



Reno-protective effect of cilostazole in a rat model of chronic kidney disease

11988

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Background: The Chronic kidney disease (CKD) is a common health burden worldwide. Progressive deterioration of kidney function in chronic kidney disease (CKD) is mediated by systemic and renal hypertension, oxidative stress, inflammation, and renal fibrosis. Renin-angiotensin blockade is commonly used to retard CKD progression. Cilostazole (phosphodiesterase III enzyme inhibitor) has been shown to exert antioxidant, antiinflammatory and anti-fibrotic effects. This study was designed to explore the possible protective effects of cilostazole versus valsartan against CKD induced by subtotal 5/6 nephrectomy in rats and to explore the underlying mechanisms. Just after surgery, cilostazole (50 and 100mg/kg/day) and valsartan (30mg/kg/day) were given daily by gavage for 10 weeks. The untreated CKD rats exhibited hypertension, tubular and glomerular damage, upregulation of proinflammatory, pro-oxidant and pro-fibrotic mediators, also increase renal inducible nitric oxide synthase (iNOS) expression and decrease in renal peroxisome Proliferation-activated receptor gamma (PPAR γ) expression. Cilostazole in its both doses was effective in improving kidney function, decreased renal malondialdehyde (MDA), nuclear factor kappa B (NF- κ B), transforming growth factor beta (TGF- β 1), renal (iNOS) expression in 5/6 nephrectomized rats, in addition to significant increase in renal level of superoxide dismutase (SOD) and renal PPAR γ expression when compared to diseased group. However, better results were observed with a dose of 100 mg/kg/day which was insignificantly different from that of valsartan 30mg/kg pretreated group as a standard therapy. Cilostazole has renoprotective effects in a rat model of CKD. These protective effects are mediated through exerting antiinflammatory, antioxidant, antifibrotic actions, increasing renal PPAR γ expression.

Keywords: Cilostazole, Valsartan, Chronic kidney disease, 5/6 Nephrectomy

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Introduction

Chronic kidney disease (CKD) is a major health problem with high mortality rate and high economic burden (Hill et al.,2016). CKD is marked by sustained kidney damage with a significant increase in the risk of cardiovascular (CV) effect being irreversible and progressive condition (Romero-González et al.,2020). Cardiovascular events are not only restricted to end stage renal disease, but early CKD stages are

also associated with variable degrees of heart failure (Sárközy et al.2018).

The main pathological processes in CKD including systemic and glomerular hypertension, oxidative stress, inflammation, and renal fibrosis. The permanent activation of these pathways plays a central role in the initiation and progression of CKD (Tucker et al.,2015). Interactions have been reported between these pathways, meaning oxidative stress causes inflammation and fibrosis through activation of the redox-sensitive pro-



inflammatory signal transduction pathways and by direct toxic effects of reactive oxygen species (ROS) in turn inflammation can cause further oxidative stress making a vicious cycle in which each factor amplifies the other causing chronic renal damage and progressive injury (**Vaziri et al.,2006**).

One effective and well-studied approach in preventing chronic renal injury is blockade of renin angiotensin aldosterone system (RAAS) . The salutary effects of RAAS blockade in slowing progression of CKD are not only caused by its antihypertensive and hemodynamic properties, but also by its ability to reduce oxidative stress, inflammation, and fibrosis (**Siragy and Carey,2010**). .

Angiotensin II exerts a vasoconstrictor effect predominantly on the post- glomerular arterioles, thereby increasing the glomerular pressure. Nonhemodynamic effect of All also important in renal disease progression .These include increased production of reactive oxygen species; upregulation of cytokines, cell adhesion molecules, and profibrotic growth factors; induction of transforming growth factor- β 1 expression, increased synthesis of extracellular matrix proteins; stimulation of plasminogen activator inhibitor-1 production by endothelial and vascular smooth muscle cells; and macrophage activations and infiltrations (**Koniari et al.,2011**).

Hence, it is not surprising that in the current study treatment of CKD animals with valsartan resulted in significant amelioration of renal oxidative stress, inflammation, fibrosis, and ultimately tubulointerstitial fibrosis and glomerulosclerosis.

Despite the broad availability of standard medications morbidity and mortality among CKD patients remained high (**Herzog et al., ,2011**).Therefore, using novel agents that ameliorate or prevent the progression of CKD is urgently needed.

Cilostazole a selective phosphodiesterase III inhibitor, has potent vasodilating and antiplatelet effects (**Jung et al., 2010**). The drug is approved for treatment of intermittent claudication in patients with peripheral vascular

diseases also in coronary arterial diseases (**Chapman et al., 2003**). Cilostazol inhibites PDE III leading to decrease degradation of intracellular 3'-5'-cyclic adenosine monophosphate (cAMP), and 3'-5'-cyclic guanosine monophosphate (cGMP), with subsequent activation of protein kinase cAMP-dependent(PKA), and protein kinase c GMP-dependent (PKG) activation, respectively (**Sholokh and Klussmann, 2021**).

Recently, studies have reported that cilostazole possess pharmacological potentials , such as antiinflammatory (**da Motta and de Brito 2016**), and antioxidant (**Chen et al. 2016**) , antifibrotic (**Han et al. 2019**) effects. These properties are related to its primary mechanism of action as well as its pleiotropic effects. The aim of the current study was to evaluate the renoprotective effects of cilostazol on CKD in rats and explore various mechanisms underlying this effect.

2. Materials and Methods

Experimental animals

Fifty six male Wistar rats weighting 200 to 250 grams were purchased from the Faculty of Veterinary Medicine, Zagazig University, Egypt. Rats were distributed three/cage and kept for one week as acclimatization period. Rats had free access to diet and water. Temperature, humidity and light/dark cycles were kept constant at the following values ($23\pm 2^{\circ}\text{C}$, $60\pm 10\%$ and 12/12 h respectively). The experimental design and animal handling performed in this study were in compliance with the National Institutes of Health for care and use of laboratory animals (NIH publications) "NO.ZU-IACUC/3/F/2/2020, revised 1996" and were approved by Zagazig University's Institutional Animal Care and Use Committee (ZU).

Drugs and chemical

Cilostazole was purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA),valsartan was purchased from Novartis, Switzerland. All other chemicals utilized were of analytical grade obtained from Sigma-Aldrich.

Induction of renal failure by subtotal 5/6 nephrectomy of total renal mass

The rats left kidneys were exposed through a lateral dorsal incision and decapsulated after



anesthetization with sodium pentobarbital (100 mg/kg, ip). with. Then, renal vessels were clamped and both poles (2/3 of the functional kidney mass) were dissected 2 weeks later, total right nephrectomy through lateral dorsal incision was done to achieve 5/6 reduction. Renal failure was significant after 10 days of surgery while heart failure and cardiac remodeling was significant after 10 weeks of surgery. Sham operated rats were anesthetized with just decapsulation of the intact kidneys bilaterally (Švíglerová et al., 2010).

Experimental Design:

Rats were randomly divided into seven groups; each group having eight rats as follows:

1. Control normal group

2. Sham group

3. Diseased group : (5/6 nephrectomy) (Švíglerová et al., 2010)

4. Diseased +vehicle group: 5/6 nephrectomized rats received carboxymethylcellulose at 0.1% (2.5 ml/kg) (**cilostazole vehicle**) once daily by gavage, for 10 weeks, immediately after surgery.

5. Cilostazole 50mg group: 5/6 nephrectomized rats received cilostazole 50 mg/kg; dissolved 0.1% carboxymethylcellulose to enhance the solubility of cilostazole once daily by gavage, for 10 weeks, immediately after surgery (El Awdanet al., 2019).

6. Cilostazole 100mg group: 5/6 nephrectomized rats received cilostazole (100 mg/kg ;dissolved in 0.1% carboxymethylcellulose once daily by gavage, for 10 weeks, immediately after surgery (El Awdanet al., 2019).

7. Valsartan group : 5/6 nephrectomized rats received valsartan (30 mg/kg once daily by gavage, for 10 weeks, immediately after surgery (Suematsu et al., 2018).

At the end of the experimental period, all animals were anesthetized with urethane (1.3 g/kg, ip). Body weights and heart weights (measured by *digital weight scale*) to assess heart /body weight ratio by the following equation [heart weight body weight ratio = heart wt. / body wt. × 100]. Blood samples were obtained from the orbital sinus of fasted rats. Blood samples were centrifuged at 3000 rpm for 10 minutes to get clear sera and the obtained serum was stored rapidly at - 20°C for later bioassay of

serum urea and creatinine which were also measured 10 days after the 5/6 subtotal nephrectomy where blood were obtained from rats tail vein. Hearts was divided into two parts one part was maintained in formalin 10% for histopathological examination, while the other part was kept in liquid nitrogen and stored at - 80°C until homogenised for biochemical evaluation of renal inflammatory mediator nuclear factor kappa B (NFκB), mediator of oxidative stress malondialdehyde (MDA) , antioxidant superoxide dismutase (SOD), fibrotic mediator transforming growth factor beta (TGF-β1) and renal expressions of inducible nitric oxide synthase (iNOS) and Peroxisome proliferation-activated receptor gamma (PPARγ) .

Measurement of blood pressure:

Blood pressure was monitored using a tail cuff blood pressure measuring system (Harvard Apparatus Ltd, Edenbridge, Kent, England). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured 10 days and 10 weeks post nephrectomy.

Colorimetric assays of serum creatinine , serum urea, renal SOD and MDA levels:

Serum creatinine and serum urea were measured using commercially available quantitative colorimetric assay kits obtained from Bio-diagnostic Co. (Egypt). All procedures were performed according to the manufacturer's instructions. SOD and MDA Colorimetric assay Kits (Bio-diagnostic company, Egypt) were used for measurement of renal SOD and MDA levels .

Enzyme linked immunosorbent assays (ELISA) of renal NFκB and TGF-β1

Renal levels of NFκB (Bio-diagnostic company, Egypt), TGF-β1 (Boster Biological Technology, Pleasanton CA, USA), were measured using rat ELISA kits. All procedures were performed according to the manufacturer's instructions.

Quantitative real time PCR for renal iNOS and PPARγ

Renal expressions of iNOS and PPARγ were assessed by quantitative real time PCR were used in which Total RNA were extracted from heart tissue using using quantitative real-time



PCR according to the manufacturer's instructions using the Qiagen tissue extraction kit (Qiagen, USA) and were reverse transcribed into cDNA using a high-capacity cDNA reverse transcription kit (Fermentas, USA). Then, using Applied Biosystems with Step One TM software version 3.1 (USA), amplification and analysis of real-time qPCR product were conducted.

The primer sequence of the gene under study include:

iNOS forward primer 5'-CACCACCCTCCTGTTCAAC-3' ,
reverse primer 5'-CAATCCACAACCTCGCTCCAA-3'.
PPAR γ forward primer 5'-TGATATCGACCAGCTGAACC-3',
reverse primer 5'-GTCCTCCAGCTGTTCGCCA-3'.

Histological analysis

Remenant kidneys tissue were harvested, fixed in 10% paraformaldehyde, embedded in paraffin, and cut into 5- μ m sections. Sections were stained with Masson's trichrome staining (**Polysciences , Inc.,USA**) in standard histological manner.

Statistical analysis

Data were expressed as mean \pm standard error of the mean (SEM). Ordinary one-way analysis of variance (ANOVA) was used to detect statistical differences among groups followed by Post-Hoc (least significant difference "LSD") tests as designated by Armitage & Berry (1994). Differences were considered significant at a $P < 0.05$. Data analysis was conducted using SPSS, Version 26 Software .

3.Results

- **Mortality during the study**
 - ❖ **In the diseased group, two rats of initial eight rats died in the first week during follow up . One animal died in cilostazole 50mg group (initial eight rats) also in the first week. No mortality was observed in other groups. Mortality was either spontaneously or due to surgery related complication. Dead rats were replaced.**
 - ❖ **Because there were no significant differences between the results coming from control normal group and sham**

group also those coming from diseased group and diseased group receiving carboxymethylcellulose (cilostazole vehicle); only results obtained from sham group and diseased group were included in our study.

Effect of cilostazole (50 and 100 mg/kg/d) on serum creatinine and serum urea levels at different time points

Baseline mean serum urea and creatinine levels were not significantly ($P > 0.05$) different among all groups . Ten days after surgery, serum urea and creatinine levels were significantly ($P < 0.05$) higher in all groups compared to sham group. Ten weeks after surgery, serum urea and creatinine levels were significantly ($P < 0.05$) increased in the diseased group compared to sham group. Cilostazole treatment in its two doses caused significant ($P < 0.05$) decrease in mean serum urea and creatinine levels as compared to diseased group. Better results were obtained with cilostazole (100 mg/kg/day) pretreated group, that was insignificantly ($P > 0.05$) different from valsartan (30 mg/kg/day) pretreated group as a standard therapy as presented in table 1 .



Table 1: Effect of cilostazole (50 and 100mg/kg/day) on kidney functions at different time points

Interval	Groups (n=8) parameter	Sham- group	Diseased - group	Treated groups		
				Cilostazole-50mg/kg	Cilostazole-100 mg/kg	Valsartan 30mg/kg
Baseline	Serum urea (mg/dl)	29.22 ±1.08	29.97 ±1.13	28.25 ± 1.23	26.77 ±1.08	27.92 ± 1.41
	Serum creatinine (mg/dl)	0.23 ±0.008	0.24 ±0.008	0.22 ± 0.006	0.23 ± 0.005	0.25 ± 0.01
10 days after surgery	Serum urea (mg/dl)	29.91 ±1.33	109.27±2.37 ^A	105.05±2.66 ^B	103.03 ± 2.54 ^B	102.82±2.83 ^B
	Serum creatinine (mg/dl)	0.26 ±0.018	0.87 ±0.021 ^A	0.85 ±0.02 ^B	0.84 ±0.02 ^B	0.84 ±0.031 ^B
10 weeks after surgery	Serum urea (mg/dl)	27.67 ± 1.47	118.96±3.22 ^A	62.66 ±2.71 ^B	50.06 ± 3.20 ^C	43.83±1.40 ^C
	Serum creatinine (mg/dl)	0.24 ± 0.015	1.64 ± 0.022 ^A	0.58 ± 0.03 ^B	0.40 ± 0.02 ^C	0.40 ± 0.02 ^C

Data represent means ± SE; n, number of rats ; significance p< 0.05

^A Significantly different from sham group

^B Significantly different from diseased

^C Significantly different from cilostazole 50mg group

Values with in the same row with different superscript capital letters are significantly (p<0.05) different

Statistical comparisons were carried out using one-way ANOVA followed by post-hoc tests using LSD method

Effect of cilostazole (50 and 100mg/kg/day) on SBP and DBP at different time points

Baseline mean SBP and DBP measurements were not significantly (P>0.05) different



among all groups . Mean SBP and DBP were significantly ($P<0.05$) higher in the diseased group compared to sham group ten days and ten weeks after surgery.

Cilostazole treatment caused non significant ($P>0.05$) decrease in mean SBP and DBP in its two doses compared to the diseased group ten days and ten weeks after surgery.

Valsartan treatment significantly ($P<0.05$) decreased SBP and DBP compared to the diseased group ten days and ten weeks after surgery as presented in table 2.

Table2: Effect of cilostazole (50 and 100mg/kg/day) on SBP and DBP at different time points

Data represent means \pm SE; n, number of rats ; significance $p< 0.05$

Interval	Groups (n=8) parameter	Sham- group	Diseased - group	Treated groups		
				Cilostazole-50mg/kg	Cilostazole-100 mg/kg	Valsartan 30mg/kg
Baseline	SBP (mmHg)	125.57 \pm 2.25	127. 51 \pm 2.76	127.20 \pm 2.51	123.17 \pm 2.32	126.20 \pm 2.51
	DBP	77.67 \pm 1.79	78.77 \pm 1.71	75.07 \pm 1.79	76.37 \pm 2.40	73.77 \pm 2.59
10 days after surgery	SBP (mmHg)	129.02 \pm 3.02	164.27 \pm 3.52 ^A	160.52 \pm 3.27 ^A	159.12 \pm 3.09 ^A	139.07 \pm 3.05 ^B
	DBP (mmHg)	78.37 \pm 1.53	102.30 \pm 1.65 ^A	97.57 \pm 1.76 ^A	98.70 \pm 1.76 ^A	86.90 \pm 3.35 ^B
10 weeks after surgery	SBP (mmHg)	127.17 \pm 2.97	172.27 \pm 3.10 ^A	168.45 \pm 3.06 ^A	166.92 \pm 3.63 ^A	141.95 \pm 2.98 ^B
	DBP (mmHg)	78.20 \pm 1.39	108.50 \pm 1.64 ^A	105.52 \pm 1.70 ^A	104.27 \pm 1.74 ^A	88.20 \pm 3.94 ^B

SBP, systolic blood pressure; DBP, diastolic blood pressure

^A Significantly different from sham group

^B Significantly different from diseased

Values with in the same row with different superscript capital letters are significantly ($p<0.05$) different



Statistical comparisons were carried out using one-way ANOVA followed by post-hoc tests using LSD method

Effect of cilostazole (50 and 100mg/kg/day) on renal MDA and SOD levels

Diseased group showed significant ($p<0.05$) increase in renal MDA and decrease in SOD levels compared to sham group. Rats pretreated with cilostazole (50 and 100 mg/kg/day) showed significant ($p<0.05$) decrease in renal MDA and increase in SOD levels compared to diseased group. Better results were obtained with the cilostazole (100 mg/kg/day) pretreated group, that were insignificantly ($p>0.05$) different from valsartan (30mg/kg/d).

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Effect of cilostazole (50 and 100mg/kg/day) on renal NF- κ B and TGF- β 1

Diseased group showed significant ($p<0.05$) increase in renal NF- κ B and TGF- β 1 levels compared sham group. Pretreatment with cilostazole (50 and 100 mg/kg/day) showed significant ($p<0.05$) decrease in their values as compared to diseased group. Better results were obtained with the cilostazole (100 mg/kg/day) pretreated group, regarding NF- κ B there were no significant ($p>0.05$) differences between cilostazole (100 mg/kg/day) and valsartan (30mg/k/d) pretreated group.



Table 3:Effect of cilostazole (50 and 100mg/kg/day) on renal MDA,SOD, NF-κB and TGF-β1

parameter	Sham- group	Diseased - group	Treated groups		
			Cilostazole-50mg group	Cilostazole-100 mg group	Valsartan 30mg-group
Renal MDA (nmol/mg protein)	23.81 ±1.59	123.80±3.23 ^A	61.80±2.80 ^B	46.06 ±1.84 ^C	45.70±1.36 ^C
Renal SOD (U/mg protein)	11.33±0.35	5.86 ±0.278 ^A	8.40±0.35 ^B	9.63±0.19 ^C	9.63±0.17 ^C
Renal NF-κB (pg/ mg protein)	88.03±1.86	248.33±3.73 ^A	174.06±3.44 ^B	122.23± 2.23 ^C	118.83±5.77 ^C
Renal TGF-β1 (Pg/mg protein)	42.80±4.81	147.03±3.16 ^A	86.0±3.91 ^B	71.0±3.77 ^C	57.96±5.16 ^D

Data represent means ± SE; n, number of rats ;significance p< 0.05

MDA, Malondialdehyde; SOD, super oxide dismutase; NF-κB, nuclear factor kappa B;TGFβ-1, transforming growth factor beta 1

^A Significantly different from sham group

^B Significantly different from diseased

^C Significantly different from cilostazole 50mg group

^D Significantly different from cilostazole 100mg group

Statistical comparisons were carried out using one-way ANOVA followed by post-hoc tests using LSD method

Values with in the same row with different superscript capital letters are significantly (p<0.05) different

Effect of cilostazole (50 and 100mg/kg/day) on renal iNOS and PPARγ expression

Diseased group showed significant (p<0.05) increase in renal iNOS and decrease in PPARγ gene expressions as compared to sham group. Pretreatment with cilostazole (50 and 100 mg/kg/day) showed significant (p<0.05) decrease in iNOS and increase in



PPAR γ expression in its two doses as compared to diseased group. Better results were obtained with the cilostazole (100 mg/kg/day) pretreated group, that were insignificantly different from valsartan (30mg/kg/d) pretreated group.

Table 4:Effect of cilostazole (50 and 100mg/kg/day) on renal iNOS and PPAR γ expressions

Data represent means \pm SE; n, number of rats ;significance $p < 0.05$

parameter	Sham- group	Diseased - group	Treated groups		
			Cilostazole- 50mg/kg	Cilostazole- 100 mg/kg	Valsartan 30mg/kg
Renal iNOS (relative expression)	1.21 \pm .123	7.57 \pm .216 ^A	3.56 \pm .216 ^B	2.73 \pm .093 ^C	2.44 \pm .188 ^C
Renal PPAR γ (relative expression)	1.04 \pm 0.018	0.365 \pm 0.02 ^A	0.671 \pm 0.019 ^B	0.806 \pm 0.02 ^C	0.833 \pm 0.03 ^C

iNOS, inducible nitric oxide synthase; PPAR γ , peroxisome proliferation-activated receptor gamma

^A Significantly different from sham group

^B Significantly different from diseased

^C Significantly different from cilostazole 50mg group

Values with in the same row with different superscript capital letters are significantly ($p < 0.05$) different

Statistical comparisons were carried out using one-way ANOVA followed by post-hoc tests using LSD method

Histo-pathological Findings

Histopathological examination of M&T stained kidney slices of diseased group showed shows marked



sclerotic changes (>75%) in the glomeruli with reduction in glomerular mass, surrounded by marked fibrosis (arrowheads) in the interstitial tissue, glomerulus, tubule. while, cilostazole treatment in its two doses showed mild sclerotic changes in the glomeruli, with no fibrosis in the interstitial tissue, glomerulus and tubule.

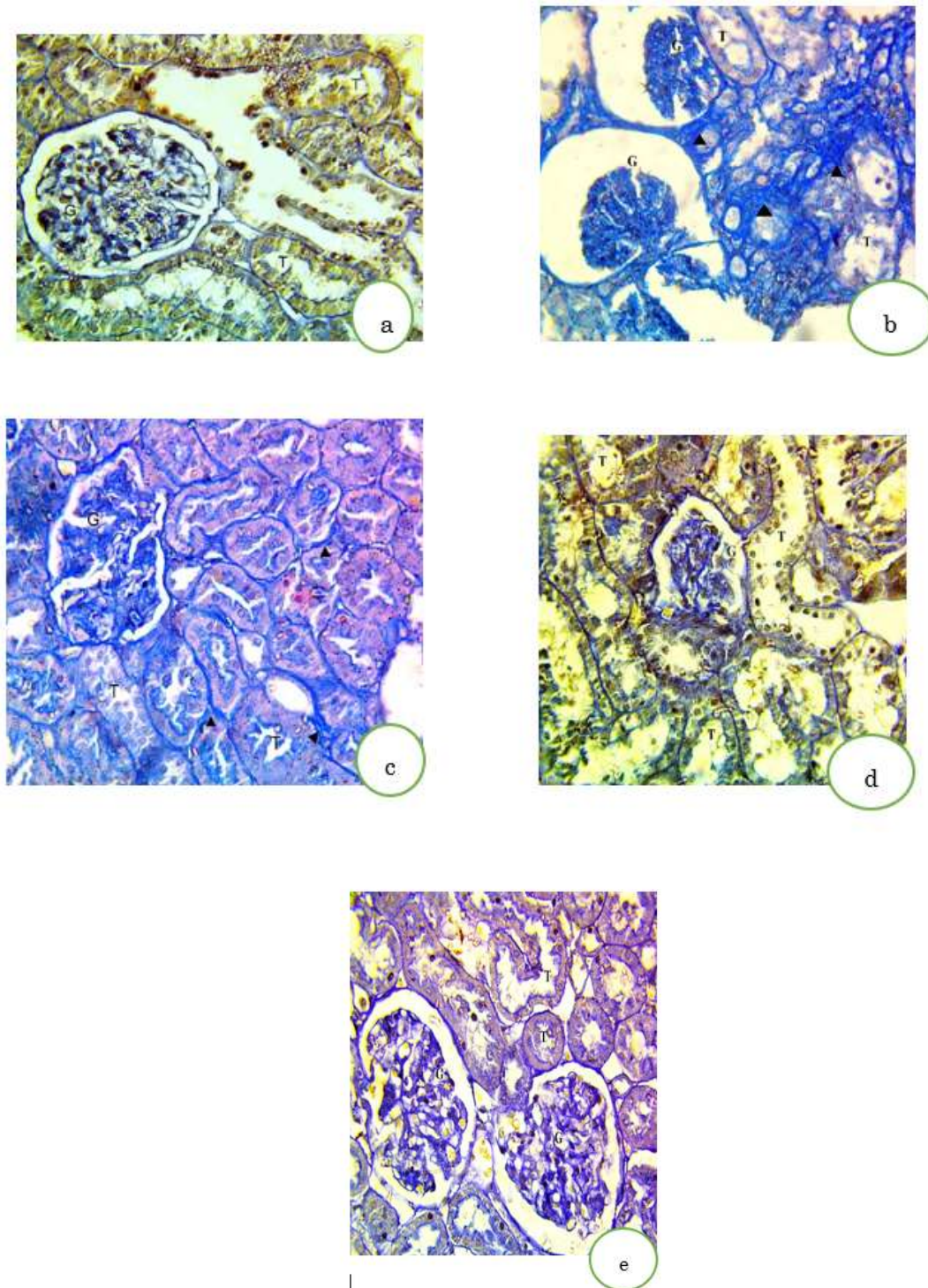


Figure 1. Photo micro-graph of renal tissue a. sham group shows normal kidney structure, b.diseased group shows marked sclerotic changes (>75%) in the glomeruli with reduction in glomerular mass, surrounded by marked fibrosis (arrowheads) in the interstitial tissue, Glomerulus (G), Tubule (T). c.cilostazole 50 group shows moderate sclerotic changes (50-75%) in the glomeruli with glomerular segmentation (lobulation), surrounded by mild interstitial fibrosis (arrowheads),Glomerulus (G), Tubule d.cilostazole 100 group showed mild sclerotic changes (25-50%) in the glomeruli, with no fibrosis in the interstitial tissue, Glomerulus (G), Tubule (T) e.valsartan group shows mild sclerotic changes (1-25%) in the glomeruli with no fibrosis in the interstitial tissue, Glomerulus (G), Tubule (T) (M &T x 400)



4. Discussion:

Chronic kidney disease (CKD) is a global health problem with serious economic effects, high morbidity and mortality rates (Evans et al., 2022). Current study was designed to study the possible renoprotective effects of cilostazole in CKD rat model induced by subtotal 5/6 nephrectomy in addition to explore the proposed underlying mechanisms. The present results revealed that cilostazole has renoprotective effects in a rat model of CKD induced by subtotal 5/6 nephrectomy. CKD was induced by performing 5/6 nephrectomy in rats, a model that induces hypertension after 10 days and cardiac remodeling after 10 weeks (Švíglerová et al., 2010). In the current work, subtotal 5/6 nephrectomy caused significant elevations in serum creatinine and urea levels in the diseased at 10 weeks after 5/6 subtotal nephrectomy compared to sham group. In chronic renal disease there is a steady and continued decrease in renal clearance or glomerular filtration rate (GFR), which leads to the gathering of urea, creatinine and other chemicals in the blood (McWilliam et al., 2017). Serum creatinine and urea are the principle indicators of renal dysfunction and nephrotoxicity (Campos et al. 2018). In accordance with our results, Mohamed et al. 2021 reported significant increase in serum creatinine and urea levels in subtotal nephrectomy group 10 weeks post 5/6 nephrectomy. Cilostazole successfully decreased serum creatinine and urea levels by its two doses compared to diseased group at 10 weeks after surgery. These findings support those of Abdelsameea et al. (2016) who demonstrated that administration of cilostazole 10mg/kg once daily for 8 days significantly reduced creatinine, urea and uric acid level in nephrotoxicity induced by gentamycin. Regarding histopathological changes of renal tissue in the present study, diseased group showed marked sclerotic changes in the glomeruli, surrounded by marked fibrosis in the interstitial tissue compared to sham group, changes that was alleviated with cilostazol treatment in its two doses. CKD is associated inflammatory responses, cell proliferation, accumulation of extracellular matrix (ECM) molecules which altogether promote further kidney injury and sclerotic changes (Sholokh and Klusmann, 2021). These findings are matched with Borges et al. (2020) who founded marked glomerular sclerosis and renal interstitial fibrosis after 60 and 120 days in untreated nephrectomy group compared to sham group in CKD model induced with 5/6 subtotal

nephrectomy. Park et al. (2017) reported that oral administration of cilostazol for 13 weeks, significantly reduced tubulointerstitial fibrosis, accumulation of ECM in the glomeruli and renal tubules in high fat diet-induced nephropathy in mice.

Our study showed significant increase in both diastolic and systolic blood pressures in the diseased group compared to sham group 10 weeks post surgery. CKD are associated with increase in the blood pressure that is related to vascular constriction, which may be a result of the activation of the (RAA) system and the sympathetic nervous system (Yamamoto et al., 2015). In accordance with our results (Švíglerová et al., 2010) showed significant increase in both diastolic and systolic blood pressures after 10 days and 10 weeks in a rat model of subtotal 5/6 nephrectomy compared to sham group. Cilostazole non significantly decreased the BP at 10 weeks after surgery. Our results matching with Chancharoenthana et al. (2017) who reported that cilostazole non significantly reduce the blood pressure in ischemia-reperfusion injury with unilateral nephrectomy mouse model (CKD model). In contrast to our results, Reddy et al. (2018) showed that cilostazole markedly suppressed the elevated SBP in obese-hypertensive mice 19 weeks after AngII infusion, they attributed cilostazole vascular relaxant effect to protein kinase A-dependent decrease in [Ca²⁺] in vascular smooth muscle cells.

Oxidative stress plays an important role in the pathogenesis of CKD. Oxidative stress-related to CKD caused by ROS production, depletion of antioxidants and impairment of the antioxidant pathways. Impairment of oxidant-antioxidant systems associated with CKD, initiates peroxidation of lipids and protein oxidation that disturb membranes (Radi, 2018). In the current study CKD disturbed the oxidative stress/antioxidant balance with increased renal level of oxidative stress biomarker MDA. Also decreased the antioxidant SOD level in the diseased group compared to sham group after 10 weeks. In the same context, Askari et al. (2018) reported progressive increase in the production of ROS with subsequent decrease in antioxidants in CKD model induced by 5/6 nephrectomy that suggested to be the key features for the pathophysiology of CKD, evidenced by increase in renal MDA with decreased SOD levels, in 5/6 nephrectomy rats 12 weeks post surgery. Cilostazole in its two doses significantly restored this balance, as it decreased renal MDA and increased SOD levels compared to diseased group that could be attributed to its free radical scavenging property or by increasing



the activity of the endogenous antioxidants. In the same context cilostazole normalized renal MDA and SOD in thioacetamide-induced nephrotoxicity in rats (**Hafez et al., 2019**). **Tawfik et al. (2021)** reported that cilostazole significantly renal increased SOD and decreased MDA levels in ischemia/reperfusion rat model. Their study supports the antioxidant capacity of cilostazol, which could be one of its renoprotective mechanistic pathway.

PPAR γ is a nuclear receptor that regulates a wide variety of down-stream signals including metabolic, inflammatory, oxidative stress and fibrotic pathways . PPAR γ expression and activity are regulated by inflammatory cytokines and ROS (**Kim and Yang, 2013**). The PPAR- γ effect in CKD depends on the modification of the various CKD related markers including NF- κ B besides its downstream target (**Wang et al., 2022**). NF- κ B functions as a central dogma of stress responses which regulate pro-inflammatory genes expression, such as cytokines ,adhesion molecules, and enzymes as iNOS (**Cerqueira et al., 2005**). Systemic inflammation plays an important role in renal disease progression (**Castro et al., 2010**).

In the current study renal PPAR- γ expression was downregulated with significant increase in NF- κ B level in the remnant kidney tissue of the diseased group compared to sham group . The origin of inflammation in CKD is multifactorial. There is evidence that activation of the RAAS and SNS, due to decreased renal filtration capacity, promotes an inflammatory response ,oxidative stress has also a central role in CKD, exerting its detrimental effects through inflammatory mediators and simultaneously being enhanced by the typical chronic inflammatory state of these patients. Uremia also contributes to high levels of inflammatory cytokines. At the same time, reduced clearance of these cytokines can itself also contribute to the characteristic low-grade inflammatory (**Hewitson et al., 2014**). In accordance with our results **Ghosh et al. (2009)** found a significant decrease in renal PPAR γ gene expression with increase in NF- κ B activity in the untreated rat group that underwent 5/6 nephrectomy compared to sham group which was attributed to significant increase in pro-inflammatory cytokine TNF- α decreasing PPAR γ expression, they hypothesized that inflammation plays a key role in progression of kidney diseases .**Maquigussa et al.(2018)** reported downregulation of renal PPAR- γ expression in CKD model induced by subtotal nephrectomy explained that by RAS system over activation associated with CKD with subsequent increase in Ang II a known inflammatory mediator

decreasing PPAR- γ expression .Matching with our results **Fang et al.(2022)** showed significant increase renal p-NF- κ B level in mice with subtotal nephrectomy 8 weeks post surgery.

Our results showed that pretreatment with cilostazole significantly increased renal PPAR γ expression that was accompanied by decrease in NF- κ B 10 weeks post nephrectomy compared to the diseased group. These results are in match with **Ragab et .al (2014)** who reported that cilostazole has renoprotective effects in renal ischemia/reperfusion rat model ,in which pretreatment with cilostazole (50 and 100mg /kg) increased PPAR- γ transcription with significant decrease in NF- κ B level confirming the link between the two parameters. **Gendy et al.2021** reported that cilostazole significantly increased the expression of PPAR- γ accompanied by reducing the expression of NF- κ B in mesenteric ischemia/reperfusion-induced lung lesion. Similary **Biscetti et al.2013** reported PPAR- γ activation with the repression of NF- κ B after the administration of cilostazole in ischemic hind limbs of streptozotocin induced diabetic mice . **Yeh et al. (2019)** showed that cilostazol prevented the progression of diabetic retinopathy in a streptozotocin-induced diabetic animal model via reduction of ROS levels reducing NF- κ B activity.

Excessive ROS associated with CKD act as signaling molecules activating genes encoding inflammation-associated molecules such as iNOS and COX-2 (**Rana et al.,2020**). iNOS activation causes DNA breaks with a subsequent activation of poly (ADP-ribose) polymerase (PARP) which is an important regulator of several pro-inflammatory mediators. iNOS triggering of inflammatory mediators has harmful biological effects on the renal systems, predisposing to functional and structural damage including glomerular hypertension, tubular-interstitial damage and renal fibrosis with further progression of CKD (**Impellizzeri et al.,2014**).

The results of this work showed that, subtotal 5/6 nephrectomy was associated with significant elevations in renal iNOS expression in the diseased group compared to sham group 10 weeks post nephrectomy. These results are in agreement with **Shirazi et al. (2019)** who showed iNOS overexpression in the 5/6 subtotal nephrectomy group of rats which have exhibited an elevated oxidative status resulting in progression of kidney disease. **Nishiyama et al.(2019)** reported increase in iNOS expression in distal colon of mice with the 5/6 subtotal nephrectomy .



The present work demonstrated that pretreatment with cilostazole in its two doses significantly decreased renal expression of iNOS compared to diseased group. Better results were observed with a dose of 100mg/kg. Matching with our results **Mohamed et al. (2018)** showed that cilostazole significantly decreased testicular iNOS expression in a dose dependant manner in streptozotocin-induced diabetes in rats after four weeks. **Park et al. (2016)** also stated that cilostazol significantly decrease lipopolysaccharide -induced iNOS expressions, and nitrite production in cultured BV-2 microglia cells.

Renal fibrosis is recognized as the final common pathway leading to CKD. TGF- β 1 is thought to be the primary driver of kidney fibrosis and parenchymal loss in CKD with a variety of functions that include increasing collagen and matrix protein formation, maintaining fibroblast viability, and inhibiting metalloproteinase production (**Pinheiro da Silva and Vaz da Silva, 2016**). The development of renal fibrosis results from an imbalance between profibrotic and antifibrotic pathways. These profibrotic processes are modulated by the upregulation of genes related to fibrosis, including Angiotensin II (Ang II), TGF- β , and Wnt, together with the downregulation of antifibrotic genes, such as PPAR- γ , and klotho (**Hu et al., 2013**).

The results of the current work showed a significant increase in renal TGF- β 1 level in the diseased group compared to the sham group. In agree with our findings **Prieto-Carrasco and coworkers (2021)** demonstrated significant increase in fibrotic markers TGF- β 1 and alpha smooth muscle actin (α -SMA) in remnant kidney over time in the 5/6Nx group compared to the control group, suggesting that 5/6Nx induces progressive reduction in mitochondrial biogenesis. Pretreatment with cilostazole in its two doses significantly decreased renal level of TGF- β 1, better results were observed with a dose of 100mg/kg. In accordance with our results **Chian et al. (2020)** reported that cilostazole deceleration of hyperglycemia-induced diabetic nephropathy in STZ induced diabetic rats is attributable to down-regulation of TGF- β , ROS reduction and NF- κ B thereby maintain of the mitochondrial function, thus preventing TGF- β -stimulated hypertrophic growth and fibrosis.

Consequently, our results revealed that cilostazol renoprotective effect in CKD model induced by subtotal 5/6 nephrectomy, via increasing SOD decreasing MDA, NF- κ B, iNOS and TGF- β 1 with relation between these parameters and cilostazole upregulation of PPAR- γ transcription, confirming

antioxidative, anti-inflammatory and antifibrotic actions of cilostazol.

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