The Salutary Effects of Diminazene, Lisinopril or Valsartan on Cisplatin – Induced Acute Kidney Injury in Rats: A Comparative Study

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Summary

Nephrotoxicity as a cause of acute kidney injury (AKI) induced by cisplatin (CP), limits its usefulness as an anticancer agent. Diminazene, an angiotensin converting enzyme 2 activator, exhibited renoprotective properties on rat models of kidney diseases. This research aims to investigate the salutary effect of diminazene in comparison with lisinopril or valsartan in CP-induced AKI. The first and second groups of rats received oral vehicle (distilled water) for 9 days, and saline injection or intraperitoneal CP (6 mg/kg) on day 6, respectively. Third, fourth, and fifth groups received intraperitoneal injections of CP on day 6 and diminazene (15 mg/kg/day, orally), lisinopril (10 mg/kg/day, orally), or valsartan (30 mg/kg/day, orally), for 9 days, respectively. 24h after the last day of treatment, blood and kidneys were removed under anesthesia for biochemical and histopathological examination. Urine during the last 24 h before sacrificing the rats was also collected. CP significantly increased plasma urea, creatinine, neutrophil gelatinase-associated lipocalin, calcium, phosphorus, and uric acid. It also increased urinary albumin/creatinine ratio, N-Acetyl-β-D-Glucos-aminidase/creatinine ratio, and reduced creatinine clearance, as well the plasma concentrations of inflammatory cytokines [plasma tumor necrosis factor–alpha, and interleukin-1beta], and significantly reduced antioxidant indices [catalase, glutathione reductase, and superoxide dismutase]. Histopathologically, CP treatment caused necrosis of renal tubules, tubular casts, shrunken glomeruli, and increased renal fibrosis. Diminazine, lisinopril, and valsartan ameliorated CP-induced biochemical and histopathological changes to a similar extent. The salutary effect of the three drugs used is, at least partially, due to their anti-inflammatory and antioxidant effects.

Keywords

Cisplatin • Diminazene • ACE2 activator • Lisinopril • Valsartan • Acute kidney injury

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Introduction

Cisplatin (CP) is a chemotherapy drug that is widely used to treat various types of cancer, including lung, ovarian, testicular, head and neck, and bladder cancer. Although CP is still considered a backbone of standard-of-care chemotherapy regimens for a variety of solid tumors, its use can often cause severe dose-limiting toxicities, especially renal toxicity. About 30 % - 40 % of cancer patients on CP-based regimens discontinue treatment due to nephrotoxicity, ending in some cases in chronic renal disease [1].

Studies on CP-induced nephrotoxicity found that the mechanisms underlying this toxicity are complex and not fully understood [2]. However, it is thought to be related to the accumulation of CP in the kidneys, which leads to the formation of reactive oxygen species (ROS)

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and the activation of pro-inflammatory pathways. In addition to its direct toxic effects on the kidneys, CP has been found to disrupt the balance of various biomarkers in the body, such as creatinine, blood urea nitrogen (BUN), and uric acid, which are indicators of kidney function [2]. Studies in animal models have shown that CP treatment results in significant increases in creatinine and BUN concentrations, indicating kidney injury [3]. Preclinical studies have also shown that different interventions can reduce CP -induced nephrotoxicity, such as antioxidant agents, ACE inhibitors, N-acetylcysteine, and natural products.

The Renin-Angiotensin System (RAS) regulates blood pressure and fluid balance. It has two main arms: a pressor and a depressor. The pressor arm releases renin, which converts angiotensinogen to angiotensin II, increasing blood pressure. Angiotensin II is associated with renal injury and chronic kidney disease. The depressor arm releases ACE2, which converts angiotensin II to angiotensin-(1-7). Angiotensin-(1-7) has beneficial effects on renal and cardiovascular diseases, such as vasodilatory, anti-inflammatory, and antifibrosis properties [4-5].

Several studies have been conducted to test the efficacy of ACE inhibitors and ARBs in preventing and treating renal injury, and the results have been promising. For example, a study by [6] found that ACE inhibitors significantly reduced the risk of developing CKD in patients with hypertension. Similarly, ARBs reduced the risk of developing renal injury in patients with hypertension and diabetes [7].

Diminazene is an antiprotozoal drug used to treat trypanosomiasis, a parasitic infection caused by the protozoan *Trypanosoma brucei*. Studies on the relationship between diminazene and angiotensin found that diminazene treatment has been shown to affect the RAS. Some studies in rats found that treatment with diminazene resulted in a significant decrease in blood pressure, as well as changes in the levels of renin and angiotensin II, two key components of the RAS [8]. Moreover, diminazene is classified as an ACE2 activator [9] and protects against kidney damage in different animal models [10].

Since few studies have addressed the nephroprotective effects of diminazene in animal models of renal injury, we aim in the current study to explore the possible ameliorative effect of diminazene in CP-induced AKI in rats, and to compare its effects with that of ACE2 inhibitor lisinopril and an ARB, valsartan.

Material and Methods

Animal subjects

Male Wistar rats weighing between 190-250 g were obtained from the Animal House of Sultan Qaboos University in Oman. They were housed in polypropylene cages and maintained under standard conditions, with a temperature of 22 ± 2 °C, a 12-hour light, and 12-hour dark cycle (lights on at 06:00 hr), and a humidity of about 60 %. The rats had access to tap water and a standard laboratory chow diet containing normal sodium purchased from Oman Mills in Muscat, Oman.

Experimental design

After a one-week acclimatization period, thirty male Wistar rats were randomly divided into 5 groups with 6 rats in each group. They were treated for 9 days as follows: Group 1 served as the control group and received oral vehicle (distilled water) for 9 days and saline injection on day 6. Group 2 received only CP and served as the positive control group. They received oral vehicle for 9 days and CP 6 mg/kg, intraperitoneal injection on day 6. Groups 3, 4, and 5 were treated with CP in combination with diminazene, lisinopril, and valsartan respectively. They were given the respective drugs orally for 9 days and received CP intraperitoneal injection on the 6th day of treatment, similar to group 2. One day before the last day of treatment, rats were placed in metabolic cages to collect urine voided in the last 24 hours.

Sample collection and analysis

Twenty-four hours after the end of the treatment, the rats were anesthetized using 75 mg/kg ketamine and 5 mg/kg xylazine, and the blood was collected from vena cava. Urine was collected from the rats while they were in the metabolic cages one day before sacrification. Plasma was obtained by centrifugation (900 g at 4 °C for 15 min). The samples were stored at -80 °C until analyzed. The rats were then sacrificed, and their kidneys were removed, weighed, and preserved for histopathological examination. The doses of CP, diminazene, lisinopril, and valsartan were chosen based on previous studies [11-12]. Diminazene aceturate was purchased from Abcam in Cambridge, UK, while lisinopril was manufactured by AstraZeneca Pharmaceuticals Co. Ltd in Cheshire, UK, and valsartan was manufactured by Novartis Pharma in Basel, Switzerland. CP was purchased from Sultan Qaboos

University Hospital Pharmacy.

Physiological and biochemical analyses were performed on the collected samples. Urine albumin and creatinine, plasma urea, creatinine, uric acid, calcium, and phosphorus were measured using an automated biochemical analyzer. Kidney superoxide dismutase, catalase, glutathione reductase, and N-acetyl-β-D-glucosaminidase activity were measured using a Biovision kit, and plasma tumor necrosis factor alpha, interleukin-1β, and neutrophil gelatinase-associated lipocalin were measured using ELISA kits from Thermo Fisher Scientific, Inc. Urine osmolality was measured using the Osmomat 3000 osmometer. Creatinine clearance (mL/min) was calculated using the following formula:

$$\frac{\text{Urinary creatinine (µmol/L) X Urine volume (mL/24 hr)}}{\text{Plasma creatinine (µmol/L) X 1440}}$$

Details of analysis methods have been published previously [13-15].

Analysis of tissue samples

The kidneys were weighed, sampled, and fixed in 10 % neutral-buffered formalin for 24-48 hours. After dehydration and clearance, they were embedded in paraffin. Kidney paraffin blocks were sectioned at a thickness of 4 micrometers and stained with either hematoxylin and eosin (H&E) or Sirius red to assess interstitial fibrosis. A blinded pathologist conducted the histopathological scoring of kidney sections using techniques previously reported [16].

For evaluation of renal tubular necrosis, kidney tissues were fixed in 10 % neutral-buffered formalin and stained with H&E and Picro-Sirius red. The extent of renal tubular necrosis was evaluated using a semi-quantitative scoring method on a scale of 0-4, as described by [16]. A total of three 40X microscopic fields were examined from each kidney section of each animal in the five groups, and the mean percentage was converted to a score value.

To evaluate fibrosis, staining with Picro-Sirus red was conducted. The method for analyzing the stained slides was based on a previous study by [17]. Three random images of the renal cortex were captured using a 40X objective lens and then analyzed using ImageJ® image analysis software. The red-stained collagen was separated by applying a hue histogram filter, and the separated area was measured as a percentage. The fibrosis index percentage was then calculated by determining the percentage of the mean red-stained collagen to the total sample area for each animal. Images were acquired from each kidney of each animal in the five groups, and the same camera and microscope settings were used for all images to ensure consistency.

Statistical analysis

The data were expressed as mean ± SEM and analyzed using GraphPad Prism version 5.03 (San Diego, CA, USA). One-way analysis of variance (ANOVA) was employed, followed by Bonferroni's multiple comparison tests for statistical analysis. Statistical significance was considered when P-value was less than 0.05.

Results

Effects on different physiological parameters

As shown in Table 1, treatment with CP led to a significant decrease in the percentage of body weight change and a significant increase in relative kidney weight, water intake and urine output, and a significant decrease in urine osmolality compared to the control group. Lisinopril treatment effectively reversed the effect of CP on water intake and urine osmolarity, while diminazene only reversed the CP-induced decreases in urine osmolarity. Treatment with valsartan failed to reverse all the observed physiological parameters.

Effects on biochemical parameters

The treatment with CP resulted in a marked rise in several plasma components including urea, creatinine, phosphorus, calcium, and NGAL, as shown in Table 2. Additionally, there was a significant increase in urinary albumin/creatinine ratio and NAG-creatinine ratio, and a decrease in creatinine clearance, as demonstrated in Fig. 1. Treatment with diminazene, lisinopril or valsartan was found to considerably mitigate the CP-induced increase in all measured parameters. Despite this, none of the three drugs were able to improve the decrease in creatinine clearance caused by CP.

Effects on plasma uric acid, TNF-α, and IL-1β

The results presented in Fig. 2 indicate that the levels of plasma uric acid, TNF-α, and IL-1β were significantly elevated by CP. However, the administration of diminazene, lisinopril, or valsartan significantly mitigated the CP-induced increase in all of the measured parameters.

Table 1. Impact of diminazene (DM), lisinopril (LS), and valsartan (VL) on some physiological parameters in rats with cisplatin (CP)-induced acute kidney injury (AKI).

Parameters/Treatment	Control	CP	CP + DM	CP + LS	CP + VL
Baseline body weight (g)	258.8 ± 9.03	258.3± 14.46	257.8 ± 21.54	260.4 ± 18.80	259.6± 10.94
Final body weight (g)	275.7 ± 9.05	250.3 ± 13.36	240.8± 19.22	245.7± 17.23	251.3± 8.40
Body weight change (%)	6.60 ± 0.99	-3.01 ± 0.97[a]	-6.28 ± 2.26[a]	-5.54 ± 1.96[a]	-2.90 ± 2.32[a]
Total kidney weight (mg)	1.65 ± 0.8	1.75 ± 0.12	1.7 ± 0.12	1.87 ± 0.13	1.8 ± 0.1
Relative kidney weight (%)	0.60 ± 0.01	0.72 ± 0.08[a]	0.70 ± 0.02	0.77 ± 0.03	0.72 ± 0.04
Water intake (mL)	19.67 ± 1.67	24.17 ± 2.39[a]	17.50 ± 1.57	21.17 ± 1.89[b]	20.5 ± 2.06
Urine output (mL)	7.00 ± 0.86	17.0 ± 1.21[a]	13.17 ± 2.17[a]	16.5 ± 0.89[a]	14.0 ± 2.19[a]
Urine osmolality	2269 ± 185.2	649 ± 35.2[a]	1003± 64.8[a, b]	929 ± 50.7[a, b]	869 ± 46.8[a]
Food intake (g)	16.53 ± 0.74	8.47 ± 1.25	10.43 ± 1.04	9.80 ± 1.24	11.7 ± 1.21
Fecal output (g)	4.87 ± 0.43	2.62 ± 0.33	2.67 ± 0.47	3.62 ± 0.28	5.10 ± 0.33

Values in the table are means ± SEM (n = 6). Acute kidney injury (AKI) was induced in rats on the 6th day of the experiment by a single intraperitoneal injection of CP (6 mg/kg), DM (15 mg/kg), LS (10 mg/kg), and VL (30 mg/kg) were administered orally to the rats throughout the experiment. On the 9th day of treatment, the rats were placed in metabolic cages to collect urine. Statistical differences between the groups were evaluated using a one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test, where $P < 0.05$. The letter "**a**" indicated the significance from the control group, while the letter "**b**" indicated the significance from CP group.

Table 2. Impact of diminazene (DM), lisinopril (LS), and valsartan (VL) on biochemical parameters in rats with cisplatin (CP)-induced Acute Kidney Injury (AKI).

Parameters/Treatment	Control	CP	CP + DM	CP + LS	CP + VL
Urea (mmol/L)	4.7 ± 0.19	29.2 ± 1.83 [a]	8.60 ± 1.41[b]	13.2 ± 4.02[a, b]	10.5 ± 1.91[a,b]
Creatinine (µmol/L)	16.2 ± 1.71	78.8 ± 8.23[a]	43.8 ± 10.19 [a, b]	42.8 ± 6.20[a, b]	47.1 ± 10.64 [a, b]
Phosphorus (mmol/L)	0.64 ± 0.04	1.61± 0.09[a]	0.73 ± 0.08 [b]	0.89 ± 0.10 [a, b]	0.96 ± 0.07 [a, b]
Calcium (mmol/L)	1.01± 0.07	0.46 ± 0.04[a]	0.84 ± 0.08[b]	0.89 ± 0.10 [b]	0.80 ± 0.06 [b]
NGAL (ng/mL)	35.1 ± 1.41	158.6 ± 16.4[a]	48.10 ± 3.63 [b]	53.71± 6.77 [b]	60.57 ± 5.40 [a, b]

Values in the table are means ± SEM (n = 6). On the 6th day of the experiment, AKI was induced in rats by administering a single intraperitoneal injection of CP at a dosage of 6 mg/kg. DM (15 mg/kg), LS (10 mg/kg), and VL (30 mg/kg) were orally administered to rats throughout the experiment. On the 9th day of treatment, the rats were placed in metabolic cages to collect urine. To assess the differences between the groups, a one-way analysis of variance (ANOVA) was conducted, followed by Bonferroni's multiple comparison test with a significance level of $P < 0.05$. In this analysis, the letter "**a**" indicated the significance from the control group, while the letter "**b**" indicated the significance from CP group.

Effects on renal oxidative stress markers

The data presented in Fig. 3 demonstrates that the activity of renal catalase, SOD, and GR was significantly reduced by treatment with CP. Additionally, the levels of renal MDA were found to be increased following treatment with CP. However, the administration of diminazene, lisinopril, or valsartan was able to significantly alleviate the CP-induced alterations in all of the measured parameters.

Histopathology

The renal tissues of rats from different groups were examined microscopically. The control group (group 1) showed normal renal histology with intact glomerular tuft and renal tubules (lesion score 0) as depicted in Fig. 4A. However, in the CP-treated group (group 2), kidney tissues showed marked renal tubular necrosis, cystic tubular dilatation, and dilatation of Bowman's capsule (Score 4) as shown in Fig. 4B. On the other hand, renal tissues of rats treated with CP plus diminazene (group 3) exhibited intact glomeruli and renal tubules except for a few necrotic tubules (Score 2) as shown in Fig. 4C. Similarly, in the CP plus lisinopril group (group 4), examined kidney tissues showed tubular necrosis, cystic dilatation of multiple renal tubules, and dilatation of Bowman's capsule (Score 3) as illustrated in Fig. 4D. In contrast, the renal tissues of rats treated with CP plus valsartan (group 5) showed cystic tubular

dilatation of a few renal tubules with intact glomeruli (Score 3) as shown in Fig. 4E. Picro-Sirus red stain was used to demonstrate the distribution of collagen fibers stained in red and non-collagen structures stained in yellow in all five groups (Fig. 4 F-J). The fibrosis index percentage was calculated and presented in Table 3.

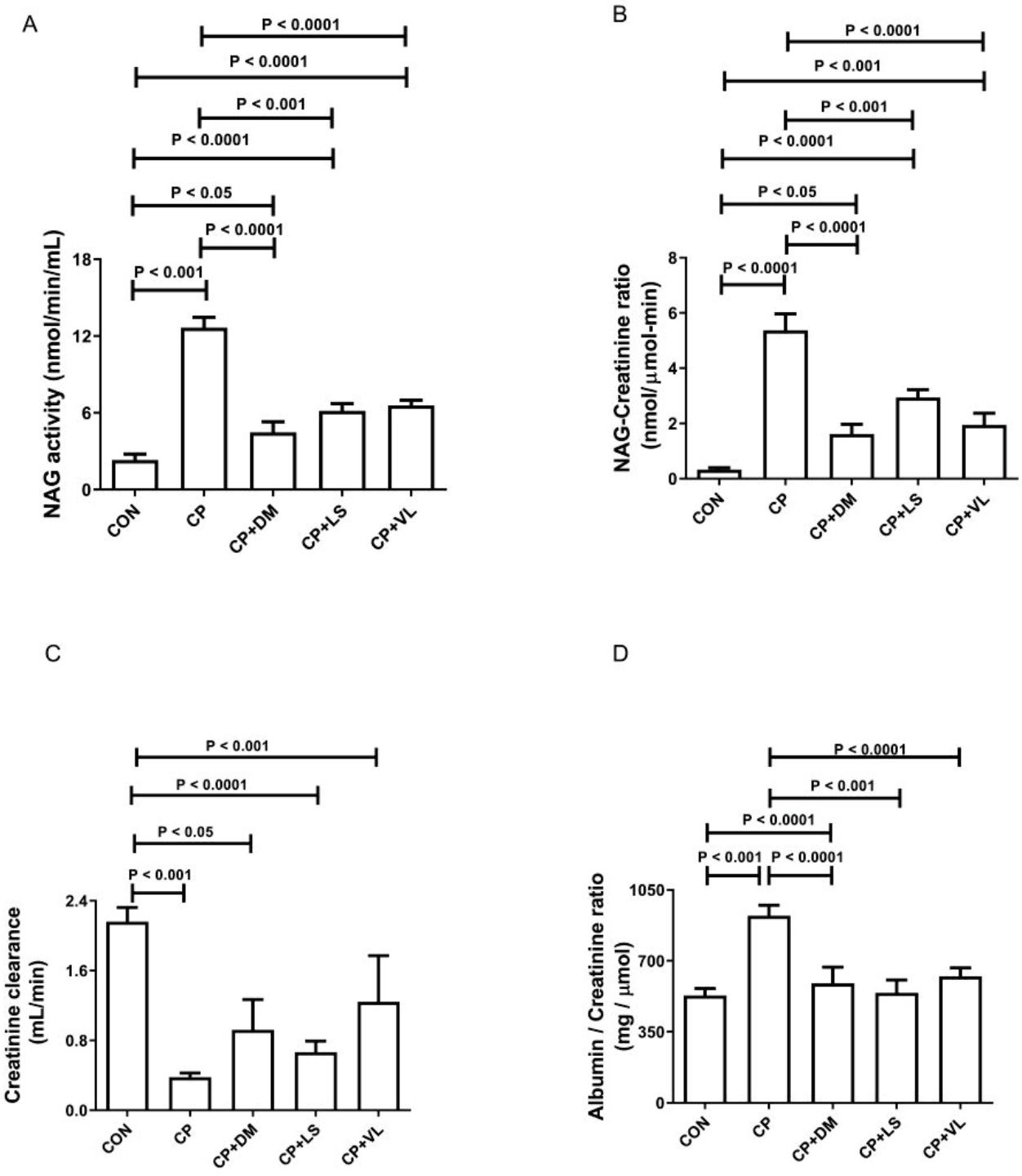

Fig. 1. The effect of diminazene (DM, 15 mg/kg), lisinopril (LS, 10 mg/kg) or valsartan (VL, 30 mg/kg) on **A)** NAG activity; **B)** NAG/creatinine ratio; **C)** creatinine clearance; **D)** albumin/creatinine ratio in rats treated with CP. Throughout the experiment, DM, LS, and VL were given orally to rats. AKI was caused by a single intraperitoneal injection of CP (6 mg/kg) on the 6th day of the experiment. On the 9th day, urine was collected from the rats while they were in metabolic cages. The data presented are the means ± SEM (n = 6).

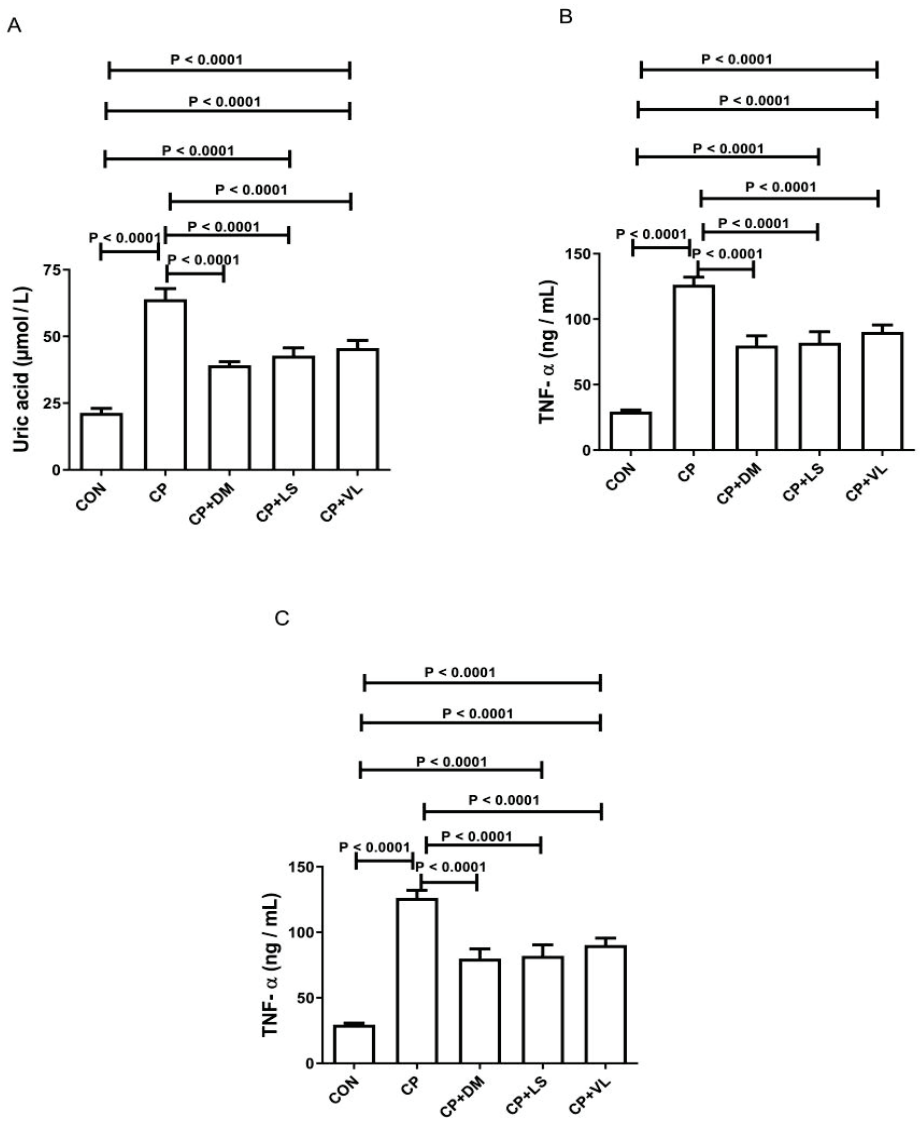

Fig. 2. The effect of diminazene (DM, 15 mg/kg), lisinopril (LS, 10 mg/kg) or valsartan (VL, 30 mg/kg) on **A)** plasma uric acid; **B)** tumor necrosis factor-alpha (TNF-α); and **C)** interleukin-1β in rats treated with CP. Throughout the experiment, DM, LS, and VL were given orally to rats. AKI was caused by a single intraperitoneal injection of CP (6 mg/kg) on the 6th day of the experiment. The data presented are the means ± SEM (n = 6).

Table 3 The impact of diminazene (DM), lisinopril (LS), and valsartan (VL) treatments on the histopathological evaluation of kidney sections in rats with acute kidney injury (AKI) induced by cisplatin (CP).

Assessment/Treatment	**Acute tubular necrosis**		**Fibrosis index**
	%	**Lesion Score**	**%**
Control	0.0 ± 0.0	0	5.54 ± 0.21
CP	77.78 ± 1.11 [a]	4	25.77 ± 0.22 [a]
CP + DM	20.0 ± 1.49 [a, b]	2	14.35 ± 0.24 [a, b]
CP + LS	35.00 ± 2.24 [a, b]	3	19.31 ± 0.24 [a, b]
CP + VL	33.33 ± 2.11 [a, b]	3	20.24 ± 0.21 [a, b]

Values in the table are means ± SEM (n = 6). AKI was induced in rats on the 6th day of the experiment through a single intraperitoneal injection of CP at a dose of 6 mg/kg. Throughout the experiment, the rats were orally administered DM (15 mg/kg), LS (10 mg/kg), or VL (30 mg/kg). On the 10th day of treatment, the rats were sacrificed to collect kidney samples. To assess differences between the groups, one-way analysis of variance (ANOVA) was performed, followed by Bonferroni's multiple comparison test. A significance level of $P < 0.05$ was considered statistically significant. The letter "**a**" indicated the significance from the control group, while the letter "**b**" indicated the significance from CP group.

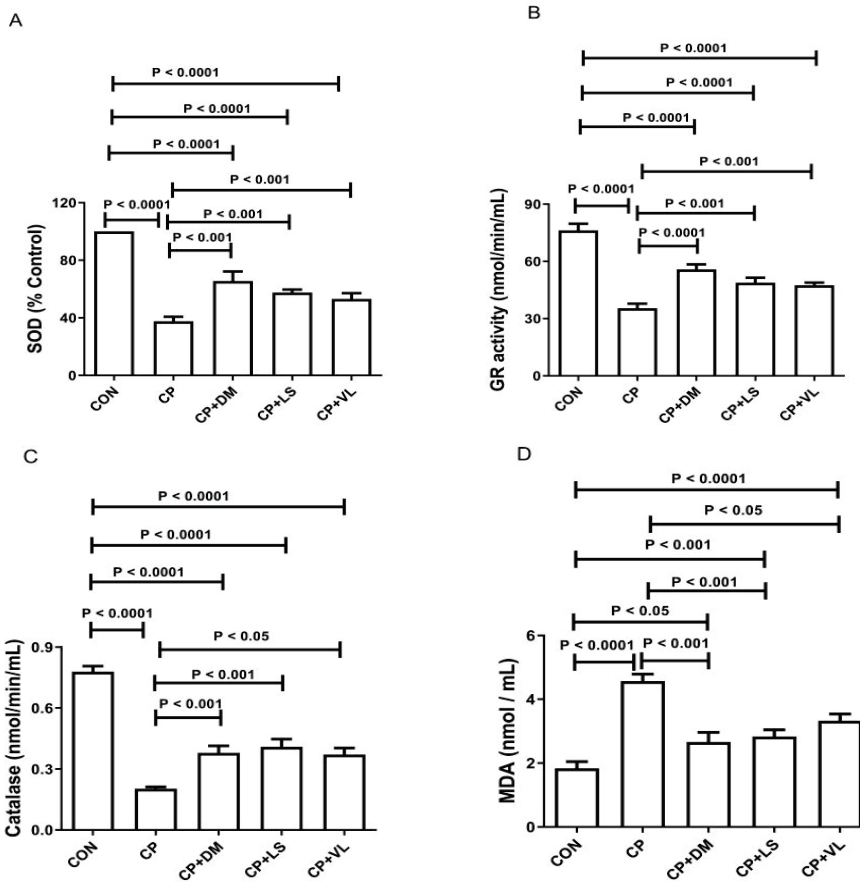

Fig. 3. The effect of diminazene (DM, 15 mg/kg), lisinopril (LS, 10 mg/kg) or valsartan (VL, 30 mg/kg) on **A)** renal superoxide dismutase (SOD); **B)** glutathione reductase (GR); **C)** catalase; and **D)** malondialdhyde (MDA) in rats treated with CP. Throughout the experiment, DM, LS, and VL were given orally to rats. AKI was caused by a single intraperitoneal injection of CP (6 mg/kg) on the 6th day of the experiment. The data presented are the means ± SEM (n = 6).

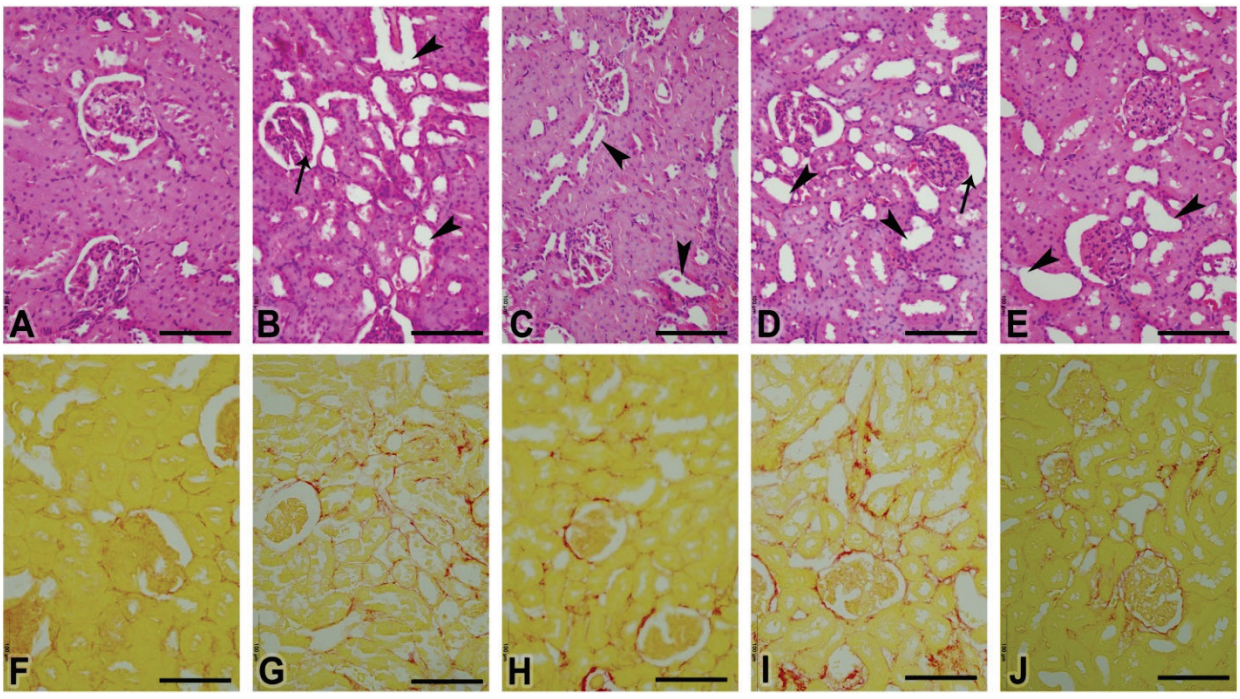

Fig. 4. Photomicrographs of the renal cortex (magnification: 100 µm) stained with H&E (A-E) and Picro-Sirius red (F-J). **A)** The control group (1) exhibited normal renal histology with intact glomeruli and renal tubules (Score 0). **B)** The CP-treated group (2) showed renal tubular necrosis with cystic tubular dilatation (arrowheads) and dilatation of Bowman's capsule (arrows) (Score 4). **C)** The CP + Diminazene-treated group (3) showed intact glomeruli and renal tubules, except for a few necrotic tubules (Score 2). **D)** The CP + lisinopril-treated group (4) showed tubular necrosis and cystic dilatation of multiple renal tubules, as well as dilatation of Bowman's capsule (Score 3). **E)** The CP + valsartan-treated group (5) exhibited cystic tubular dilatation of a few renal tubules with intact glomeruli (Score 3). **F-J)** The distribution of collagen fibers stained in magenta red and the non-collagen structures stained in yellow are shown for all groups (1-5).

Discussion

In this study, we have presented evidence suggesting that daily oral administration of diminazene at a dose of 15 mg/kg/day for 9 days can significantly mitigate several biochemical and histopathological changes induced by CP in the kidney of rats. These changes include inflammation, oxidative stress, and kidney injury, without causing any overt adverse side effects. These findings are supported by several other studies looking at protective effects of diminazene. For instance, in an experimental sepsis model, diminazene exhibited a protective effect against sepsis-induced cardiomyopathy by influencing the Angiotensin Receptor (AR) system. It accomplished this by enhancing ACE2 expression and restoring Angiotensin-(1-7) levels [18]. Angiotensin-(1-7) stimulates the Mas receptor, which leads to vasodilation, potentially counteracting or reversing vascular dysfunction [19]. Furthermore, research indicated that both acute and chronic intravenous administration of diminazene to spontaneously hypertensive rats resulted in a reduction in mean arterial pressure through the activation of ACE2 [20]. In a study involving rats, diminazene significantly decreased right ventricular systolic pressure in rats with monocrotaline-induced pulmonary hypertension. This effect was attributed to increased ACE2 expression and higher Mas receptor levels in lung tissue, affirming diminazene's protective effects on the cardiopulmonary system [21]. These findings were corroborated by another study, which revealed a link between diminazene and ACE2, with the induction of Angiotensin-(1-7) and the Mas receptor axis playing a role in activating functions relevant to vasoprotection in CD34+ cells [22]. In an additional study, diminazene was associated with a reduction in renal cortical ACE activity and an increase in renal cortical ACE2 activity [23]. In a rat model of diabetes, diminazene restored ACE2 and AT2 receptor expression in glomeruli, lowered Angiotensin II levels, and boosted Angiotensin-(1-7) expression. These findings suggest that diminazene has a renoprotective effect possibly through the ACE2/Angiotensin-(1-7)/AT2 pathway [24]. Notably, the ameliorative actions of diminazene were comparable to those of the antihypertensive drugs lisinopril (10 mg/kg/day) and valsartan (30 mg/kg/day), which have previously been shown to abrogate CP nephrotoxicity [25-26]. The doses used for all three agents were broadly similar to those previously reported to be effective and safe [10, 25-26].

Numerous studies in humans and animals have demonstrated that CP treatment can alter both physiological and biochemical parameters in patients and animal models [27]. In our study, CP treatment resulted in several physiological alterations, including a significant reduction in body weight, water intake, urine volume, and an increase in relative kidney weight. The reduction in water intake may have been caused by the gastrointestinal toxicity inflicted by CP [28], which resulted in modified eating and drinking habits. This, in turn, led to the reduction of urine output. Since there was no significant change in the absolute kidney weight, it is most likely that the observed increase in the relative kidney weight is attributed to the decrease in body weight. The decrease in body weight seen in CP-treated rats may be attributed to the gastrointestinal toxicity of the drug [28] or possibly due to the injured renal tubules, and the resultant inability of the tubular cells to reabsorb water, increased urine volume excreted, leading to dehydration and decrease of body weight [29].

Plasma creatinine, urea, creatinine clearance, and urine biochemical profile are established biochemical indices for nephrotoxicity. These indices were significantly altered by CP treatment and normalized to a similar extent with the treatment of the three agents used.

In our study, we have confirmed that CP, at the dose used, caused significant histopathological structural alterations in the kidney. This is consistent with findings observed in several previous studies investigating CP-induced nephrotoxicity (e.g., [30]). However, treatment with the three agents mitigated the damage observed in the CP-treated group. This ameliorative action may be due to the reduction of CP-related inflammation and oxidative stress, which has been attributed to the reduction of platinum accumulation in the renal tissue.

Our results that show that diminazene (15 mg/kg/day) for 9 days has an anti-oxidant action in cisplatin induced nephrotoxicity rats are not in accordance with the study of Baldiserra *et al.* [31] that showed that a single dose of diminazene (3.5 mg/kg) increased oxidative stress in healthy rats. The reason for the discrepancy may be due to the different rat models and dosage regimens in the two experiments or to other unknown reasons.

We have studied here, the role of oxidative stress and inflammation on CP nephrotoxicity. However, CP-induced renal damage may also involve various

cellular and molecular mechanisms, including DNA damage, mitochondrial dysfunction, oxidative stress, and endoplasmic reticulum stress. Additionally, stress responses such as autophagy, cell-cycle arrest, senescence, apoptosis, programmed necrosis, and inflammation have been implicated [4]. Thus, additional investigations are necessary to elucidate the precise molecular pathways underlying the protective effects of diminazene, lisinopril, and valsartan against CP-induced nephrotoxicity.

Ethical approval

The study was performed using rats. It was approved by the University Animal Ethical Committee of Sultan Qaboos University, Oman (IG/MED/PHAR/21/02). All procedures involving animals and their care were carried out in accordance with international laws and policies (EEC Council directives 2010/63/EU, 22 September 2010 and NIH Guide for the Care and Use of Laboratory Animals, NIH Publications, 8th edition, 2011).

Authors´ contributions

Al Suleimani Y, Ali BH, Abdelrahman A: Conceptualization, Data curation, Methodology, Validation. Al Suleimani Y, Abdelrahman A, Manoj P, Ali H: Formal analysis. Manoj P, Ali H, Almashaiki K: Investigation. Al Suleimani Y, Abdelrahman A: Project administration, Supervision. Al Suleimani Y, Ali BH: Writing – original draft, Writing – review and editing.

Conflict of Interest

There is no conflict of interest.

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