ABSTRACT

The purpose of this study was to investigate the possible protective effect of SIL against CP-induced nephrotoxicity. Twenty four male Wistar albino rats were classified into four equal groups; normal group, SIL group which received SIL twice (10 mg/kg; i.p. twice; 30 minutes and just before saline administration), CP group which received a single dose of CP (7.5 mg/kg; i.p.) and treated with saline, and CP + SIL group which was treated with the same previous doses. Treatment with SIL was given 2 days before and 4 days after CP administration. On day 4 after CP administration, inulin and para-aminohippurate (PAH) clearances were performed and blood was withdrawn for determination of urea and creatinine. Thereafter, rats were euthanized and kidneys were dissected out for determination of the tissue levels of reduced glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPx) as well as TNF-α level. CP induced renal injury was evidenced decrease in both inulin and PAH clearances and manifested by significant increase in urea and creatinine levels. Moreover, renal injury was associated with decreased renal tissue activities of SOD, and GPx as well as GSH level. Moreover, renal tissue contents of MDA and TNF-α level were increased. Alterations in these biochemical indices of oxidative stress and inflammation due to CP were attenuated by SIL treatment. In conclusion, SIL treatment can protect rats from CP-induced nephrotoxicity through improving glomerular filtration rate and renal blood flow. This ameliorative effect can be partially attributed to its antioxidant and antiinflamatory effects.
**Key words:** Sildenafil, Cisplatin, oxidative stress, antioxidant, anti-inflammatory, glomerular filtration rate, renal blood flow.

1. **INTRODUCTION**

Cisplatin (CP) is a most commonly used broad-spectrum anti-neoplastic agent against different types of human tumors, particularly solid tumors. However, severe side effects of CP such as nephrotoxicity, neurotoxicity, ototoxicity, greatly hamper its chemotherapeutic efficacy (1). The therapeutic efficacy of this drug is often associated with severe toxic effects including nephrotoxicity which is a dose-limiting factor in therapy (2).

Previous studies showed that CP decreased renal blood flow (RBF), glomerular filtration rate (GFR), transglomerular pressure and effective filtration pressure (3). Several mechanisms have been proposed for CP cytotoxicity in renal tubule cells, including formation of reactive oxygen species (ROS), lipid peroxidation, lowering the activities of antioxidant enzymes, depletion of reduced glutathione (GSH) (4), and activation of TNF-α apoptotic pathways (5).

Sildenafil (SIL) is a selective inhibitor of phosphodiesterase-5 (PDE5), which degrades cyclic guanosine monophosphate (cGMP) and has a relaxant effect on the smooth muscle cells of the arterioles (6). Several studies have shown that, in addition to treating erectile dysfunction, SIL has an ameliorative effect on tissue injury including renal ablation model of kidney damage (7) and isoprenaline-induce cardiotoxicity in rat hearts (8). Moreover, it has been found that SIL reduces oxidative stress (9) and has anti-inflammatory effect (7) through NO/cGMP pathway. Another study has shown that SIL has a renoprotective potential against oxidative stress and inflammation in diabetic rats (10). Therefore, the current study was designed to investigate the possible protective effect of SIL against CP-induced nephrotoxicity.

2. **MATERIALS AND METHODS**

2.1. Chemicals and drugs

Sildenafil (Viagra; Pfizer, PA, France) tablets (50 mg) were purchased from Alnahdi Pharmacy and dissolved in tap water. Drug solution was administered to the animals in a volume of 0.5 ml by means of an orogastric tube. Cisplatin (CP) was purchased from Hospira Australia (Melbourne, Australia) as solution (1mg/ml). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).
2.2. Animals
The study was conducted according to the guidelines of the Biochemical and Research Ethical Committee at King Abdulaziz University, Jeddah, Saudi Arabia. Twenty four male Wistar albino rats (250-300 g) were housed in a well-ventilated, temperature controlled (22+3 °C) with 12 hours light/dark cycles. They had free access to water ad libitum and a normal rat chow diet. All experimental procedures were performed between 8-10 a.m. and care was taken to avoid all stressful conditions.

2.3. Experimental design and treatment
One week after acclimatization, rats were divided into four equal groups (6 rats in each group) in separate metabolic cages. Group I served as normal control group received saline, 1ml/kg; intraperitoneally (i.p.). Group II (SIL group) received SIL alone, 10 mg/kg; i.p. twice; 30 minutes and immediately before saline administration (instead of CP). The dose of SIL is based on preliminary study. Group III (CP group) received saline (instead of SIL), 1ml/kg, 30 minutes and immediately before CP administration, 7.5 mg/kg once i.p.(11). Group IV (SIL + CP group) received both SIL and CP in the same previous doses. In groups II, III and IV, the treatment with saline or SIL was administered 2 days before and 4days after saline or CP administration.

After inulin and para-aminohippurate (PAH) clearance studies, the blood samples was obtained directly from carotids and was centrifuged at 3000 xg for 10 minutes to obtain clear sera which were stored at -80 °C for further analysis. Thereafter, rats were euthanized using ether anesthesia the abdomen of each rat was opened and kidneys were rapidly dissected out, washed in ice-cold isotonic saline and blotted between two filter papers. For subsequent analysis, one kidney was immediately removed, immersed in liquid nitrogen and kept at -20°C.

2.4. Assessment of glomerular filtration rate (GFR) and renal blood flow (RBF)
GFR and RBF was assessed through measurement of inulin and para-aminohippurate (PAH) clearances. On day 4 after Cis or saline injection, inulin and PAH clearances were calculated by using inulin and PAH concentrations in plasma and urine samples as recently described by Morales et al. (12). Rats were anesthetized with thiopental sodium (60 mg/kg ip). In the anesthetized animal, the trachea was cannulated with a PE-240 catheter, and spontaneous breathing was maintained. To allow blood sampling, a PE-60 catheter was inserted into the right carotid artery, while the left jugular vein was cannulated with a PE-60 catheter to inject
inulin and PAH (1 mg of each substance solved in 0.25 ml 0.9% NaCl), followed by constant infusion of both substances (5 mg/h) using perfusor Secura FT (Braun, Netherlands). After suprapubic incision, the urine bladder was cannulated with a PE-240 catheter to obtain urine samples. A total of three urine samples were collected at 30-min intervals. Blood samples were obtained at the beginning and the end of the experiment and were centrifuged. Inulin concentrations in urine and plasma were determined by fluorescence spectrometry (Bioscience Tech., NY, USA), whereas PAH concentrations were measured by photospectrometry (Dynatech Laboratories, Inc., Guernsey, UK). Calculations of inulin and PAH clearances were performed according to the equations: inulin clearance = \( \frac{I_U \times V_U}{I_P \times t} \); PAH clearance = \( \frac{PAH_U \times V_U}{PAH_P \times t} \); where \( I_U \) is inulin concentration in urine; \( PAH_U \) is PAH concentration in urine; \( I_P \) is inulin concentration in plasma; \( PAH_P \) is PAH concentration in plasma; \( V_U \) is urine volume; and \( t \) is time of measurement.

2.5. Biochemical assessment of renal function

Blood urea nitrogen (BUN) and serum creatinine were determined by commercial diagnostic kits using automated blood analyzer (Han Fang Medical Instrument Co., Ltd, Shan Dong - China)

2.6. Assessment of renal oxidant/antioxidant status

Reduced glutathione (GSH) was determined according to the method of Moron et al. (13) based on the formation of a yellow-colored complex with Ellman’s reagent. Lipid peroxidation was determined by measuring thiobarbituric acid reactive substances (TBARS) in tissue homogenates referring to the malondialdehyde (MDA) standard calibration curve according to the method of Uchiyama and Mihara (14).

Superoxide dismutase (SOD) levels in the kidney tissue were determined according to the modified method of Kakkar et al. (15). Glutathione peroxidase (GPx) activity was determined by the method of Hafeman et al. (16) based on the degradation of \( \text{H}_2\text{O}_2 \) in the presence of GSH.

2.7. Assessment of TNF-\( \alpha \) in renal tissue

The level of TNF-\( \alpha \) in the kidney tissue homogenate was assessed using a Precoated rat TNF-\( \alpha \) ELISA kit (RayBiotech, Inc., GA, USA) according to the manufacturer's instructions. Results are expressed as pg/mg protein
2.8. Assessment of protein content
The protein content of tissue homogenates was determined by the Lowry protein assay using bovine serum albumin as a standard (17).

2.9. Statistical analysis
All data were expressed as means ± SEM. Assessment of these results was performed using one-way ANOVA procedure followed by Tukey-Kramer multiple comparisons tests using Software GraphPad Prism, Version 5. Results were considered significant when P < 0.05.

3. RESULTS
3.1. Effect on inulin and PAH clearances
Single injection of CP significantly decreased the GFR as measured by renal clearance of inulin (figure 1A). Treatment with SIL has improved this decreased GFR to nearly normal level value. Furthermore, PAH clearance study revealed decrease in the RBF after CP administration (figure 1B) and this decrease was ameliorated by SIL treatment. In addition, figure 1B showed that SIL alone has increased significantly the RBF in normal treated rats.

![Figure 1](image1.png)

Figure 1 Effect Sildenafil (SIL) treatment on cisplatin (CP)-induced alterations in inulin and para-aminohippurate (PAH) clearances. Data are the mean ± SEM of 6 rats in each group. *p < 0.05 vs. normal group. #p < 0.05 vs. CP group

3.2. Effect on BUN and serum creatinine
Administration of CP to rats significantly increased BUN and serum creatinine levels, which are used as index of nephrotoxicity. This was diminished significantly by SIL treatment (figure 2A & B)
Figure 2 Effect Sildenafil (SIL) treatment on cisplatin (CP)-induced alterations in blood urea nitrogen (BUN) and serum creatinine. Data are the mean ± SEM of 6 rats in each group. *p < 0.05 vs. normal group. #p < 0.05 vs. CP group

3.3. Effect on oxidant/antioxidant parameters in kidney tissue

In CP rats, significant depletion of GSH and increase in MDA levels were observed compared to the normal group. Treatment with SIL significantly attenuated these changes in SIL + CP group compared to CP group (table 1). Moreover, renal tissue activities of the antioxidant enzymes; SOD and GPx, were significantly decreased in CP group compared to normal group. Treatment with SIL significantly elevated these levels (table 1).

Table 1 Effect Sildenafil (SIL) treatment on cisplatin (CP)-induced alterations in oxidant/antioxidant parameters including: reduced glutathione (GSH), malonyldialdehyde (MDA), Superoxide dismutase (SOD), and Glutathione peroxidase (GPx).

<table>
<thead>
<tr>
<th></th>
<th>GSH (µ mol/g protein)</th>
<th>MDA (nmol/g protein)</th>
<th>SOD (U/mg protein)</th>
<th>GPx (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2.63 ± 0.19</td>
<td>28.98 ± 1.51</td>
<td>65.00 ± 1.64</td>
<td>35.52 ± 1.66</td>
</tr>
<tr>
<td>SIL</td>
<td>2.90 ± 0.20</td>
<td>30.33 ± 1.65</td>
<td>68.40 ± 1.63</td>
<td>34.82 ± 2.61</td>
</tr>
<tr>
<td>CP</td>
<td>0.97 ± 0.17*</td>
<td>76.30 ± 3.95*</td>
<td>41.60 ± 1.63*</td>
<td>18.20 ± 1.35*</td>
</tr>
<tr>
<td>SIL+CP</td>
<td>2.10 ± 0.12*</td>
<td>62.67 ± 3.65*</td>
<td>59.00 ± 2.12*</td>
<td>28.58 ± 2.37*</td>
</tr>
</tbody>
</table>

Data are the mean ± SEM of 6 rats in each group.

* p < 0.05 vs. normal group

#p < 0.05 vs. CP group
3.4. Effect on TNF-α level in kidney tissue

Rats treated with CP showed marked increase in TNF-α level. This increase has been ameliorated in CP rats treated with SIL (figure 3).

![Figure 3](image_url)

Figure 3 Effect Sildenafil (SIL) treatment on cisplatin (CP)-induced alterations in TNF-α level in tissue homogenate. Data are the mean ± SEM of 6 rats in each group. *p < 0.05 vs. normal group. #p < 0.05 vs. CP group.

4. DISCUSSION

Acute renal failure caused by CP in rats is associated with the loss of renal functions, including severe reductions in GFR and RBF and increased levels of serum creatinine and BUN (18). The results of the present study showed that single dose of CP 7.5 mg/kg i.p produced acute renal failure in the rat, and the changes in renal function were characterized by increases in the BUN and serum creatinine concentrations as well as reduced inulin and PAH clearances indicating reduced both GFR and RBF. These findings agree with a previous study using CP-induced nephrotoxicity model of acute renal failure (19).

The present study showed that these indicators of acute renal failure have been attenuated by using SIL treatment. These ameliorative effects indicate that SIL has improved both GFR and RBF which may be attributed to its vasodilator effect of SIL. It has been found that SIL is a selective and potent inhibitor of cGMP-specific PDE-5, which catalyzes the hydrolysis of cGMP and has a relaxant effect on the smooth muscles of the arterioles (6). Moreover, Santos et al. (20) has shown that SIL provides effective protection against indomethacin-induced gastropathy in rats increasing gastric blood flow.
The exact mechanism of CP-induced nephrotoxicity is not completely understood. However, oxidative stress and production of reactive oxygen species (ROS) has been found to be involved in the pathogenesis of CP-induced renal damage (21, 22). Significant decline in antioxidant enzymes activities and increase in free radicals have been observed both experimentally and clinically during CP treatment (23). Mansour et al. (24) demonstrated that CP induces ROS by depleting the antioxidant system including GPx and GSH. These findings are in accordance with the results of the present study. Treatment with SIL obviously ameliorated these alterations in the antioxidant system.

The present study supports the hypothesis that the mechanism of CP toxicity is related to exhaustion of antioxidant defense system. A significant increase in renal MDA and decrease in the activities of antioxidant enzymes were reported by many researchers following CP treatment of rats (25, 26). In the current study, CP elevated tissue MDA level indicating lipid peroxidation and oxidative damage in the renal tissue. Treatment with SIL inhibited this increase in MDA levels which was in accordance with Yildirim et al. (27) who observed a significant decrease in tissue MDA levels in the SIL-treated lung fibrosis group.

In addition to oxidative stress, there is strong evidence that in the pathogenesis of CP-induced renal damage, many pro-inflammatory mediators are involved including TNF-α (28). This was in accordance with the current study that revealed a significant increase in the level of TNF-α after CP administration. This increased level of TNF-α was ameliorated by SIL administration which was in accordance with the results of the study conducted by Cadirci et al. (29) which found that SIL treatment attenuates lung and kidney injury through maintenance of the oxidant-anti-oxidant status and decrease in the level of TNF-α. Moreover, the current study showed that SIL may preserve tubular integrity through vasodilator effects as well as, via attenuation of inflammation, may explain the partial tubular function maintenance observed upon co-treatment with CP. Furthermore, because inflammation is known to induce renal vasoconstriction and to reduce RBF and GFR (30, 31) prevention of inflammation should also result in a better filtration.

5. CONCLUSION

In conclusion, the present study showed that SIL treatment attenuates CP-induced renal injury and preserve the renal function through its vasodilator effect and antioxidant mechanism as well as elevating the level of TNF-α.
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Conflict of interests: None.

REFERENCES


