Research Article

Ameliorative Effects of Phosphodiesterase (PDE) Inhibitors in Potassium Dichromate-Induced Acute Renal Failure in Rats

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ABSTRACT

Heavy metal as potassium dichromate (PD) is nephrotoxic xenobiotic that lead to acute tubular necrosis. The aim of this study was to examine the possible renoprotective effect of members in the phosphodiesterase (PDE) inhibitors on potassium dichromate induced acute renal failure (ARF) and oxidative stress in rats. ARF was induced by subcutaneous (s.c) injection of a single dose (15 mg/kg) of PD. Rats were randomly allocated into 5 groups as follows: Group I: Normal control group received saline. Group II: Rats injected s.c with PD and served as renal failure group. Group III, IV and V: Rats received daily Sildenafil (0.5 mg/Kg), Vardenafil (3 mg/Kg) and Tadalafil (10 mg/Kg), respectively, prior PD injection for 14 days. Estimation of serum creatinine, blood urea nitrogen (BUN) and total protein, kidney tissue glutathione peroxidase (GPx), malondialdehyde (MDA), nitric oxide (NO) and tumer necrosis factor alpha (TNF-α) contents as well as histopathological examination were carried out. Injection of PD to rats induced a marked renal failure, characterized with a significant increase in serum creatinine urea and total protein. PD group had lower kidney GPx content and higher MDA, NO and TNF-α contents while PDE inhibitors therapy improves kidney function, GPx content and ameliorates MDA, NO and TNF-α contents. PDE inhibitors may reduce or delay the emergence of PD nephrotoxicity.

Keywords: Acute renal failure; Oxidative stress; phosphodiesterase (PDE) inhibitors; Rat.

INTRODUCTION

Acute renal failure (ARF) is characterized by azotemia (the accumulation of nitrogenous waste (urea) and other solutes that progresses over several hours or days), with or without oliguria. ARF is common in hospitalized patients and also has a poor prognosis with the mortality ranging from 10%-80% dependent upon the patient population studied. Patients, who present with uncomplicated acute kidney injury (AKI), have a mortality rate of up to 10%. In contrast, patients presenting with AKI and multiorgan failure have been reported to have mortality rates of over 50%. If renal replacement therapy is required the mortality rate rises further to as high as 80%[1].

Heavy metals are nephrotoxic xenobiotics that may lead to acute tubular necrosis[2]. Previous studies showed that dichromate exposure increases the concentration of reactive species[3], and provokes oxidative damage in hepatocytes[4], the brain[5] and kidney[6]. Another explanation of renal injury-induced by dichromate is mediation of inflammatory process as seen by increased pro-inflammatory cytokine renal TNF-α content and myeloperoxidase (MPO)[7].

Phosphodiesterase (PDEs) are important regulators of the intracellular cAMP concentration, which is a central second messenger that affects a multitude of intracellular functions[8]. PDEs are found in high concentration in smooth muscle cells of the peripheral arterial and venous vessels as well as coronary and pulmonary circulation, and in platelets. It is specific for the hydrolysis of cGMP that plays an important role in regulation of intracellular calcium levels, modulation of platelet function and induces vasodilatation in renal injury[9].

The PDE-5 inhibitors, including Sildenafil (Viagra®), Vardenafil (Levitra®), Tadalafil (Cialis®) and Avanafil (Stendra®) have been approved by the Food and Drug Administration (FDA) for the treatment of erectile dysfunction (ED). Sildenafil and Tadalafil have also been approved for the management of pulmonary arterial hypertension[10].

Therefore, the main goal of this study was to investigate the protective effect of PDE-5 inhibitors against potassium dichromate induced renal failure in rats.

MATERIALS AND METHODS

Animals

Adult male Wister albino rats weighing 120 – 140g purchased from the animal house colony of the National Research Centre (Dokki, Giza, Egypt) and were kept in the animal house under conventional laboratory conditions. Experiments were performed according to the National Regulations of Animal Welfare and Institutional Animal Ethical Committee (IAEC).

Chemicals

Potassium dichromate was obtained from National Research Centre (Dokki, Giza, Egypt).

Drugs

a) Sildenafil (Viagra®, Pfizer Egypt).

b) Vardenafil (Levitra®, Bayer Pharma AG, Germany).

c) Tadalafil (Cialis®, Eli Lilly and Company, USA).

Experimental design

ARF was induced by administration of a single dose of PD (15 mg/kg, s.c)[11]. Animals were divided into 5 groups as follows: Group I: Normal control group received saline. Group II: Rats injected s.c with PD and served as renal failure group. Group III, IV and V: Rats received daily Sildenafil (0.5 mg/Kg/day, p.o.)[12], Vardenafil (3 mg/Kg/day, p.o.)[13] and Tadalafil (10 mg/Kg/day, p.o.)[14], respectively for 14 days prior to the induction of ARF with PD. 2 days following the last treatment, blood samples were taken from the abdominal aorta and used for determination of creatinine, BUN and total protein levels. The animals were sacrificed under light ether anesthesia by cervical dislocation, and one kidney from each rat were immediately dissected out, washed with ice-cooled physiological saline and homogenized in 0.15M KCl solution. Aliquots of the homogenate were prepared for determination of tissue contents of GPx, MDA, NO and TNF-α.

Biochemical analysis

The following parameters, indicating glomerular, tubular and oxidative kidney damage, were measured 2 days after induction of renal failure. Creatinine, BUN and total protein levels were determined in serum samples using commercially available kits (Biodiagnostic, Egypt) according to the method by Bartles[15], Fawcett, and Soctt[16] and Gornal[17], respectively. GPx, MDA, and NO contents in kidney tissue were determined using commercially available kits (Biodiagnostic, Egypt) according to Beutler[18], Uchimaya and Mihara[19] and Miranda[20].

TNF-α in kidney tissue was also determined using commercially available ELIZA kit (KOMA BIOTECH, Korea) according to Brouckaert[21].

Histopathological examination of kidney

Kidney was immediately removed and washed in saline solution. The kidney was fixed in 10% phosphate buffered formalin. Following an overnight fixation, slices (3–4mm) of kidney tissue were dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax (58-60°C). Blocks were made and sectioned of 5 µm thickness with microtome. The tissue sections were stained with hematoxylin and eosin and observed under the light microscope. The slides were observed for histopathological changes and microphotographs were taken using a microscope system (Olympus, Japan).

Immunohistochemistry for caspase-3

Immunohistochemical staining of anti-caspase-3 antibodies was performed by streptoavidin–biotin. Four-micrometer–thick sections were deparaffinized and incubated with fresh 0.3 % hydrogen peroxide in methanol for 30 min at room temperature. The specimens were then incubated with anti-caspase-3 antibody as the primer antibody at a 1:100 dilution. The specimens were counterstained with H&E. Negative controls were prepared by substituting normal mouse serum for each primary antibody.

Data analysis

All the values are presented as means ± standard error of the means (SE). Comparisons between different groups were carried out using one way analysis of variance (ANOVA) followed by least significant difference test (LSD). Difference was considered significant when $p < 0.05$. Graph Pad prism® software (version 5) was used to carry out these statistical tests.

RESULTS

Effect of pre-treatment with Sildenafil, Vardenafil and Tadalafil on serum creatinine, BUN and total protein

Induction of ARF by PD significantly increased serum creatinine, BUN and total protein levels by 3.67%, 113.61% and 13.08%, respectively, 2 days after induction of ARF, as compared with normal control group. While the pre-treated animals with Sildenafil significantly decreased serum creatinine, BUN and total protein levels by 1.46%, 17.30% and 1.93%, respectively, animals pre-treated with Vardenafil, also, significantly decreased serum creatinine, BUN and total protein levels by 2.97%, 26.11% and 6.89%, respectively, moreover animals pre-treated with Tadalafil significantly decreased serum creatinine, BUN and total protein levels by 4.94%, 27.87% and 5.51%, respectively, 2 days after induction of ARF, as compared with PD group (Table 1).

Table 1: Effect of pre-treatment with Sildenafil, Vardenafil and Tadalafil on serum creatinine, blood urea nitrogen (BUN) and total protein

Groups / Parameters	Normal Control (Saline)	Pottasium dichromate PD (15 mg/kg)	Sildenafil (0.5 mg/Kg)	Vardenafil (3 mg/Kg)	Tadalafil (10 mg/Kg)
Creatinine (mg/dl)	1.79±0.14	1.86±0.04^{a}	1.83±0.07^{ab}	1.80±0.10^{b}	1.77±0.02^{b}
BUN (mg/dl)	5.13±0.23	10.95±0.39^{a}	9.06±0.29^{ab}	8.09±0.43^{ab}	7.90±0.19^{ab}
Total protein (g/dl)	4.94±0.12	5.58±0.05^{a}	5.48±0.05^{ab}	5.20±0.02^{ab}	5.28±0.06^{ab}

Five groups were used in the present experiment, each consisted of 10 rats. Group 1 received saline and served as normal group.

Group 2 received PD (15 mg/kg, s.c.) and served as kidney damaged group. Groups III, IV and V received Sildenafil (0.5 mg/Kg/day, p.o.), Vardenafil (3 mg/Kg/day, p.o.) and Taladafil (10 mg/Kg/day, p.o.) respectively for 14 days prior to the induction of ARF with PD. Blood samples were collected from all groups for estimation of serum creatinine, BUN and total protein levels.

Data were expressed as mean ± SE.

Statistical analysis was carried out by one-way ANOVA followed by LSD test. [a]Significantly different from normal control (Saline) at *P<0.05*. [b]Significantly different from kidney damaged group (PD) at *P<0.05*.

Effect of pre-treatment with Sildenafil, Vardenafil and Tadalafil on kidney GPx, MDA, and NO contents

Induction of ARF by PD significantly decreased kidney GPx by 66.75% and increased MDA and NO contents by 78.43% and 112.78%, respectively after 2 days of induction of ARF, as compared with normal control group.

Animals pre-treated with Sildenafil, vardenafil and Tadalafil significantly increased kidney GPx content by 56.83%, 38.13% and 97.84%, respectively, decreased MDA content by 30.04%, 38.67 % and 39.64%, respectively, and decreased NO content by 51.95%, 39.94% and 44.44%, respectively, 2 days after induction of ARF, as compared with PD group (Fig 1a, b &c).

Effect of pre-treatment with Sildenafil, Vardenafil and Tadalafil on kidney TNF- α content

Induction of ARF by PD significantly increased kidney TNF-α content by 82.26%, 2 days after of induction of ARF, as compared with normal control group.

Whereas animals pre-treated with Sildenafil, Vardenafil and Tadalafil significantly decreased kidney TNF-α content by 30.06**%**, 34.83% and 40.44%, respectively, 2 days after induction of ARF, as compared with PD group (Fig 1d).

Five groups were used in the present experiment, each consisted of 10 rats. Group 1 received saline and served as normal group. Groups. Group 2 received PD (15 mg/kg, s.c.) and served as kidney damaged group. Groups III, IV and V received Sildenafil (0.5 mg/Kg/day, p.o.), Vardenafil (3 mg/Kg/day, p.o.) and Taladafil (10 mg/Kg/day, p.o.) respectively for 14 days prior to the induction of ARF with PD. Kidney tissues were collected and homogenized for estimation of GPx, MDA, NO and TNF-α contents. Data were expressed as mean ± SE.

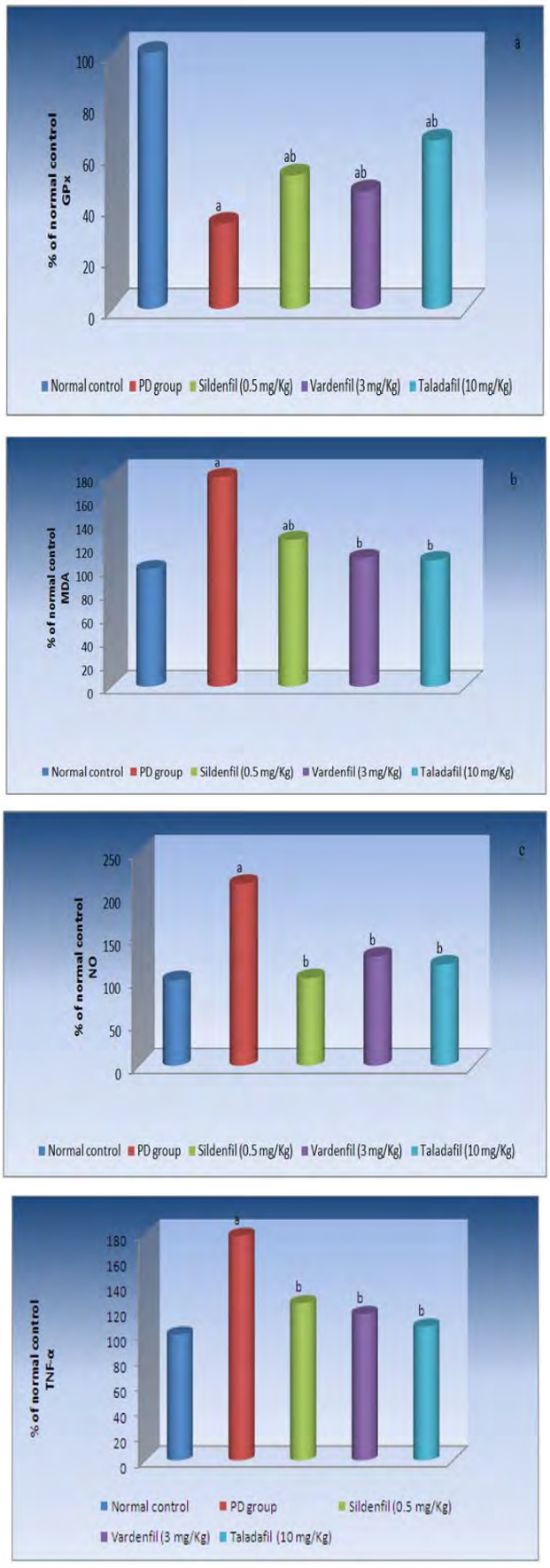

Figure 1: Effect of pre-treatment with Sildenafil, Vardenafil and Tadalafil on kidney a) GPx, b) MDA, c) NO and d) TNF-α contents.

Statistical analysis was carried out by one-way ANOVA followed by LSD test. [a]Significantly different from normal control (Saline) at *P<0.05*. [b]Significantly different from kidney damaged group (PD) at *P<0.05*.

Histopathological Examination

Kidneys of control rats showed normal architecture of renal tissue, being composed of a number of glomeruli with proximal and distal convoluted tubules (Fig 2A).

Microscopical examination of kidney sections administrated PD showed various pathological lesions characterized by glomerular atrophy, extension of the renal glomerulus capsular space and periglomerular aggregation of mononuclear lymphocytes cells. The renal glomeruli revealed thickening of the glomerular basement membrane.

Additional, necrosis, vacuolation in the renal epithelia and some tubular cells had pyknotic, karyolytic nuclei and apoptotic cells. Renal cast was observed in the lumina of some tubules and renal interstitial haemorrhage (Fig 2B & C).

Rats Pre-treated with Sildenafil (0.5 mg/kg/day, p.o. for 14 days) and subjected to PD showed partial reduction of the histopathological features of renal injury. Renal glomerulus capsular space was still dilated with moderate vacuolation in the renal epithelia (Fig 2D).

Sections of kidneys of rats pre-treated with Vardenafil (3 mg/kg/day, p.o. for 14 days) and subjected to PD showed mild renal glomerulus capsular space still dilated and mild vacuolation in the renal epithelia (Fig 2E).

Rats pre-treated with Tadalafil (10 mg/kg/day, p.o. for 14 days) and subjected to PD was associated with more reduction in injury almost similar to control rat kidney. Only a slight degree of degenerative changes among the tubular linings was marked along with a decrease in the cellular necrosis with renal glomerulus capsular space still dilated (Fig 2F).

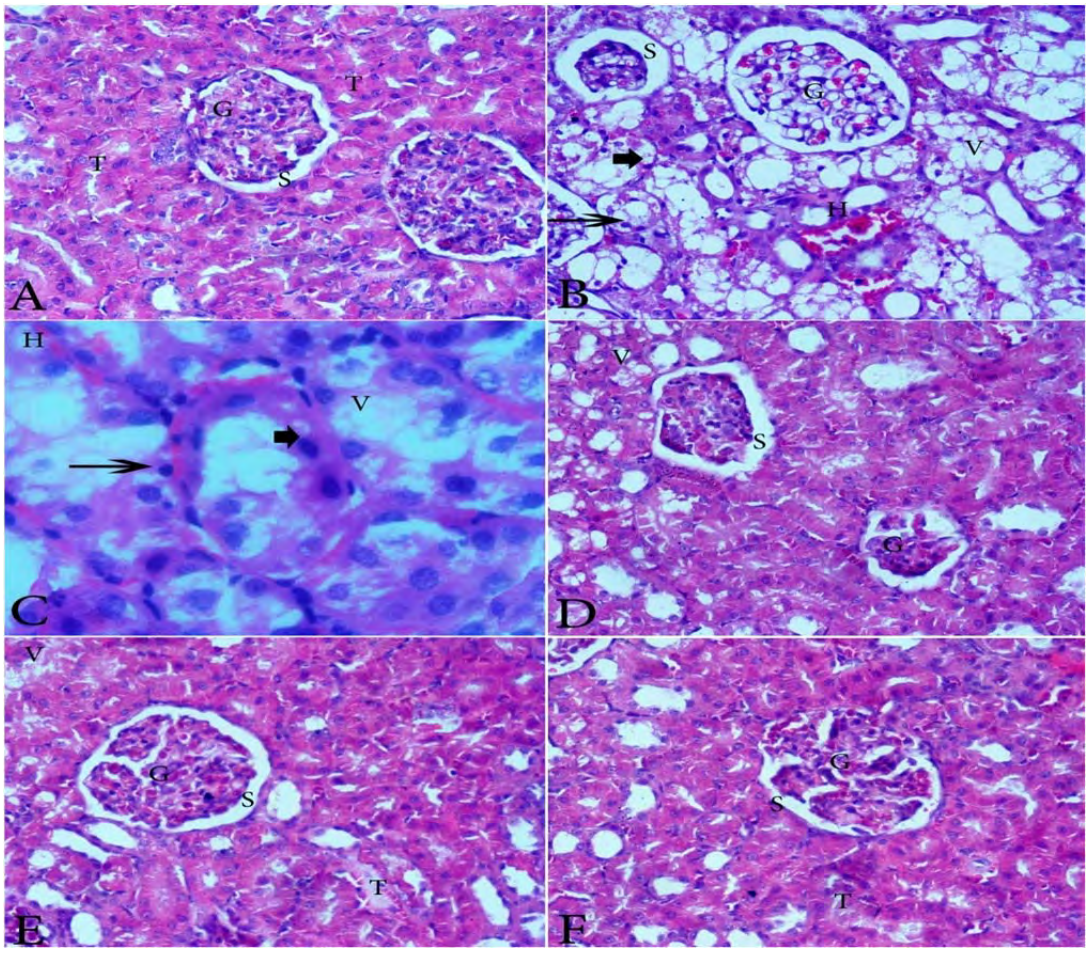

Figure 2: A. Photomicrograph of the kidney of control rat showing normal architecture of glomeruli and renal tubules **(H and E, X 400).**

B. Photomicrograph of the kidney of rat treated with Potassium dichromate (15 mg/kg) showing glomerular atrophy (G), extension of the renal glomerulus capsular space (S) and renal interstitial haemorrhage (H). Necrosis, vacuolation in the renal epithelia (V) and some tubular cells were pyknotic (arrowhead). Apoptotic cells (arrow) were also observed **(H and E, X 400).**

C. Photomicrograph of the kidney of rat treated with PD (15 mg/kg) showing necrosis, vacuolation in the renal epithelia (V), some tubular cells were pyknotic (arrowhead). Apoptotic cells (arrow) and renal interstitial haemorrhage (H) were also observed **(H and E, X 1000).**

D. Photomicrograph of the kidney of rat treated with Sildenafil (0.5 mg/Kg/day, p.o.) for 14 days prior to PD (15 mg/kg) showing renal glomerulus capsular space still dilated (S). Moderate vacuolation (V) in the renal epithelia was observed **(H and E, X 400).**

E. Photomicrograph of the kidney of rat treated with Vardenafil (3 mg/Kg/day, p.o.) for 14 days prior to PD (15 mg/kg) showing renal glomerulus capsular space still dilated (S). Mild vacuolation in the renal epithelia (V) was apparent (**H and E, X 400).**

F. Photomicrograph of the kidney of rat treated with Taladafil (10 mg/Kg/day, p.o.) for 14 days prior to PD (15 mg/kg) showing renal glomerulus capsular space still dilated (S) and slight degrees of degenerative changes among the tubular linings (T) **(H and E, X 400).**

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Immunohistochemical results

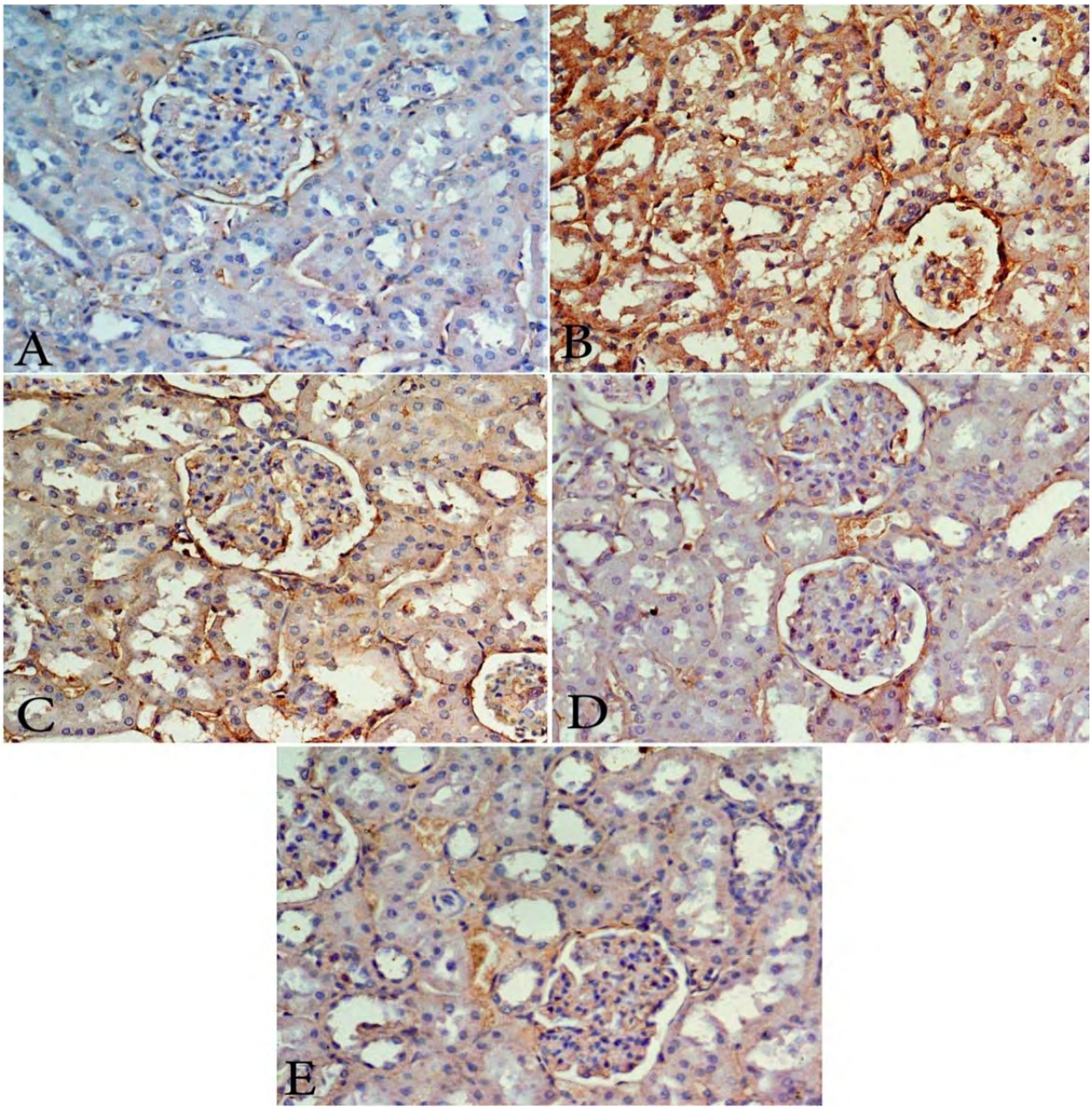

Figure 3: A. Photomicrograph of a section of a kidney from the normal control group showing negative caspase-3 immunostaining of the glomeruli and tubular cellcytoplasm **(Caspase-3 immunohistochemistry, hematoxylin counterstain×400)**.

The kidney of the control rat showed a negative immune reaction to caspase-3 (Fig 2A). The expression of renal caspase-3 was positive in glomerular and renal tubular epithelial cell and that effect was observed in the PD group (brownish staining in the cytoplasm of glomerular and tubular cells) (Fig 3B).

The expression of renal caspase-3 in Slidenafil group showed mild increase of glomerular and tubular epithelial cell compared with PD control group (Fig 3C). However, minimally expressed caspase-3 reaction in the glomerular and tubular epithelial cell was observed in the Vardenafil and Taladafil groups (Fig 3D&E).

DISCUSSION

Acute renal failure is defined as an abrupt decline in Glomerular filtration rate (GFR) resulting from toxic injury to the kidney[22]. Creatinine is filtered in the glomerulus and its clearance (CCr) is a marker of GFR. Over years there has been increasing recognition that relatively small rises in serum creatinine in a variety of clinical settings are associated with worse outcomes[23]. Our results showed elevation in serum creatinine, BUN and total protein, 2 days after dichromate administration, this elevation has been related to tubular obstruction by cell debris and cylinders. In line with our findings, Salama[11] reported that induction of kidney damage by PD produced a significant increase of serum creatinine, BUN and total protein, 2 days after PD administration.

A single subcutaneous injection of potassium dichromate (15mg/kg) resulted in a significant increase of markers related to oxidative stress, nitrosative stress, necrosis and inflammation. Potassium dichromate is a strong oxidant and our results showed that dichromate exposure caused oxidative damage in the kidney, 2 days after its administration. The role of oxidative stress in dichromate-induced kidney damage has been supported by the present work and previous studies reported by Sahu[24]. This data was further confirmed by the histopathological picture of the kidneys after administration of PD that showed glomerular atrophy, extension of the renal glomerulus capsular space and renal interstitial hemorrhage. Necrosis, vacuolation in the renal epithelia, some pyknotic tubular cells as well as apoptotic cells were also apparent.

ARF is no longer considered to be an innocent. It has been demonstrated to be an independent risk factor for mortality. The cause of this is unclear but is possibly associated with an increased risk of “non-renal” complications such as bleeding and sepsis[25]. An alternative explanation may lay in experimental work that has demonstrated the "distant effects" of ischemic AKI on the other organs. In experimental models isolated ischemic AKI up-regulates inflammatory mediators in other organs including the brain, lungs and heart[26].

In our study, pre-treatment with PDE inhibitors; Sildenafil, Vardenafil and Taladafil, for 14 days significantly decreased creatinine, BUN and total protein in rats treated with PD when compared to renal failure group (PD group). Previous study showed that Taladafil was effective in reducing serum creatinine[27]. In addition to PDE inhibitors amelioration of changes in renal functions, they afforded protection against the renal effects of dichromate through other mechanisms, such as modulation of the response to oxidative stress through increasing GPx and decreasing MDA. Other studies showed that treatment with PDE inhibitors significantly attenuated brain oxidative stress levels in rat model of Streptozotocin diabetes induced vascular dementia[28] and Sildenafil increased GSH levels significantly in Sepsis (a systemic inflammatory response to infection)[29].

Exploration of other underlying renoprotective mechanisms of PDE inhibitors revealed that they attenuated renal expression of inducible nitric oxide synthase (iNOS) and participated in depressing inflammatory processes through decreasing renal content of TNF-α. These results are in line with other studies which reported that PDE5 inhibitors could act as potent anti-inflammatory drugs[30] and Tadalafil therapy ameliorates circulating inflammatory cytokines and chemokines in a diabetic animal model[31]. Moreover, Sildenafil is a highly protective agent in preventing lung and kidney damage via maintenance of the oxidant–anti-oxidant status and decrease in the level of TNF-α[29].

Tadalafil has the advantage because it is long acting whereas the durations of action of Sildenafil and Vardenafil are generally 4 to 8 h. Moreover, Tadalafil is a highly selective inhibitor of PDE with 10,000-fold selectivity for PDE-5 over PDE-1 to PDE-4 and approximately 700-fold selectivity for PDE-5 over PDE-6. Tadalafil is also the only PDE-5 inhibitor whose activity is unaffected by food and has a relatively short time to onset of action (16–17 min)[32].

Our data was further supported by histopathological picture of the kidney tissues pre-treated with Sildenafil, Vardenafil or Taladafil that showed improvement in histopathological picture of the kidney tissues of rats. Taladafil administration prior to the renal ischemia/reperfusion injury attenuated morphological disarrangements[9].

The immunohistochemical examination of the kidney tissue in the present study showed that the dichromate causes acute tubular necrosis and glomerulonephritis due to expression of renal caspase-3. This is in line with El-Mahalaway[33]. Moreover, the immunohistochemical examination of the kidney tissue of rats pre-treated with Sildenafil, Vardenafil or Taladafil for 14 days prior to PD administration showed varying degrees of improvement in expressed caspase-3 reaction in the glomeruli and cytoplasm of tubular cells.

CONCLUSION

We have provided the evidence that the beneficial effect of PDE inhibitors (Sildenafil, Vardenafil and Taladafil) are related to amelioration of changes in the renal function and structure and inflammatory cytokines in ARF model. The present study demonstrated that these PDE inhibitors produced renoprotective effect against PD-induced ARF and oxidative stress in rat.

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