Chemopreventive Effect of Tadalafil in Cisplatin-Induced Nephrotoxicity in Rats

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Summary: Nephrotoxicity remains a common untoward effect of cisplatin therapy with limited effective chemopreventive options available till date. This study aims to evaluate the possible chemopreventive effect and mechanism(s) of action of 2 mgkg\(^{-1}\) and 5 mgkg\(^{-1}\) of Tadalafil in cisplatin-induced nephrotoxic rats. In this study, twenty-five male Wistar rats were randomly divided into five groups (n = 5 rats per group) and daily pretreated with oral doses of distilled water (10 mLkg\(^{-1}\)), ascorbic acid (100 mgkg\(^{-1}\)), Tadalafil (2 mgkg\(^{-1}\) and 5 mgkg\(^{-1}\)) for 7 days before cisplatin (5 mgkg\(^{-1}\), intraperitoneal) was administered. 72 hours post-cisplatin injections, rats were sacrificed humanely and blood samples for serum electrolytes, urea and creatinine and renal tissues for reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and malonialdehyde dehydrogenase (MAD) assays and histopathology were collected. Results showed that cisplatin injection caused significant decreases in the serum sodium (Na\(^{+}\)), potassium (K\(^+\)), bicarbonate (HCO\(_3\)), calcium (Ca\(^{2+}\)), phosphate (PO\(_4\)^{3-}\)) and concomitant significant increases in the serum urea and creatinine levels. In addition, there were significant decreases in the renal tissue GSH, SOD, CAT and increased MAD and GSH-Px levels which were corroborated by histopathological features of tubulonephritis. However, these histobiochemical alterations were significantly attenuated by ascorbic acid and Tadalafil pretreatments. Overall, results of this study showed the chemopreventive potential of Tadalafil against cisplatin-induced nephrotoxicity which was possibly mediated via antioxidant and anti-lipoperoxidation mechanisms.

Keywords: Cisplatin-induced nephrotoxicity, Renal function parameters, Oxidative markers, Histopathology
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INTRODUCTION

Cisplatin [cis-diamminechloroplatinum (II)] is a divalent, inorganic, water-soluble platinum-coordination complexes alkylating antineoplastic drugs used for the treatment of solid tumors such as testicular, ovarian, bladder, head and neck, lung and colon tumors (Kintzel, 2001; Morgan et al., 2012; Ozkok and Edelstein, 2014). Its chief dose-limiting extramedullary side-effects include amongst others peripheral motor and sensory neuropathy, ototoxicity and nephrotoxicity with 20% of patients on high-dose cisplatin treatment eventually developing severe renal dysfunction (Go and Adjei, 1999; Tew et al., 2001; Ekborn et al., 2003; Yao et al., 2007).

The mechanism for cisplatin-related renal cell injury has been the focus of intense investigation for many years, and recent studies suggest that the in vivo mechanisms of cisplatin nephrotoxicity are complex and involve oxidative stress, apoptosis, inflammation, and fibrogenesis of the renal proximal convoluted tubules (Lieberthal et al., 1996; Li et al., 2014). Reactive oxygen species (ROS) which are produced via the xanthine-xanthine oxidase system, mitochondria, and reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in renal cells act directly on the renal cell components, including lipids, proteins, and DNA, and compromise renal cell integrity (Kawai et al., 2006). Cisplatin induces glucose-6-phosphate dehydrogenase and hexokinase activity, which increase free radical production and decrease antioxidant production (Yilmaz et al., 2004). Cisplatin also increases intracytoplasmic calcium level which activates NADPH oxidase and stimulates ROS (such superoxide anion, hydrogen peroxide and hydroxyl radical) production within the damaged mitochondria of the cisplatin-treated kidneys (Shino et al., 2003; Kawai et al., 2006). These free radicals damage the lipid components of the cell membrane by peroxidation and denature proteins, leading to enzymatic inactivation as well as mitochondrial dysfunction (Yilmaz et al., 2004). However, vigorous hydration with saline and simultaneous administration of mannitol before, during, and after cisplatin administration, has been reported to reduce...
cisplatin-induced nephrotoxicity. This strategy has been accepted as standard clinical practice in the prevention of cisplatin-related nephrotoxicity (Carnelison and Reed, 1993) although another randomized trial demonstrated that saline alone or with furosemide offers better renal protection than saline plus mannitol (Santoso et al., 2003). Other chemotherapeutic drugs used clinically in ameliorating cisplatin-related nephropathy include amifostine (Capizzi, 1999), procarcinamide (Viale et al., 2000), interleukin-10 (Deng et al., 2001), N-acetylcysteine (Nisar and Feinfeld, 2002), salicylate (Li et al., 2002), fibrate (Nagothu, 2008), allopurinol and ebselen (Lynch et al., 2005), serum thymic factor (Kohda et al., 2005), glutamine (Mora et al., 2003), melatonin (Kilic et al., 2013), captopril (El-Sayed et al., 2008), capsaicin (Shimeda et al., 2005), lipoic acid (Somani et al., 2008) and most recently metformin (Li et al., 2016) and trimetazidine (El-Sherbeeny and Attia, 2016). Despite availability of these therapeutic strategies, they are costly and have limited effectiveness as some patients on these therapies still progress to develop severe cisplatin-related renal dysfunctions and/or other systemic toxicities (Vermeulen et al., 1993). In view of these drawbacks, the current study has been designed primarily at exploring the possible chemopreventive effect and mechanism(s) of action of 2 mg/kg and 5 mg/kg of Tadalafil, a long-acting phosphodiesterase-5 (PDE-5) inhibitor, against cisplatin-induced nephrotoxicity in Wistar rats, outside its current therapeutic uses in the management of erectile dysfunction and pulmonary arterial hypertension. This is with the view of discovering and developing a new effective chemopreventive drug available for cisplatin-related renal toxicities and without significantly altering its antineoplastic activity. Doses of Tadalafil and cisplatin used in the present study were chosen based on results of previous studies (Greggi-Antunes et al., 2000; Ajiboye and Oluwole, 2012) and results of the preliminary study conducted.

MATERIALS AND METHODS

**Drugs and Chemicals**

Ascorbic acid (Sigma Chemical Co., St. Louis, Missouri, U.S.A.), Tadalafil (Tadalis-20®, Evans Medical Plc, Agbara Industrial Estate, Ogun State, Nigeria), Cisplatin (Cisplatyl® 50 mg, Laboratoire Roger Bellon, France).

**Experimental Animals**

A total of twenty-five young adult male Wistar rats were procured from the Rat Colony of Animal House, College of Medicine of the University of Lagos, Ibadan, Nigeria, in the month of August, 2012, after institutional ethical clearance has been obtained for this study. The rats were allowed to acclimatize under standard laboratory conditions for 14 days at the Rat Colony of the Animal House, Lagos State University College of Medicine, Ikeja, Lagos State, Nigeria. Rats were cared for and handled according to existing guidelines on the Use and Care of Experimental Animals as prescribed by United States National Institutes for Health (1985). They were allowed free access to standard rat feed and potable drinking water for 2 weeks before being used for experimentation.

**Induction of cisplatin-induced nephrotoxicity and drug treatment**

Twenty-five adult male Wistar rats were randomly allotted to five groups (Groups I-V) of five rats per group such that the weight difference between and within groups does not exceed ±20% of the mean sample population. The rats were orally pretreated with distilled water, ascorbic acid and Tadalafil at between 07:00 hour and 09:00 hour once daily for 7 days. Twenty-four hours after the last oral treatment with 10 mLkg⁻¹ distilled water, 100 mgkg⁻¹ of ascorbic acid and 2 mgkg⁻¹ and 5 mgkg⁻¹ of Tadalafil, rats in Groups II-V were treated with 5 mgkg⁻¹ of cisplatin given through the intraperitoneal route as described by Greggi-Antunes et al. (2000) and Chen-zhe et al. (2012). Details of drug treatments of rats are as follows:

- **Group I**: oral treatment with 10 mLkg⁻¹ of distilled water for 7 days + 1 mLkg⁻¹ of distilled water i.p. on 8th day
- **Group II**: oral treatment with 10 mLkg⁻¹ of distilled water for 7 days + 5 mgkg⁻¹ of cisplatin i.p. on 8th day
- **Group III**: oral treatment with 100 mgkg⁻¹ of ascorbic acid for 7 days + 5 mgkg⁻¹ of cisplatin i.p. on 8th day
- **Group IV**: oral treatment with 2 mgkg⁻¹ of Tadalafil in distilled water for 7 days + 5 mgkg⁻¹ of cisplatin i.p. on 8th day
- **Group V**: oral treatment with 5 mgkg⁻¹ of Tadalafil in distilled water for 7 days + 5 mgkg⁻¹ of cisplatin i.p. on 8th day

**Blood collection and measurement of renal function parameters**

Seventy-two hours after induction with 5 mgkg⁻¹ of cisplatin, rats were sacrificed humanely under inhaled halothane and blood samples were collected directly from the heart chambers using 21 G needles mounted upon a 5 mL syringe (Unique Pharmaceuticals, Sango Otta, Ogun State, Nigeria). Each blood sample obtained for each rat was collected into a well labeled 10 mL capacity plain sample bottle. The blood samples were allowed for complete clotting for about 1-2 hours before they were centrifuged with Uniscope Laboratory Centrifuge (Model SM 112, Surgifriend Medicals, England) at 2000 revolutions per minute for 15 minutes. This was aimed at separating the sera from clotted blood cells. The sera were carefully separated into new, well labeled, corresponding plain
sample bottles at room temperature 23-26 °C. The sera were assayed for serum sodium, potassium, bicarbonate, urea and creatinine. Serum creatinine and blood urea were assayed using Rando Diagnostic kits (Randox Laboratories Ltd., Crumlin, U.K.) by methods of Varley and Alan (1984) and Tietz et al. (1994).

Serum levels of sodium, potassium, chloride, calcium, bicarbonate and phosphate were determined using the ISE 6000 BYF SFRI spectrophotometer. The machine when powered on carries out self-calibration for all parameters. When calibration was complete, the serum sample was placed into the probe and the tune button on the machine was pressed on the screen of the machine. The machine aspirated the sample and beeped with a screen display “remove sample”. The machine then processed the sample and displayed the test result. The machine then printed out the result of the test showing all the required electrolyte levels namely: sodium, potassium, chloride, bicarbonate, calcium and phosphate.

**Collection of kidney for renal tissue oxidative stress markers assay**

After blood collection through cardiac puncture, a deep longitudinal incision was made into the ventral surface of the rat abdomen. The kidneys were identified and thereafter, harvested from each animal. One of the kidneys was rinsed in 1.15% potassium chloride (KCl) solution in order to preserve the oxidative enzyme activities of the kidney before being placed in a clean sample bottle which itself was in an ice-pack filled cooler. This was to prevent the breakdown of the enzymes for kidney function enzyme biomarkers.

**Determination of renal tissue superoxide dismutase activity**

Superoxide dismutase (SOD) activity in renal homogenate was determined according to the method of Sun and Zigma (1978) and Usoh et al. (2005). This method is based on the generation of superoxide anions by pyrogallol autoxidation, detection of generated superoxide anions by nitro blue tetrazolium (NBT) formazan color development and measurement of the amount of generated superoxide anions scavenged by SOD (the inhibitory level of formazan color development). The homogenate was centrifuged at 105,000 for 15 minutes at 4 °C. 0.25 mL of supernatant, 0.5 mL of tris cacodylic buffer, 0.1 mL of 16% triton X-100 and 0.25 mL NBT were added. The reaction was started by the addition of 0.01 mL diluted pyrogallol. Incubation was maintained for 5 minutes at 37 °C. The reaction was stopped by the addition of 0.3 mL of 2 M formic acid. The formazan color developed was determined spectrophotometrically (Spectronic 501, Shimadzu). Enzymatic activity was expressed as Umg⁻¹ protein.

**Determination of renal tissue catalase activity**

Serum catalase activity was determined using the method of Gaetani et al. (1989) by measuring the decrease in absorbance at 240 nm due to the decomposition of hydrogen peroxidase (H₂O₂) in UV recording spectrophotometer. The reaction mixture (3 mL) contained 0.1 mL of serum in phosphate buffer (50 mM, pH 7.0) and 2.9 mL of 30 mM of H₂O₂ in the phosphate buffer pH 7.0. An extinction coefficient for H₂O₂ at 240 nm of 40.0 M⁻¹ cm⁻¹ according to Aebi (1984) was used for calculation. The specific activity of catalase was expressed as moles of reduced H₂O₂ per minutes per mg protein (Umg⁻¹ protein).

**Determination of renal tissue glutathione peroxidase activity**

The renal tissue glutathione peroxidase activity was determined using the method described by Gaetani et al. (1989). Glutathione peroxidase (GSH-Px) was determined in renal homogenate according to the method of Lawrence and Burk (1976). This method is based on measuring the oxidation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) using hydrogen peroxide as the substrate. A reaction mixture of 1 mL contained 50 mM potassium phosphate buffer (pH = 7), 1 mM disodium ethylene diamine tetra acetic acid (EDTA), 1 mM sodium trinite (NaN₃), 0.2 mM NADPH, 1 unit/mL oxidized glutathione reductase and 1 mM GSH was prepared. The homogenate was centrifuged at 105,000 for 15 minutes at 4 °C. 0.1 mL of the supernatant was added to 0.8 mL of the reaction mixture and the solution was incubated for 5 minutes at 25 °C. 0.1 mL of 0.25 mM hydrogen peroxide solution was added to initiate the reaction. Absorbance was measured at 340 nm for 5 minutes, and an extinction coefficient of 6.22 × 10⁻³ was used for calculation. The results were expressed as μmolmin⁻¹ g⁻¹ tissue. The changes in the absorbance at 340 nm were recorded at 1 min interval for 5 min. The results were expressed as Umg⁻¹ protein.

**Determination of renal tissue reduced glutathione activity**

In the renal homogenate, reduced glutathione (GSH) was determined according to the methods of Ellman (1959) and as adopted by Sedlak and Lindsay (1968). The method is based on the reduction of Ellman’s reagent [5,5’-dithio-bis- (2-nitrobenzoic acid)] by SH groups to form 1.0M of 2-nitro-5-mercaptopbenzoic acid per mole of SH. The nitro-mercaptopbenzoic acid has an intense yellow color and can be determined spectrophotometrically. To 0.5 mL of 10% trichloroacetic acid, 6 mM disodium EDTA, 0.5 mL of homogenate was added and shaken gently for 10-15 minutes. This was followed by centrifugation at 2,000 rpm for 5 minutes. 0.2 mL of the supernatant
was mixed with 1.7 mL of 0.1M potassium phosphate buffer (pH = 8). At least a duplicate was made for each sample. 0.1 mL of Ellman’s reagent was added to each tube. After 5 minutes the optical density was measured at 412 nm against a reagent blank. The results were expressed as μmol·mg⁻¹ protein.

**Determination of renal tissue malondialdehyde activity**

Malondialdehyde (MDA) levels in renal tissue homogenates were determined spectrophotometrically using the method of Buege and Aust (1978). 0.5 mL of tissue homogenate was shaken with 2.5 mL of 20% trichloroacetic acid in a 10 mL centrifuge tube. To the mixture, 1 mL of 0.67% thiobarbituric acid was added, shaken and warmed for 30 minutes in a boiling water bath followed by rapid cooling. Then 4 mL of n-butyl-alcohol was added and shaken. The mixture was centrifuged at 3,000 rpm for 10 minutes. The resultant n-butyl-alcohol layer was taken and MDA content was determined from the absorbance at 535 nm. The results were expressed as μmol·mg⁻¹ protein.

**Histopathological studies of rat kidneys**

After the animals were sacrificed, postmortem examination was performed. The rat kidneys were identified and carefully dissected out en bloc for histopathological examination. After rinsing in normal saline, sections were taken from each harvested kidney. The tissue was fixed in 10% formal saline, dehydrated with 100% ethanol solution and embedded in paraffin. It was then processed into 4-5 μm thick sections stained with hematoxylin and eosin and observed under a photomicroscope (Model N - 400ME, CEL-TECH Diagnostics, Hamburg, Germany).

**Table 1.** Effect of 2 mg·kg⁻¹ and 5 mg·kg⁻¹ of Tadalafil and 100 mg·kg⁻¹ of ascorbic acid on the serum electrolytes, urea and creatinine in cisplatin-treated rats

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺ (mmol/L)</td>
<td>141.60 ± 1.21</td>
<td>121.60 ± 1.17ᵇ</td>
<td>130.40 ± 1.75ᵈ</td>
<td>126.60 ± 1.57ᶜ</td>
<td>135.60 ± 1.29ᵉ</td>
</tr>
<tr>
<td>K⁺ (mmol/L)</td>
<td>6.94 ± 0.20</td>
<td>3.90 ± 0.22ᵇ</td>
<td>5.60 ± 0.14ᵇ</td>
<td>5.12 ± 0.09ᵇ</td>
<td>6.54 ± 0.19ᵇ</td>
</tr>
<tr>
<td>Cl⁻ (mmol/L)</td>
<td>100.30 ± 2.25</td>
<td>79.80 ± 2.87ᵇ</td>
<td>96.60 ± 1.83ᵉ</td>
<td>92.80 ± 1.53ᵉ</td>
<td>98.40 ± 0.51ᵉ</td>
</tr>
<tr>
<td>HCO₃⁻ (mmol/L)</td>
<td>14.02 ± 0.53</td>
<td>7.88 ± 0.32ᵇ</td>
<td>9.84 ± 0.17ᵈ</td>
<td>9.02 ± 0.12ᵈ</td>
<td>9.74 ± 0.12ᵈ</td>
</tr>
<tr>
<td>Ca²⁺ (mmol/L)</td>
<td>1.73 ± 0.05</td>
<td>0.64 ± 0.09ᵇ</td>
<td>1.32 ± 0.09ᵉ</td>
<td>1.10 ± 0.07ᵉ</td>
<td>1.38 ± 0.07ᵉ</td>
</tr>
<tr>
<td>PO₄³⁻ (mmol/L)</td>
<td>1.44 ± 0.06</td>
<td>0.68 ± 0.09ᵇ</td>
<td>1.94 ± 0.05ᵈ</td>
<td>0.90 ± 0.04ᶜ</td>
<td>1.16 ± 0.09ᵉ</td>
</tr>
<tr>
<td>Urea (mg·dl⁻¹)</td>
<td>6.74 ± 0.18</td>
<td>17.28 ± 1.35ᵈ</td>
<td>9.50 ± 0.24ᶜ</td>
<td>7.88 ± 0.25ᶜ</td>
<td>0.70 ± 0.18ᵗ</td>
</tr>
<tr>
<td>Creat (mg·dl⁻¹)</td>
<td>65.40 ± 1.58</td>
<td>102.00 ± 4.69ᵃ</td>
<td>81.70 ± 1.97ᵗ</td>
<td>85.80 ± 2.42ᶜ</td>
<td>74.40 ± 3.42ᵗ</td>
</tr>
</tbody>
</table>

ᵃ andᵇ represent significant increases and decreases at p<0.0001, respectively, when compared to Group I values; ᶜᵈ andᵉ represent significant increases at p<0.05, p<0.001 and p<0.0001, respectively and ¹ represent a significant decreases at p<0.0001 when compared to Group II values. Group I: oral treatment with 10 mL·kg⁻¹ of distilled water + 1 mL·kg⁻¹ of distilled water i.p. Group II: oral treatment with 10 mL·kg⁻¹ of distilled water + 5 mg·kg⁻¹ of cisplatin i.p. Group III: oral treatment with 100 mg·kg⁻¹ of ascorbic acid + 5 mg·kg⁻¹ of cisplatin i.p. Group IV: oral treatment with 2 mg·kg⁻¹ of Tadalafil in distilled water + 5 mg·kg⁻¹ of cisplatin i.p. Group V: oral treatment with 5 mg·kg⁻¹ of Tadalafil in distilled water + 5 mg·kg⁻¹ of cisplatin i.p.

**Statistical Analysis**

Data were expressed as mean ± standard error of the mean (SEM) of five rats and analyzed using One-way Analysis of Variance followed by Newman-Keuls test as post hoc test on statistical software package, GraphPad Prism (Graph Pad Software; version 5.0, Graph Pad Software Inc., La Jolla, California, U.S.A.). Significant levels were considered at p<0.05, p<0.001, p<0.0001.

**RESULTS**

**Effect of 2 mg·kg⁻¹ and 5 mg·kg⁻¹ of Tadalafil on serum electrolytes, urea and creatinine in cisplatin-induced nephrotoxic rats**

Single intraperitoneal injection of 5 mg·kg⁻¹ of cisplatin was associated with significant (p<0.0001) decreases in the serum levels of sodium, potassium, chloride, bicarbonate, calcium, phosphate and significant (p<0.0001) increases in the serum urea and creatinine concentrations in the untreated model control (Group II) rats when compared with untreated control (Group I) rats (Table 1). However, daily oral pre-treatment with 2 mg·kg⁻¹ and 5 mg·kg⁻¹ of Tadalafil for 7 days before cisplatin injection significantly (p<0.05, p<0.0001) attenuated decreases in the serum sodium, potassium, chloride, bicarbonate, calcium and phosphate concentrations in a dose-dependent fashion when compared to untreated model control (Group II) values (Table 1). In the same pattern, 2 mg·kg⁻¹ and 5 mg·kg⁻¹ of Tadalafil also significantly (p<0.05, p<0.0001) attenuated significant elevation in the serum urea and creatinine concentrations when compared to Group II values (Table 1). In addition, the protective effects of 5 mg·kg⁻¹ of Tadalafil on serum electrolytes, urea and

Tadalafil prevents cisplatin-induced nephrotoxicity in rats 4
Table 2. Effect of 2 mg kg\(^{-1}\) and 5 mg kg\(^{-1}\) of Tadalafil and 100 mg kg\(^{-1}\) of ascorbic acid on oxidative stress markers in cisplatin-treated kidney tissues.

<table>
<thead>
<tr>
<th>Group</th>
<th>GSH (µmol/mg(^{-1}) protein)</th>
<th>SOD (U mg(^{-1}) protein)</th>
<th>CAT (U mg(^{-1}) protein)</th>
<th>MDA (µmol/mg(^{-1}) tissue)</th>
<th>GSII-Px (U mg(^{-1}) protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.68 ± 0.07</td>
<td>12.92 ± 1.25</td>
<td>54.65 ± 4.39</td>
<td>0.15 ± 0.02</td>
<td>02.42 ± 0.39</td>
</tr>
<tr>
<td>II</td>
<td>0.20 ± 0.03(^{a})</td>
<td>05.54 ± 0.43(^{b})</td>
<td>23.66 ± 1.05(^{b})</td>
<td>0.44 ± 0.03(^{c})</td>
<td>04.86 ± 0.48(^{c})</td>
</tr>
<tr>
<td>III</td>
<td>01.02 ± 0.07(^{d})</td>
<td>16.35 ± 1.06(^{d})</td>
<td>65.78 ± 3.24(^{d})</td>
<td>0.27 ± 0.02(^{e})</td>
<td>03.56 ± 0.18(^{e})</td>
</tr>
<tr>
<td>IV</td>
<td>01.37 ± 0.11(^{d})</td>
<td>11.90 ± 0.85(^{d})</td>
<td>45.30 ± 2.41(^{d})</td>
<td>0.29 ± 0.03(^{e})</td>
<td>03.62 ± 0.16(^{f})</td>
</tr>
<tr>
<td>V</td>
<td>02.28 ± 0.15(^{d})</td>
<td>19.59 ± 1.11(^{d})</td>
<td>50.13 ± 3.24(^{d})</td>
<td>0.21 ± 0.02(^{e})</td>
<td>03.06 ± 0.25(^{f})</td>
</tr>
</tbody>
</table>

\(^{a}\) and \(^{b}\) represent significant decreases at \(p<0.001\), \(p<0.0001\), respectively, while \(^{c}\) represents a significant increase at \(p<0.0001\) when compared to Group I values; \(^{d}\) represent a significant increase at \(p<0.0001\) while \(^{e}\) and \(^{f}\) represent significant decreases at \(p<0.05\), \(p<0.001\) and \(p<0.0001\), respectively, when compared to Group II values.

Group I: oral treatment with 10 mL kg\(^{-1}\) of distilled water + 1 mL kg\(^{-1}\) of distilled water i.p.
Group II: oral treatment with 10 mL kg\(^{-1}\) of distilled water + 5 mg kg\(^{-1}\) of cisplatin i.p.
Group III: oral treatment with 100 mg kg\(^{-1}\) of ascorbic acid + 5 mg kg\(^{-1}\) of cisplatin i.p.
Group IV: oral treatment with 2 mg kg\(^{-1}\) of Tadalafil in distilled water + 5 mg kg\(^{-1}\) of cisplatin i.p.
Group V: oral treatment with 5 mg kg\(^{-1}\) of Tadalafil in distilled water + 5 mg kg\(^{-1}\) of cisplatin i.p.

Figure 1. Transverse sections of rat kidney in (A) showing normal renal architecture in Normal rats treated with 10 mL kg\(^{-1}\) distilled water; (B) 5 mg kg\(^{-1}\) cisplatin-treated rats showing remarkable proximal tubular swellings and degenerative necrosis of the proximal tubules; (C) 100 mg kg\(^{-1}\) ascorbic acid-pretreated, cisplatin-treated kidney showing few areas of early tubular necrosis and hemorrhages; (D) 2 mg kg\(^{-1}\) Tadalafil-pretreated, cisplatin-treated kidney showing few patchy proximal tubular necrosis of the kidney, and; (E) 5 mg kg\(^{-1}\) Tadalafil showing glomeruli with no remarkable changes but few insignificant, patchy proximal tubular necrosis. (X400, H and E)
Histopathological studies of daily oral pretreatment with 2 mg/kg\textsuperscript{1} and 5 mg/kg\textsuperscript{1} of Tadalafil on cisplatin-treated renal tissue

Figure 1A depicts architecture of normal rat kidney showing intact glomerulus and tubules. However, single intraperitoneal injection of 5 mg/kg\textsuperscript{1} of cisplatin was associated with remarkable proximal tubular swellings and degenerative necrosis of the proximal tubules (Figure 1B) when compared to normal renal architecture of the kidney (Figure 1A). In rat kidneys pretreated with 100 mg/kg\textsuperscript{1} of ascorbic acid, the proximal tubule showed few areas of tubules with early necrosis and hemorrhages (Figure 1C) while rat kidneys pretreated with 2 mg/kg\textsuperscript{1} and 5 mg/kg\textsuperscript{1} of Tadalafil, glomeruli showed no remarkable glomerular changes but few insignificant and patchy proximal tubular necrosis of the kidneys (Figures 1D and 1E, respectively).

DISCUSSION

Drugs are a common source of acute renal injury resulting in drug-induced nephropathy, a clinical condition that is often reversible when detected early since its clinical signs may not be apparent in its early phases until it reaches an advanced stage when acute deterioration of the renal function or chronic renal insufficiency manifest (Modesti et al., 2003). Drugs notorious for causing nephropathies include penicillins and cephalosporins, cimetidine, diuretics, allopurinol, NSAIDs, angiotensin converting enzyme inhibitors, cyclosporine, aminoglycosides, lithium, amphotericin B, radiocontrast agents, cisplatin, quinolones, among others (Paller, 1990; Hoitsma et al., 1991; Naughton, 2008; Kodama et al., 2014). Cisplatin as a causative agent for drug-related nephropathy which may manifest as pre-renal azotemia, fluid and electrolyte imbalance, acute tubular necrosis, acute interstitial nephritis, and chronic interstitial nephritis, is well documented (Miller et al., 2010). Drugs involved in causing nephrotoxicity exert their deleterious effects through one or more pathophysiologic mechanisms which include altered intraglomerular haemodynamics, tubular cell toxicity, inflammation, crystal nephropathy, rhabdomyolysis, and thrombotic microangiopathy (Zager, 1997; Perazella, 2003; Schetz et al., 2005; Naughton, 2008; Miller et al., 2010, El-Sherbeeny and Attab, 2016).

Serum electrolytes, uric acid, urea and creatinine are considered reliable indirect markers of renal function test parameters and profound alterations in the serum levels of these markers are diagnostic of nephropathy (Hakim and Lazarus, 1988; Gowda et al., 2010; Saka et al., 2012), although most recently, Cystatin C and β-trace protein are considered more effective markers of glomerular filtration rate and better diagnostic parameters of nephropathy (Gowda et al., 2010). In the present study, nephrotoxicity was reliably established with single intraperitoneal injection of 5 mg/kg\textsuperscript{1} of cisplatin to treated rats and this nephrotoxicity was associated with profound reductions in the serum electrolytes and profound increases in the serum urea and creatinine levels which are consistent with those earlier reported (Greggi-Antunes et al., 2000; Prabhu et al., 2013). However, the significant attenuations of reductions in the serum Na\textsuperscript{+}, K\textsuperscript{+}, Cl\textsuperscript{-}, HCO\textsubscript{3}\textsuperscript{-}, Ca\textsuperscript{2+} and PO\textsubscript{4}\textsuperscript{2-} and concomitant elevations in the serum urea and creatinine in rats pretreated with 2 mg/kg\textsuperscript{1} and 5 mg/kg\textsuperscript{1} of Tadalafil are indicative of the possible protective effect of Tadalafil against cisplatin-induced nephrotoxicity.

Another notable finding of this study was the effect of cisplatin on oxidative stress markers. Literature has shown that cisplatin-induced nephrotoxicity to be significantly associated with significant reductions in the renal tissue levels of GSH, CAT and SOD and concomitant elevations in the tissue activities of MAD and GSH-Px (Nisar and Feinfeld, 2002; Kadikoylu et al., 2004; Karimi et al., 2005) and our results are consistent with those previously reported. Cisplatin is known to induce nephrotoxicity via generation of toxic, highly reactive oxygen-free radicals resulting increase in lipid peroxidation and a decrease in the activity of enzymes protecting the body against lipid peroxidation as well as decrease in the body’s antioxidant status (Sadzuka et al., 1992a; Sadzuka et al., 1999b; Karimi et al., 2005). In addition, reactive nitrogen species have also been implicated in the mechanism of cisplatin-induced nephrotoxicity resulting in an increase in the renal content of peroxynitrite and nitric oxide (Sadzuka et al., 1994; Yildirim et al., 2003). Peroxynitrite causes changes in protein structure and function, lipid peroxidation, chemical cleavage of DNA, and reduction in cellular defenses by oxidation of thiol pools resulting in cisplatin-induced nitrosative stress and nephrotoxicity (Chirino et al., 2004). However, reactive/oxidative stress is measured by the activities of oxidative enzyme markers such as catalase, superoxide dismutase and glutathione levels while that of lipid peroxidation is measured through malonaldehyde dehydrogenase and glutathione peroxidase activities (Nisar and Feinfeld, 2002; Kadikoylu et al., 2004; Karimi et al., 2005). Also, cisplatin is known to primarily cause tubulo-interstitial lesions affecting the proximal tubules, specifically the S3 segment of the outer medullary stripe (Tanaka et al., 1986; Vickers et al., 2004). Again, the histological features of tubulo-interstitial nephritis induced by cisplatin in this study is also in strong agreement with that previously reported by Tanaka et al. (1986) and Vickers et al. (2004).

Tadalafil is a potent, highly selective and long-acting type-5 cyclic guanosine monophosphate (cGMP) phosphodiesterase-5 inhibitor which has been widely reported to be highly efficacious and well tolerated by a broad population of men with...
erectile dysfunction (Padma-Nathan, 2003; Eardley et al., 2004; Broderick et al., 2006; Porst et al., 2006; La Vignera et al., 2011) and to control symptoms and signs of benign prostatic hypertrophy (Egerdie et al., 2012). Tadalafil is clinically useful for the prevention of erectile dysfunction after radiotherapy for prostate cancer (Pisansky et al., 2014). It inhibits PDE-5 in the corpus cavernosum to help achieve and maintain penile erection (Latties, 2009). It is also approved for World Health Organization group I pulmonary arterial hypertension (PAH) to improve exercise ability (Humbert et al., 2004). Sildenafil, another prototype of PDE-5 inhibitor, has been reported to boost human erythrocyte antioxidant status by enhancing activities of erythrocyte superoxide dismutase and catalase (Perk et al., 2008) as well as prevent vascular oxidative stress in insulin resistant rats by increasing NO release and regulating vascular superoxide release (Oudot et al., 2009). Tadalafil has equally been reported to possess antioxidant activity mediated via increased tissue/serum levels of nitric oxide and increased serum activity of SOD (Serarslan et al., 2010; Koka et al., 2010; La Vignera et al., 2012). A recent study has reported the protective effect of 10 mgkg⁻¹ of Tadalafil pretreatment against high intra-abdominal pressure (IAP)-induced renal failure in Congestive Heart Failure (Bishara et al., 2012) while another recent study reported the relaxant effect of Tadalafil in the isolated KCl pre-contracted ileum of both normal and diabetic rat which was mediated via NO and PDE mechanisms (Hekmat et al., 2013). Attenuation of profound reductions in the tissue levels of GSH, CAT and SOD as well as concomitant elevations in the renal tissue levels of MAD and GSH-Px strongly indicate the possible antioxidant and anti-lipoperoxidative effects of Tadalafil. However, this protective effect by Tadalafil was corroborated by the unremarkable histological alterations in the tubulo-interstitial architecture in rats pretreated with 2 mgkg⁻¹ and 5 mgkg⁻¹ of Tadalafil before cisplatin treatment, thus, suggesting protection from cisplatin-induced tubulonephritis.

Overall, the biochemical and histopathological results of this study strongly support the dose-related chemopreventive effect of the daily oral 2 mgkg⁻¹ and 5 mgkg⁻¹ of Tadalafil in cisplatin-induced nephrotoxic rats and this was mediated via antioxidant and anti-lipoperoxidative mechanisms.

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