oral dose with adenine at 50 mg/kg for 28 days caused significant reduction in haematocrit value and plasma erythropoietin levels with significant increase in serum creatinine. On the other hand, coadministration of nicorandil with adenine prevented the reduction in body mass and Hb concentrations. However, the elevation in Hb contents in Nicorandil group was minimal and not reversed completely the drop in Hb contents to the normal level suggesting the effect of nicorandil on erythropoietin secretion is partial not complete.

Also, in agreement with previous studies, we found that high adenine intake caused marked elevation of mean arterial blood pressure (ABP) and PRA with significant reduction in HR (Kim et al. 2013; Zhao et al. 2014; Hussein et al. 2016). The significant increase in PRA suggests involvement of renin angiotensin system (RAS) in elevation of ABP. In addition, the significant reduction in HR suggests intact ba-

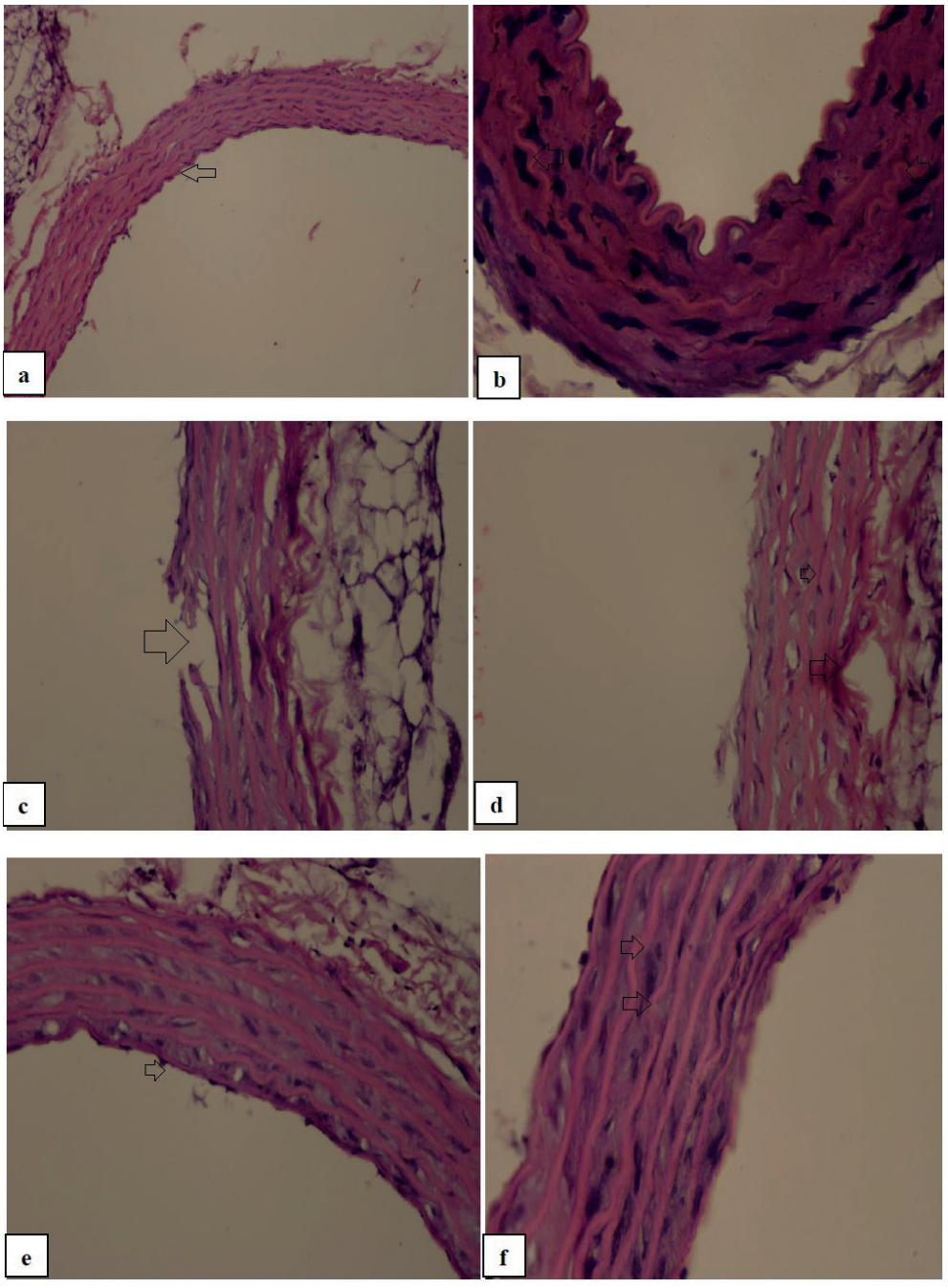


Figure 3. Specimen of aortic tissues showing intact aortic endothelium (**a**) and wavy collagen fibers (**b**) in Control group, disrupted aortic endothelium (**c**) with loss wavy collagen fibers (**d**) in Adenine group and intact endothelium of aorta (**e**) and wavy collagen fibers (**f**) in Nicorandil group. H&E, ×200 (a), ×400 (b–f).

roreceptor reflex in rats with adenine-induced-nephropathy. However, a study by Nguy and his colleagues (2012) demonstrated in a rat model of adenine-induced nephropathy, significant reduction in PRA and HR in adenine-treated rats, suggesting no role for RAS in adenine-induced nephropathy and impairment of baroreceptor reflex in adenine-induced nephropathy. This discrepancy in results might be due to the duration of adenine intake. In our study, adenine was given for 4 weeks and in Nguy et al. (2012) study, adenine was given for a longer time (11 weeks). On other hand, pretreatment with nicorandil in adenine-treated rats caused significant improvement in ABP and HR with minimal reduction in PRA. The dose of nicorandil in the present study was chosen because it was not associated with any change in the blood pressure or serum aldosterone or PRA (Sudo et al. 2008).

Regarding the renal effects of adenine on kidney functions and morphology, the present study showed significant increase in serum creatinine and urinary protein excretion in Adenine group suggesting impairment of glomerular and tubular kidney functions. Moreover, the histopathological examination showed significant damage in the renal tubules, glomerulosclerosis and interstitial inflammatory cells and fibrosis. These findings are in agreement with those reported by previous studies (Aminzadeh et al. 2013; Kim et al. 2013;

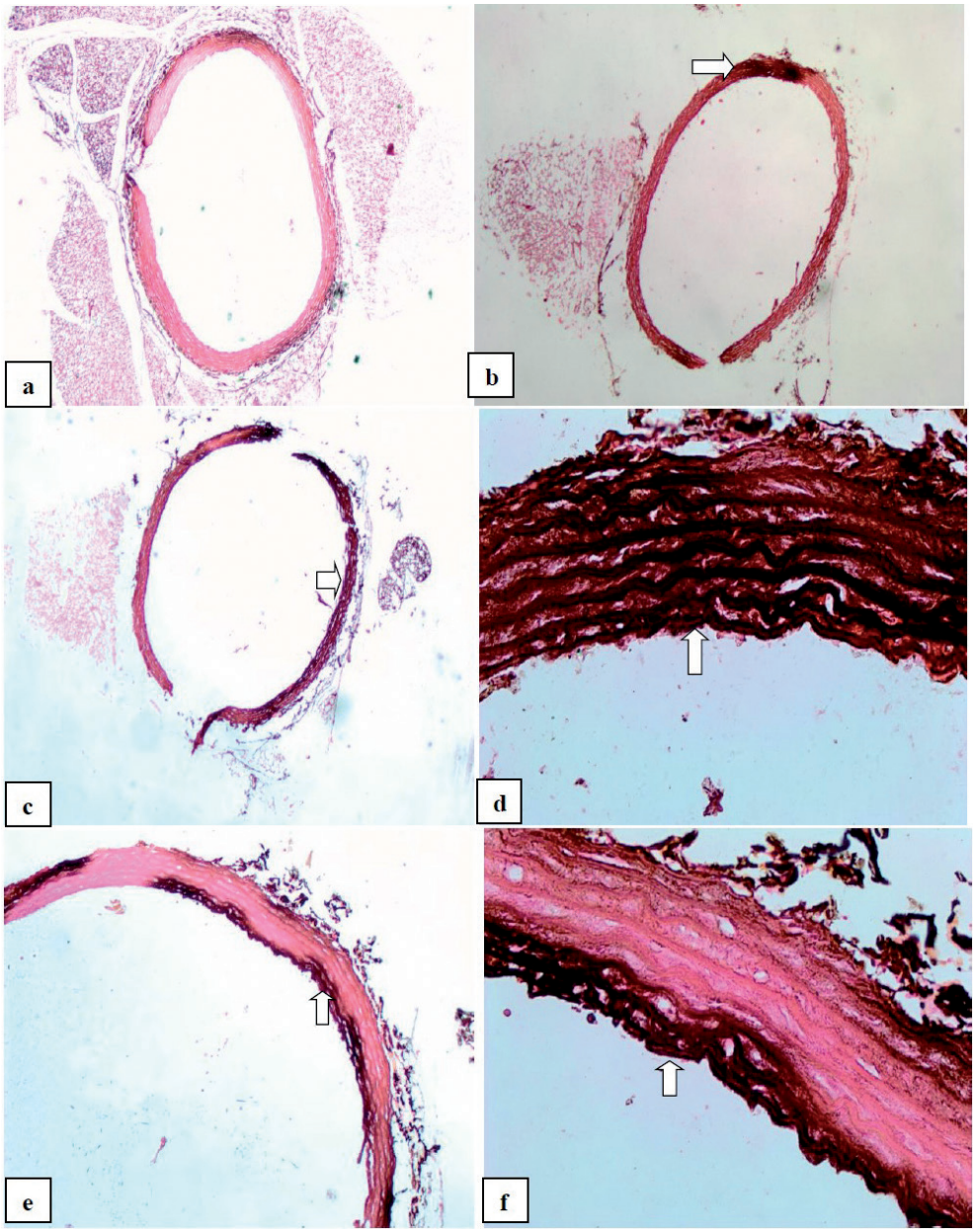


Figure 4. Specimen of aortic tissues showing no vascular calcifications by von Kossa stain in Control group (**a**), marked aortic vascular calcifications in Adenine group in patches (**b**), half of the aorta (**c**) and with high power (**d**) and minimal calcifications in Nicorandil group (**e, f**). ×40 (a, b, c, e), ×400 (d), ×200 (f).

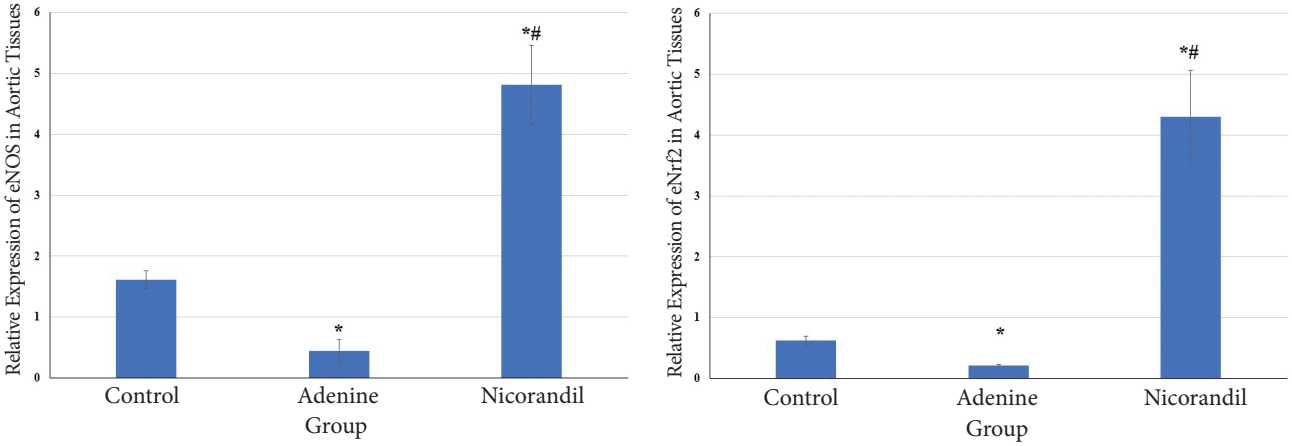


Figure 5. Relative expression of mRNA of eNOS (**A**) and Nrf2 (**B**) in aortic tissues in different groups. One-way ANOVA followed by Tukey's *post hoc* test for multiple comparison.* significant *vs.* Control group, # significant *vs.* Adenine group.

Ali et al. 2014; Hussein et al. 2016). Moreover, we found in the present study normalization of the kidney functions and morphology by co-administration of nicorandil with adenine suggesting renoprotective role for nicorandil against adenine-induced nephropathy. This renoprotective effect for nicorandil was shown in rat models of renal I/R injury (Shimizu et al. 2011) and partial unilateral ureteral obstruction (Ozturk et al. 2017). In addition, Tamura et al. (2012) demonstrated the renoprotective effects of nicorandil on a rat remnant kidney model of chronic kidney disease. Moreover, Shiraishi et al. (2014) demonstrated that nicorandil might synergize with renoprotective effects of enalapril in chronic kidney disease.

In the present study, we found aortic calcifications in Adenine group. These results are in line with previous studies (Subhash et al. 2015; Ravindran et al. 2017a, 2017b). VC are not passive process, but it is an active process, in which the vascular cells are transformed to osteoblast like cells. Calcium and phosphorous levels in blood and in tissues are the key players of VC seen in patients with chronic kidney disease (Patsalas et al. 2007). In the present study, we found electrolyte changes in the form of elevated serum K^+, Na^+ and PO_4 with significant decrease in serum Ca^{2+} caused by adenine administration for 4 weeks. These electrolytes disturbances are in line with previous studies (Jia et al. 2013; Kim et al. 2013; Zhao et al. 2014; Hussein et al. 2016), and due to impairment of tubular functions by adenine administration (Ikeda et al. 2010; Kim et al. 2013). Hypernatremia shown in the present study could be explained by reduction in the filtered load of Na^+ due to reduction of glomerular filtration (GFR) and activation of renin angiotensin system, which enhance Na^+ retention. Also, we found in this work significant improvement in disturbed electrolyte imbalance induced by adenine *via* nicorandil administration suggesting renoprotective role for nicorandil; a mechanism by which nicorandil prevented VC.

Previous studies suggested a relation between mitochondrial oxidative stress and VC. Ravindran et al. (2017b) demonstrated that high adenine diet (0.75%) for 4 weeks (28 days) caused significant elevation in the calcium, phosphorus product in the brain with enhanced oxidative stress in the cytoplasm and mitochondria of cerebral neurons (elevated TBARS and decline in the antioxidant enzymes) suggesting a relation between oxidative stress and VC. Mitochondrial dysfunction is the common pathological features in the diseases related to calcification (Ahn et al. 2010). Mitochondria play significant role in process of VC where insoluble calcium phosphate salts are deposited in the mitochondrial matrix (Millane et al. 1994). Oxidative stress activates several endogenous cytoprotective and cellular antioxidant proteins and enzymes that limit the degree of tissue injury and dysfunction. Nrf2 factor is a transcription factor that regulates the basal activity and the induction of numerous genes that encode various antioxidants and phase 2 detoxifying enzymes (Kobayashi et al. 2006; Wakabayashi et al. 2010). In the present study, we found reduction in the expression of *nrf2* in aortic tissues at the levels of mRNA by Adenine group suggesting the high adenine diet impaired the activity of *nrf2* in aortic tissues. Previous studies reported downregulation of *nrf2* in adenine-induced renal failure (Aminzadeh et al. 2013; Hussein et al. 2016).

Moreover, Subhash and others (2015) demonstrated a direct link between the arterial calcification in major arteries and vascular brain disease where common link is believed to be mitochondrial K_{ATP} channel. The link between the ATP sensitive K_{ATP} channel and ROS clarifies the intracellular mechanism of vascular calcification and may allow exploration of mitochondrial K_{ATP} channel modulator and

antioxidants as therapeutic agents for vascular calcification. In the present study, nicorandil (K_{ATP} channel opener) pretreatment caused upregulation of nrf2 in aortic tissues obtained from rats treated with high adenine diet at the level of mRNA suggesting improvement of oxidative stress in aortic tissues might reduce the aortic calcifications. The antioxidant action of nicorandil was demonstrated in a rat model of remnant kidney disease (Tamura et al. 2012; Shiraishi et al. 2014), rat model of renal I/R injury (Ozturk et al. 2017) and brain injury after cardiac arrest in pigs (Zhu et al. 2018).

Endothelial dysfunctions in chronic kidney disease was demonstrated in high adenine rat model and occurred in the form of imbalance between the vasodilators (serum nitric oxide) and vasoconstrictors (serum endothelin) (Peng et al 2013; Ali et al. 2015). In the present study, we found down-regulation of eNOS that enhanced the production of vasodilator (nitric oxide) in aortic tissues in adenine-treated group, indicating that adenine impaired the vasodilator responses of the vascular endothelium. Nicorandil pretreatment caused significant increase in the expression of *eNOS* in the aortic tissues of adenine-treated and normal rats, suggesting that upregulation of eNOS might be a potential protective mechanism for nicorandil against adenine-induced VC. To the best of our knowledge, this is the first study that examined the role of eNOS as a potential mechanism for the improvement of adenine with nicorandil. However, Date et al. (2005), Hongo et al. (2005) and Tashiro et al. (2015) showed that nicorandil increased endothelium-derived NO production by activation of eNOS, inhibited endothelial cell death, and exerted anti-inflammatory and antioxidative effects and this may contribute to the protective effect of nicorandil.

Conclusions

High adenine diet (0.75%w/w) for 4 weeks caused significant deteriorations of kidney functions and morphology, and aortic VC with downregulations of nrf2 and *eNOS*. Nicorandil (K_{ATP} channel opener) ameliorated the vascular and renal harmful effects of adenine, which might be due to improvement of the renal glomerular and tubular functions, correction of electrolyte imbalance and upregulation of *nrf2* and *eNOS* in aortic tissues.

Conflict of interest. Authors declared that there was no conflict of interest

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Received: April 29, 2019
Final version accepted: August 8, 2019
First published online: October 7, 2019