Nicorandil Mitigates Renal Oxidative Damage, Apoptotic, and Fibrotic Changes Induced by Cyclosporine-A in Rats

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A B S T R A C T

Cyclosporine A (CsA) is one of the immunosuppressive drugs broadly used in transplantation rejection. However, nephrotoxicity arising from its long-term use constrained its efficacy. This study investigated the advantageous role of nicorandil against CsA nephrotoxicity in rats. Rats were assigned into 5 groups (n= 6 each): control group, nicorandil group, CsA group, CsA+nicorandil group, CsA+nicorandil+glibenclamide group. CsA induced Nephrotoxicity orally for 21 days. The results exhibited that CsA nephrotoxicity manifested by increasing serum urea, creatinine, and uric acid in the orchestra with histopathological changes. There was a significant rise in oxidative damage parameters; malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) without relevant effect on nitric oxide (NO) level. CsA increased the expression of transforming growth factor beta (TGF-β) and collagen 1A1 by reverse transcriptase (RT-PCR) method with reduction of immunohistochemical expression of the Bcl2 level and increasing caspase-3 level. Administration of nicorandil significantly ameliorated oxidative changes with elevation in NO level. Nicorandil prevented the elevated serum markers associated with significant improvement in TGF-β, collagen IA1 and endothelial nitric oxide synthase (eNOS) expression. Besides, immunohistochemical expression of Bcl2 significantly augmented with a reduction in caspase-3 in comparison with CsA. Glibenclamide did not significantly have a role when combined with CsA and nicorandil. The renal protective effect of nicorandil was thought to be in part through anti-oxidant, anti-fibrotic, and anti-apoptotic mechanisms. These results may offer additional nephroprotective mechanisms for nicorandil independent on its vasodilator effect.

Key Words: Cyclosporine; nicorandil; nephrotoxicity; glibenclamide.

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1. INTRODUCTION

Cyclosporine-A (CsA) is a calcineurin inhibitor applied widely as the immunosuppressive agent in organ transplantation and autoimmune illnesses (Halloran, 1996; Yong-Gang et al., 2014). However, its therapeutic benefits are often limited by serious organ toxicity; the most important of which is nephrotoxicity (Takasu et al., 2015). It was suggested that the possible mechanisms underlying short- and long-term nephrotoxicity induced by CsA is atrophy, fibrosis, inflammation, and sclerosis. Several factors contributed; one of the main causative factors is the increased generation of free radical species and lipid peroxidation products (Sereno et al., 2014, Ateyya, 2015). The pathogenesis of CsA toxicity on renal tissues comprises afferent arteriolar vasoconstriction with pro-fibrotic effect in its chronic stage and inhibition of intra-renal nitric oxide (NO)(Mazali et al., 2012). Renal damage progresses through activation of the immune mediators such as transforming growth factor (TGF-β) which is known to be an important cytokine in the pathogenesis of glomerulosclerosis and interstitial fibrosis in CsA toxicity (Kim et al., 2016). Deregulation of apoptosis has been involved additionally in the pathogenesis of CsA-induced nephrotoxicity by promoting caspase-3 activation (Lai et al., 2015). Nicorandil an ATP-sensitive potassium channel opener with NO donation property has been shown to have a significant protective effect against ischemic heart diseases; it exhibits vasodilation in the vascular system (Zhang et al., 2015). Former reports demonstrated renal defense in mesangioproliferative glomerulonephritis, and ischemia reperfusion injury but its role in CsA nephrotoxicity was not investigated (Sudo et al., 2009). Beneficial effects of nicorandil have been reported in various organs that were linked to its anti-oxidant and...
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The aim of the current study was to evaluate the possible protective role of nicorandil on chronic nephrotoxicity induced by CsA exploring putative antioxidant, anti-fibrotic, and anti-apoptotic mechanisms.

2. MATERIALS AND METHODS

2.1. Animals

The present research was conducted on adult male albino rats weighing 150–200 g. Rats were gotten from the National Research Center, El-Giza, Egypt. Rats were permitted an ordinary diet of commercial rat food and water and were adjusted to laboratory conditions for a period of 2 weeks. The experiments were done in accordance with the Guide for the Care and Use of Laboratory Animals of the National research council. The protocol was accepted by the Faculty of Medicine, Minia University Ethics Committee for the Care and Use of Laboratory Animals.

2.2. Chemicals and antibodies

Cyclosporine A (Sandimmun® soft gelatin capsules; 50 mg/ml) was obtained from Novartis (Basel, Switzerland) and dissolved in olive oil. Nicorandil was purchased from (Merck Ltd., Cairo, Egypt) dissolved in distilled water. Anti-Bcl2 and caspase-3 polyclonal antibodies were purchased from Thermo Fisher Scientific Inc. /Lab Vision (Fermont, CA, USA).

2.3. Experimental design

The rats were assigned randomly into 5 groups (n = 6 at each group) and treated every day for 21 days. Group 1 served as control group and received (1ml/rat/day, p.o.) olive oil (vehicle). Group 2: Rats were given CsA (25 mg/kg, p.o.) diluted in olive oil (Chandramohan and Parameswari, 2013). Group 3: Rats were treated with nicorandil (15 mg/kg, p.o.) dissolved in distilled water. Anti-Bcl2 and caspase-3 polyclonal antibodies were purchased from Thermo Fisher Scientific Inc. /Lab Vision (Fermont, CA, USA).

2.4. Biochemical analysis

2.4.1. Measurement of renal functions

Serum urea, creatinine, and uric acid were estimated by enzymatic colorimetric kits according to previous techniques (Fawcett and Scott, 1960; Schirmeister et al., 1964).

2.4.2. Measurements of oxidative stress parameters

2.4.2.1. Lipid peroxidation product

MDA, a lipid peroxidation indicator, was analyzed by a spectrophotometric method that built on a reaction with thiobarbituric acid (Buege and Aust, 1978). Colored complexes were obtained when extracted with n-butanol/pyridine. Sample absorbance was measured at 532 nm and calculated using the absorbance of the standard using 1, 1, 3, 3-tetramethoxy propane.

2.4.2.2. Measurement of nitric oxide in renal tissue

Nitric oxide production was measured by levels of total nitrite and nitrate (NO2⁻ and NO3⁻) as indicators. Total nitrite and nitrate were estimated after the reduction of nitrate to nitrite by copperized cadmium particles in glycine buffer at pH 9.7. A concentration of NO2⁻ was based on the Griess reaction, in which a color substance with absorbance at 540 nm is formed by reaction of nitrite with a mixture of naphthylethylenediamine and sulfanilamide, method described by Ridnouret al. (Ridnour et al., 2000). Sodium nitrite and nitrate solutions were used for standard measurements.

2.4.2.3. Renal SOD activity

It was measured in accordance with the method described by (Marklund and Marklund, 1974) with a minor modification. This method is based on inhibition of pyrogallol autoxidation by SOD which activity was estimated at 420 nm.

2.4.2.4. Measurement of GSH in renal tissue

According to Moron et al. (1979) method, GSH was estimated. The reaction mixture including 0.25 mL of supernatant, 1 mL of 0.2 M Tris-HCl (containing 1mM EDTA, pH 8.9), and 0.05 mL of 0.01 M 5,5-dithiobis-(2-nitrobenzoic acid) in absolute methanol was preserved at room temperature for 5 min. Yellow color was measured spectrophotometrically at 412 nm. Glutathione concentration in tissue

\[ \text{GSH (mg/g tissue)} = \frac{\text{A sample} \times 66.66}{\text{g. tissue used}} \]

2.5. Reverse transcriptase (RT-PCR) analysis for detection of TGF-β, collagen 1A1 and eNOS mRNA expression

Total RNA was purified from homogenized kidney tissues using RiboZol RNA Extraction reagent
enzyme mix (Hot Star Taq DNA polymerase, Omniscript of total RNA template, 1 μl dNTPs mix (100 mM), 1 μl primers for TGF-β, Coll1A1, eNOS and GAPDH, 5 μg containing 5 μl of RT-PCR buffer 5×, 10 pM of specific Scientific, UK). Reaction tube of RT-PCR was determined by spectrophotometer (Genova Plus, Bibby (Amresco, Solon, USA) following the manufacturer’s instructions. Nucleic acid concentrations were determined by spectrophotometer (Genova Plus, Bibby Scientific, UK). Reaction tube of RT-PCR was containing 5 μl of RT-PCR buffer 5×, 10 pM of specific primers for TGF-β, Coll1A1, eNOS and GAPDH, 5 μg of total RNA template, 1 μl dNTPs mix (100 mM), 1 μl enzyme mix (Hot Star Taq DNA polymerase, Omnисcript and Sensiscript reverse transcriptions). PCR amplification was performed in a DNA thermal cycler (Progene; Teclne Ltd., Duxford, United Kingdom). Rat TGF-β PCR amplification was performed as follow: 5 min, at 95°C (initial denaturation); 94°C for 20 s; 51°C for 30 s; 72°C for 40 s (30 cycles); and 72°C for 5 min (final extension). The MgCl₂ concentration used for cDNA amplification was 2.0 mM for rat TGF-β. The RT-PCR condition for rat Coll1A1, rat eNOS and GAPDH was the same as rat TGF-β except that the annealing temperature was 50°C, 55 °C and 54°C respectively. Primers used and amplicon size are as follows, rat TGF-β: Sense: 5’ TGTGCTCTTCAACAACACAA 3’ and Antisense: 5’ GCTTGGCAACCGTAGTA 3’; 103bp. rat Coll1A1 Sense: 5’ TTCCCTTGGACCTAAGGGA 3’ and Antisense: 5’ TTGAGCTCAGCTGCCC 3’; 114bp. rat eNOS sense: 5’-CCA GATATC TTC AGT CCC AAG C-3’ and antisense 5’-GTG GAT TTG CTG CTC TCT TCT AGG-3’; 135bp. PCR was performed with rat GAPDH as an internal standard (Thermo scientific) USA. Sense5’:CAGTGCCAGCCTGCTCAT-3’; antisense 5’:GGGGCCATCCACA GTCTTC-3’, 595bp. The intensity of the PCR product bands were quantified using gel documentation system software (Biometra GmbH, Germany).

2.6. Detection of PCR Product
Polymerase chain reaction products (5 μl) were detected by electrophoresis on a 1.5% agarose gel containing ethidium bromide. Location of a predicted product was confirmed by using 100 bp ladder (Gene Ruler, Thermo scientific, USA)) as a standard size marker.

2.7. Histopathological changes in renal tissues
The kidney specimens were fixed in 10% formalin for at least 24 h. Then, kidney tissues were dehydrated with a sequence of ethanol solutions, embedded in paraffin, cut into 5 μm thickness, and stained with hematoxylin and eosin dye. These sections were then examined by a pathologist for randomly selected 10 fields from each rat. The histopathological scoring analysis was performed, the assessment was expressed as the sum of the individual score grades from 0 (no findings), 1 (mild), 2 (moderate), to 3 (severe) for each of the following 4 parameters from kidney sections: tubular cell swelling, cellular vacuolization, pyknotic nuclei and medullary congestion (Singh and Chopra, 2004).

2.8. Immunohistochemical localization of Bcl2and caspase-3 proteins in renal tissue
Five μm sections placed on positively charged slides (CITOGLAS) were prepared. Sections were deparaffinized with xylene, hydrated through 99.9%, 95%, and 70% ethanol. For antigen retrieval, sections were treated for 20 minutes in citrate buffer (pH 6.0) by microwave, and then allow cooling. The slides then treated with 3% hydrogen peroxide for 30 minutes to inactivate endogenous peroxides, washed in phosphate-buffered saline (PBS) solution. Primary antibodies were incubated overnight in a humidity chamber using caspase-3 and Bcl2 primary antibodies (Polyclonal rabbit antibody, 7ml Ready to use, Lab Vision Laboratories), then washed with PBS before applying the biotinylated secondary antibody (Lab Vision Laboratories) for 30 min. Sections washed in PBS, incubated with the streptavidin-biotin complex reagent (Lab Vision Laboratories) for 30 min. A brown color was developed with 3, 3-diaminobenzidinetera hydrochloride (DAB, Lab Vision Laboratories) for 5 min, then washed in distilled water, counterstained with Mayer’s haematoxylin, dehydrated, cleared in xylene, mounted and covered slipped.

2.8.1. Scoring of immunostaining
Screening of sections was done under light microscope magnification X200. Positivity for Bcl2protein family occurs in the cytoplasm. The immunoexpression of Bcl2 was evaluated based on stain intensity, scored from 0 to 3, with 0 considered negative, 1 as weak, 2 as intermediate and 3 as strong. The number of positive cells was evaluated on a scale of 0 to 3, where 0 corresponded to 0 to 10% of cells, 1 = 11 to 25%, 2 = 26 to 50% and 3 = more than 51% of cells. A score was obtained for each reaction by multiplying the intensity of the reaction by the percentage of positive cells (Krajewska et al., 1996). Regarding caspase-3, tissue section considered positive when > 10% of the cells displayed cytoplasmic staining (Zhang et al., 2009).

2.9. Statistical analysis
Data were presented as the means ± S.E.M. The results were analyzed using the One-way ANOVA followed by Tukey’s multiple comparison tests. Statistical analysis was performed using Graph Pad Prism software (version 5). Differences with P value < 0.05 were considered significant.

3. RESULTS

3.1. Effect of nicorandil and CsA on renal function
CsA administration significantly increased serum urea, creatinine and uric acid as compared with control group. These parameters were significantly improved CsA+nicorandil and CsA+ nicorandil+glibenclamide as compared with CsA (Table 1).
3.2. Effect of nicorandil on CsA induced nitrosative stress

Administration of CsA didn’t significantly affect NO level in renal tissue as compared with control group. Treatment with nicorandil in CsA intoxicated rats increased NO level significantly compared with control (Table 2).

3.3. Effect of nicorandil on CsA induced oxidative changes

CsA-intoxicated rats showed significant elevation in MDA level and reduction in SOD and GSH activities in renal tissue as compared with control group. CsA-treated group either with nicorandil or with nicorandil+glibenclamide significantly decreased MDA or increased GSH as compared with CsA (Table 2). SOD activity was raised only in CsA+ nicorandil compared with CsA.

3.4. Effect on renal histopathological changes

Kidney of rats intoxicated with CsA showed evident histological changes in the form of epithelial cell swelling and vacuolization with many pyknotic nuclei and medullary congestion. Treatment with nicorandil either alone or in combination with glibenclamide improved renal damage as compared with CsA (Figure 1).

3.5. Immunohistochemical expression of Bcl2 and caspase-3 proteins in renal tissue

The expression level of Bcl2 protein in renal tissue was markedly decreased in CsA-intoxicated rats while caspase-3 was increased as compared with control group. However, CsA+ nicorandil and CsA+ nicorandil+glibenclamide groups showed significant increase in expression of Bcl2 and decrease caspase-3 expression compared with CsA group (Figure 2 and 3).

3.6. RT-PCR localization of TGF-β, Collagen Iα1 and eNOS mRNA in renal tissue

CsA caused significant up-regulation of TGF-β (Figure 4) and Collagen Iα1 (Figure 5) expression as well as down-regulation of eNOS expression (Figure 6) in renal tissue in comparison with control groups. There was significant reduction in TGF-β and collagen Iα1 with significant elevation in expression level of eNOS in CsA+ nicorandil and CsA+ nicorandil+glibenclamide groups as compared with CsA.

Table (1): Effect of cyclosporine A (CsA) and nicorandil on serum renal function

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>38.84±3.39</td>
<td>0.54±0.02</td>
<td>1.50±0.08</td>
</tr>
<tr>
<td>Nicorandil</td>
<td>29.60±2.08</td>
<td>0.54±0.02</td>
<td>1.40±0.05</td>
</tr>
<tr>
<td>CsA</td>
<td>62.33±3.44</td>
<td>1.00±0.08</td>
<td>3.81±0.09</td>
</tr>
<tr>
<td>Nicorandil + CsA</td>
<td>30.20±2.45a</td>
<td>0.58±0.01b</td>
<td>1.80±0.07b</td>
</tr>
<tr>
<td>Nicorandil+glibenclamide+CsA</td>
<td>28.17±2.73b</td>
<td>0.68±0.03b</td>
<td>1.50±0.03b</td>
</tr>
</tbody>
</table>

All parameters are expressed as means ± S.E.M. (n=5). a, b significantly different (at P< 0.05) from normal control group and CsA group, respectively. CsA: cyclosporine

Table (2): Effect of Cyclosporine (CsA) and nicorandil on oxidative stress parameters in renal tissue

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (nmol/g tissue)</th>
<th>Nitrate/nitrite level (nmol/ml)</th>
<th>SOD (U/g tissue)</th>
<th>GSH (mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>101.30±7.34</td>
<td>106.71±4.81</td>
<td>1237.10±56.50</td>
<td>103.20±5.11</td>
</tr>
<tr>
<td>Nicorandil</td>
<td>113.51±6.94</td>
<td>275.01±10.82a</td>
<td>1129.20±33.49</td>
<td>122.21±4.48</td>
</tr>
<tr>
<td>CsA</td>
<td>159.54±11.41a</td>
<td>158.80±5.20</td>
<td>445.80±18.25a</td>
<td>71.22±2.30a</td>
</tr>
<tr>
<td>Nicorandil + CsA</td>
<td>120.42±8.47b</td>
<td>246.70±4.10ab</td>
<td>1193.01±55.28b</td>
<td>97.80±3.65b</td>
</tr>
<tr>
<td>Nicorandil+gliben+CsA</td>
<td>109.22±5.53b</td>
<td>252.81±8.21ab</td>
<td>754.83±20.35</td>
<td>105.82±4.33b</td>
</tr>
</tbody>
</table>

All parameters are expressed as means ± S.E.M. (n=5). a, b significantly different (at P< 0.05) from normal control group and CsA group, respectively. CsA: cyclosporine; MDA: malondialdehyde; NO: nitric oxide; SOD: superoxide dismutase; GSH: reduced glutathione.
Figure 1: Histopathological findings in renal sections stained with H&E

(A): Control group with normal renal histology. (B): Nicorandil group has normal renal histology. (C): CsA group showed swelling and vacuolization in epithelial cells of renal tubules, the structure of the epithelial cells was unclear. Blue arrow indicates cellular vacuolization, Black arrow cellular swelling. Many pyknotic nuclei and medullary congestion were also observed. (D) and (E): CsA+Nicorandil group and CsA+Nicorandil+glibenclamide group showing histological improvement (Original magnification 100x). (F): Data are expressed as means ± S.E.M. (n = 5). ** significantly different at (P < 0.05) from normal control group and CsA group; respectively. (CsA): cyclosporine; (Glib): glibenclamide.
Figure 2: Bcl2 immunostaining results in renal tissue
Reduced expression of Bcl2 was noted in the CsA group (C) when compared with control group (A). Furthermore, a more significant increase in its expression was observed after administration of either nicorandil (D) or with glibenclamide (E) in comparison with CsA. (F): Data are expressed as means ± S.E.M. (n = 5). ** significantly different at (P < 0.05) from normal control group and CsA group, respectively. (CsA): cyclosporine; (Glib): glibenclamide.
Figure 3: Caspase-3 immunostaining results in renal tissue
(A): Control group show no immunostaining (200x). (B): Nicorandil group with a trivial expression (200x). (C): CsA group showing high immunostaining (200x). (D) and (E): CsA+ Nicorandil group and CsA+Nicorandil+glibenclamide group showing low expression as compared with CsA group (200x). (F): Data are expressed as means ± S.E.M. (n = 5). "*" significantly different at (P < 0.05) from normal control group and CsA group respectively. (CsA): cyclosporine; (Glib): glibenclamide.
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Figure 4: Analysis of TGF-β gene expression. (A, B) represent RT-PCR gel of TGF-β expression. TGF-β mRNA expression was significantly elevated in CsA group compared to control group. There was significant reduction in expression level of TGF-β in CsA groups treated with nicorandil and nicorandil+glibenclamide. Data are expressed as means ± S.E.M. (n = 5). *# significantly different at (P < 0.05) from normal control group and CsA group, respectively. (Nic): nicorandil; (CsA): cyclosporine; (Glib): glibenclamide; (GAPDH): glyceraldehyde-3-phosphate dehydrogenase.

Figure 5: Analysis of collagen 1A1 gene expression. (A, B) represent RT-PCR gel of collagen 1A1 expression. Collagen1A1 mRNA expression was significantly elevated in CsA group compared to control group. There was significant reduction in expression level of collagen 1A1 in CsA groups treated with nicorandil and nicorandil+glibenclamide. Data are expressed as means ± S.E.M. (n = 5). *# significant difference at (P < 0.05) from normal control group and CsA group, respectively. (Nic): nicorandil; (CsA): cyclosporine; (Glib): glibenclamide; (GAPDH): glyceraldehyde-3-phosphate dehydrogenase.
Figure 6: Analysis of eNOS gene expression. (A, B) represent RT-PCR gel of eNOS expression. eNOS mRNA expression was significantly decreased in CsA group compared to control group. There was significant increase in expression level of eNOS in CsA groups treated with nicorandil or nicorandil+glibenclamide. Data are expressed as means ± S.E.M. (n = 5). *# significantly different at (P < 0.05) from normal control group and CsA group, respectively. (Nic): nicorandil; (CsA): cyclosporine; (Glib): glibenclamide; (eNOS): endothelial nitric oxide synthase; (GAPDH): glyceraldehyde-3-phosphate dehydrogenase.

4. DISCUSSION

Nicorandil, a K<sub>ATP</sub> channel opener, and NO donor could ameliorate CsA toxicity in renal tissue by enhancement of anti-oxidant capacity and damping of inflammatory process as well as decrease pro-fibrotic genes. The results of the present study demonstrated that mechanisms involved in this protection were not likely to couple with activation of K<sub>ATP</sub> channels. In the present work, CsA induced toxicity on the renal tissue was obvious biochemically and histopathologically. Elevated renal urea and creatinine were in harmony with renal morphological changes in the form of tubular degenerations and vacuolization (Korolczuk et al., 2014; Takasu et al., 2015). Furthermore, CsA-induced hyperuricemia was observed in the current study. Development of hyperuricemia may be secondary to a reduction in uric acid excretion that mediates an ischemic vasoconstriction and acts as a pro-oxidant; playing a deleterious role in renal diseases (Mazali et al., 2012; Tamura et al., 2012). Nicorandil offered a significant improvement in renal function and morphology in CsA-intoxicated rats. This was supported by other clinical and experimental studies, which demonstrated its beneficial role in renal insufficiency disorders (Shiraishi et al., 2014; Fan et al., 2016) and renal ischemia reperfusion injury (Shimizu et al., 2011). Mechanisms underlying CsA nephrotoxicity are still not clarified, however; oxygen free radicals still one of the injurious factors. The role of oxidative damage by CsA on the renal tissue was clear in the present study. CsA induced elevation of lipid peroxidation product, MDA, and reduction of antioxidant defense system GSH and SOD levels. Previous studies related CsA-induced reactive oxygen species by NADPH and xanthine oxidases, with cellular injury and reduction in GSH and SOD (Takasu et al., 2015). Anti-oxidant enzymes GSH and SOD are considered major defense mechanisms against oxidative stress associated with CsA administration (Sereno et al., 2014; Korolczuk et al., 2014). In the present work, nicorandil provides its renoprotective effect in part through amelioration of oxidative stress and increasing anti-oxidant system together with indirect action on pro-oxidant system such as uric acid level, this was supported by previous studies in different organs (Shiraishi et al., 2014; Elshazly, 2015). Our finding revealed no detectable effect for glibenclamide, a K<sub>ATP</sub> channels blocker, on resultant
nicorandil protection against CsA nephrotoxicity. That is may exclude the possible role of potassium channel activation. Complementary, the role of NO in both CsA toxicity and protection was further examined. NO is a vasorelaxant agent that maintains vascular tone, decreases renal ischemia, mesangial cell proliferation and inflammatory cell infiltration (Yoon and Yang 2009). CsA administration in the present study significantly down-regulated the expression of renal eNOS mRNA but the overall effect on NO level had no change. This could be explained theoretically by accompanying the rise in iNOS production. Level of NO varies among different models in CsA nephrotoxicity. This difference is due to the balance between its different NOS isoforms (Shin et al., 2012; El-Kashef et al., 2016). The beneficial role of nicorandil in our model is thought to be mediated by NO/cGMP signaling pathway. This was evident by the significant elevation in NO level with eNOS up-regulation in CsA+nicorandil-treated rats. NO/cGMP was proved to inhibit the overexpression of inflammatory and fibrogenic mediators such as TGF-β and collagen proteins (Sudo et al., 2009; Tashiro et al., 2015). In accordance with others (Naensens et al., 2009; Mazali et al., 2012), administration of CsA was associated with increased TGF-β and collagen 1A1 mRNA expression in renal tissues. TGF-β, a growth factor released by macrophages, implicated in chronic CsA toxicity (Mazali et al., 2012) by promoting interstitial fibrosis and induction of various forms of renal injury (Ling et al., 2003). Treatment with nicorandil attenuated the overexpression of TGF-β and collagen 1A1 mRNA in CsA-intoxicated rats indicating its role as an anti-fibrotic agent. Our findings confirmed that apoptosis represents one of the mechanisms of renal injury induced by CsA. Apoptosis results in tubular injury and loss of functional mass. Apoptosis is regulated by different caspases. Caspases mediate cell injury and anti-apoptotic agents responsible for cell survival (Yoon and Yang 2009). Caspase-3 that is specific for apoptosis was significantly reduced in CsA group with a significant reduction in Bcl2 level, responsible for cell survival, which is in agreement with previous reports (Yang et al., 2002; Lai et al., 2015). Nicorandil treatment was able to protect tubular cells against CsA-induced apoptosis by regulation of apoptosis process. Immunostaining for caspase-3 was significantly attenuated in rats treated with nicorandil associated with significant increase in anti-apoptotic marker; Bcl2 level. In support of our results, other findings demonstrated that nicorandil inhibited apoptosis in different organs (Li et al., 2015; Zhang et al., 2015).

5. CONCLUSION

The present study demonstrated the promising defensive role of nicorandil against CsA-induced nephrotoxicity with different proposed mechanisms. Nicorandil exerts its protection through anti-oxidant role by attenuating lipid peroxidation and increasing anti-oxidant enzymes. In addition; significant anti-apoptotic and anti-fibrotic mechanisms were also explored.

Declaration of interest

The authors declare no conflict of interest.

6. REFERENCES


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