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Original Article

Quercetin improves survival rate of cisplatin-induced acute nephrotoxicity: A renal clearance and histopathological study

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Abstract

The protective effects of quercetin in cisplatin-induced nephrotoxic rats were investigated. Renal functions using clearance techniques, histopathological analysis, and the oxidative status were determined on days 3, 7, and 14 after a cisplatin (7.5 mg/kg, intraperitoneal) injection. In cisplatin-treated rats, the survival rate decreased to 59% and 50% on days 7 and 14, respectively. Survived rats had a significant reduction in glomerular filtration rate and an increase in urinary Na^+ and K^+ excretion. Histopathological scoring revealed both glomerular and severe tubular damage along with a significant increase in renal malondialdehyde (MDA) and a reduction in catalase activity. Oral administration of quercetin (50 mg/kg, twice at 24 h and 10 min before cisplatin injection) not only increased the survival rate to 75% but also suppressed the elevation of renal MDA and improved both glomerular and tubular function. The responsible mechanism of the renal protection by quercetin may be via its antioxidant properties.

Keywords: quercetin, cisplatin, nephrotoxicity, renal tubular damage, glomerular filtration rate

1. Introduction

Cisplatin (*cis*-diaminedichloroplatinum II) is one of the effective chemotherapeutic agents and the drug of choice in treatment of various cancers. It has been demonstrated that the high incidence of nephrotoxicity after cisplatin treatment limits the use of higher doses to maximize its clinical chemotherapeutic effects. Acute and sub-chronic treatment of cisplatin in experimental rats resulted in both structural and functional alterations of the kidney. Three and five days after intraperitoneal injection of cisplatin (6 mg/kg) the renal proximal tubule, particularly the S3 segment located in the outer stripe of the outer medulla (OSOM), was the most damaged region compared to other parts of the nephron. These tubular damages included a loss of brush border, swelling, chromatin condensation, and necrosis (Dobyan *et al.,* 1980). Daily administration of cisplatin (0.4 mg/kg) for 8 weeks caused glomerular atrophy and the dilatation of proximal convoluted tubules with sloughed epithelium and cell debris (Ravindra *et al.,* 2010). At higher doses, cisplatin (7 and 7.5 mg/kg, intraperitoneal [i.p.]) caused histological alterations in the renal cortical tubules observed during 3-10 days after the injection. The damage included vacuolization, swelling, desquamation, casts, apoptosis, and necrosis along with an increase in plasma creatinine and blood urea nitrogen (BUN) (Atessahin *et al.,* 2005; Atessahin *et al.,* 2006; Chirino *et al.,* 2004; Guerrero-Beltran *et al.,* 2010; Yildirim *et al.,* 2003).

Proteinuria and an increase in urinary N-acetyl-β-D-glucosaminidase were also reported suggesting both glomerular and tubular impairment (Chirino *et al.,* 2004; Guerrero-

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Beltran *et al.,* 2010). Urinary excretion of electrolytes was found to increase in days 4, 7, and 9 along with a decrease in renal blood flow (RBF) and glomerular filtration rate (GFR) after cisplatin (5 and 6 mg/kg, i.p) injection (Bagnis *et al.,* 2001; Khan *et al.,* 2007; Salman *et al.,* 2009). Moreover, an impairment of urine concentrating ability was observed with similar doses and duration after cisplatin administration (Bae *et al.,* 2009; Ecelberger *et al.,* 2001; Kang *et al.,* 2004).

Generation of reactive oxygen species (ROS) and oxidative stress have been recognized as one of the factors that contribute to cisplatin nephrotoxicity. In freshly isolated porcine proximal tubular cells, cisplatin (5-100 μM) caused a time- and concentration-dependent elevation of ROS (Kruidering *et al.,* 1997). Renal proximal tubular cells incubated with cisplatin (20 μM) resulted in an elevation of hemeoxygenase-1 (HO-1) concentration, leading to p53 activation, tubular cell apoptosis, and nephrotoxicity (Jiang *et al.,* 2007). Cisplatin (7.5 mg/kg, i.p.) injection in rats also caused an increase in oxidative markers such as 4-hydroxy-2-nonenal and malondialdehyde (MDA) in renal tissue homogenate observed in days 3 and 5 (Guerrero-Beltran *et al.,* 2010; Yousef *et al.,* 2009).

Quercetin is a plant bioflavonoid and has been shown to possess antioxidant properties in both *in vitro* and *in vivo*. Quercetin suppressed superoxide anion radical ($O^{\bullet-}_2$) production *in vitro* due to an inhibition of xanthine oxidase (Cos *et al.,* 1998) and inhibited the production of the hydroxyl radical ($HO^{\bullet}$) induced by H_2O_2 in iron-preloaded Madin-Darby canine kidney cells (Vlachodimitropoulou *et al.,* 2011). Quercetin exerted its antioxidant property *in vivo* by the upregulation of antioxidant defense systems. Single dose administration of quercetin (50 mg/kg, i.p.) can reduce renal oxidative stress induced by ischemia and reperfusion by enhancing antioxidant enzyme activities including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (Inal *et al.,* 2002).

According to its robust antioxidant property, quercetin has been researched as a renoprotective substance in various nephrotoxic models including cisplatin-induced renal failure in either non-tumor or tumor rat model. Oral administration of multiple dosages of quercetin in cisplatin-induced nephrotoxicity at a cisplatin dose of 5 mg/kg body weight in non-tumor rats improved the alterations of renal MDA level, plasma creatinine, urine volume and osmolarity, acute tubular necrosis, intraluminal casts, tubular swelling, and interstitial inflammation (Behling *et al.,* 2006). In tumor-bearing rats, quercetin (50 mg/kg, p.o. daily for 9 days) prevented the nephrotoxic effect of cisplatin (4 mg/kg, i.p.) without affecting its anti-tumor activity (Sanchez-Gonzalez *et al.,* 2011).

From previous studies, a single dose of cisplatin injection (4 and 5 mg/kg) can induce nephrotoxicity in experimental rats. The renal damaging effects were pronounced from day 2 to day 8 after the injection and then recovered to control level in day 10 (Nishikawa *et al.,* 2001; Sanchez-Gonzalez *et al.,* 2011). In accordance with a previous study, cisplatin injections of 4.5, 6, 7.5, and 9 mg/kg in rats could induce nephrotoxicity. However, cisplatin at the dose of 7.5 mg/kg was the minimal dose that could cause the reduction of GFR by >60%, an increase in urine sodium and water excretion by >50% and an elevation in the BUN level more than 4-fold within 3 days after the injection (Kunworarath *et al.,* 2014).

In this study, cisplatin at the single dose of 7.5 mg/kg, i.p. was chosen to induce renal glomerular and tubular damage. From our preliminary data, the distinct renal protective effects of quercetin could be noticed at the dose of 50 mg/kg compared to the lower doses. We, therefore, investigated the protective effect of quercetin (50 mg/kg, p.o., twice at 24 h and 10 min before cisplatin injection) on days 3, 7, and 14 after the administration. The animal survival rate was also monitored during 14 days after cisplatin treatment along with the renal clearance study, histopathological analysis in the cortex and medulla, renal lipid peroxidation, and an antioxidant status determination.

2. Materials and methods

2.1 Animals

Male Wistar rats weighing 220-260 g were obtained from the Southern Laboratory Animal Facility of Prince of Songkla University, Songkhla, Thailand. All rats were housed under controlled conditions (temperature 23-25ºC, relative humidity 50-55%, and 12-h light/dark cycle). They were given commercial animal feed (S.W.T., Thailand) and free access to tap water. All experimental rats were maintained and handled according to the approval of the Prince of Songkla University Animal Ethics Committee (project license number: MOE 0521.11/678).

2.2 Experimental design

Animals were randomly assigned to three main groups according to the number of days after cisplatin injection (days 3, 7, and 14). Each main group was divided into three subgroups (vehicle control, cisplatin, and cisplatin + quercetin). The number of animals in each subgroup was between 4 and 11.

Nephrotoxicity in the rats was induced by an i.p. injection of cisplatin dissolved in 0.9% NaCl (Kemoplat, Fresenius Kabi Oncology, Distt. Solan, India) at a dose of 7.5 mg/kg body weight. Isotonic saline solution (an equal volume of cisplatin) was used as the vehicle in the control groups. Quercetin (quercetin dihydrate, Sigma, Steinheim, Germany) was dissolved in corn oil and administered orally twice at 24 h and 10 min before the cisplatin injection at a dose of 50 mg/kg body weight each time. An equal volume of corn oil was also administered in the control and cisplatin groups.

2.3 Experimental protocol

The survival rates of the animals subjected to each treatment was monitored daily for 14 days after the cisplatin injection. All groups of the survived rats were subjected to two identical protocols: 1) renal clearance and histopathological study and 2) determinations of renal MDA, antioxidant enzymes, and glutathione (GSH) concentration.

2.4 Renal clearance study

On the day of the experiments, the rats were anaesthetized with Nembutal® (pentobarbital sodium; 60 mg/kg body weight, i.p.; Ceva Sante Animale, Libourne, France)

and placed on a thermostatically controlled heated table to maintain body temperature at 37 ºC. A tracheostomy was performed and the right carotid artery was cannulated for blood sampling and arterial blood pressure recording (Grass Polygraph model 7DAG; Grass Instrument Co., Quincy, MA, USA) throughout the experiment. The left jugular vein was then cannulated and infused with 0.9% NaCl containing 1% inulin and 0.5% para-aminohippuric acid (PAH) at the rate of 1.6 ml/h/100 g body weight. Urine samples were collected in preweighed tubes through a cannula placed in the bladder via a suprapubic midline incision. A one-hour equilibration period was allowed in order to obtain the steady state of plasma inulin and PAH concentrations before clearance measurements were undertaken.

After the equilibration period, all rats were subjected to an identical protocol in which six consecutive 30-min urine collections were made. Three arterial blood samples (500 µl) were taken at the mid-point of the first, fourth, and sixth urine sample. A small amount of the blood sample was used to determine the hematocrit value. The remainder of the blood sample was centrifuged and the plasma was collected and stored frozen for determination of the clearance markers, electrolytes, and BUN. Blood cells were resuspended in 300 µl isotonic saline and returned to the animal via the jugular vein cannula. At the end of the renal clearance experiment, both kidneys were removed, blotted, weighed, and then fixed in formalin for histopathological examination.

Renal clearance of PAH and inulin were calculated according to the clearance equation and were taken as the indices of renal plasma flow (RPF) and GFR, respectively. RPF was calculated assuming a 90% extraction of PAH. RBF was calculated according to the equation RBF = RPF/(1-Hematocrit). Renal vascular resistance (RVR) was calculated using the mean arterial blood pressure (MABP) divided by RBF. Inulin and PAH concentrations were estimated by spectrophotometric method (Davidson & Sackner, 1963; Smith *et al.*, 1945). Urine output was determined gravimetrically assuming a density of 1 g/ml. The hematocrit was measured by microcapillary method. Na^+ and K^+ concentrations were measured by an inductively coupled plasma-optical emission spectrophotometer (Optima 4300 DV, Perkin Elmer, MA, USA) using NaCl and KCl as the standard solutions, respectively. Fractional excretion of Na^+ (FE_{Na}) and K^+ (FE_K) were calculated according to the equation: $FE_x = (C_x/GFR) \times 100$, where FE_x is the fractional excretion of Na^+ and K^+ and C_x is the clearance of Na^+ and K^+. The BUN level was estimated by enzymatic method using a urease enzyme kit (Humana, Wiesbaden, Germany).

2.5 Renal histopathological examination

The degree of glomerular and tubular damage were scored by histopathological examination. After removing both kidneys at the end of the renal clearance studies, the samples were fixed in 10% buffer neutral formalin for at least 24 h and then dehydrated in a series of graded concentrations of ethanol and embedded in paraffin wax (Tyco/Healthcare, Manfield, USA). The tissue block was cut into 6 µm-thick sections using a rotary microtome (model 820, Tucson, AZ, USA). Paraffin sections were stained with hematoxylin and eosin for light microscope examination. The sections were viewed and photographed on an Olympus light microscope (Olympus BX51, Tokyo, Japan) with an attached camera (Olympus DP 50, Olympus Optical Co. Ltd., Japan). Ten sections were prepared from each kidney. All sections were evaluated for glomerular atrophy and tubular injury. Tubular injury was defined as intratubular hyaline cast formation, tubular dilatation, renal tubular nuclear swelling, pyknosis and karyorrhexis. Hyaline cast formations were examined in the renal cortex and medulla while the others were performed in the renal cortex and OSOM.

Each kidney slide was examined and assigned for severe changes using scores described by Atessahin *et al.* (2006): 0 = no tubular, glomerular injury; 1 = ≤10%; 2 = 11-25%; 3 = 26-50%; 4 = 51-75%, and 5 = >75% injury of the tubules and glomeruli.

2.6 Determination of renal lipid peroxidation

The concentration of renal MDA was used as a marker of lipid peroxidation. On the day of experiment, the abdomen of anaesthetized rat was opened and a cannula was inserted into the abdominal aorta beneath the left renal artery and used to retrograde perfuse both kidneys simultaneously with phosphate buffered saline solution, pH 7.4, to remove red blood cells and clots. Kidney homogenates, prepared from both kidneys, were used for the determination of protein content and MDA concentration according to the method by Ohkawa *et al.* (1979) which is based on the reaction with thiobarbituric acid. The renal MDA level was expressed as a percentage of the control value.

2.7 Determination of renal antioxidant status

The determination of CAT and SOD activity and GSH content was performed using an assay kit (Cayman Chemical Company, MI, USA). The activity of CAT, SOD, and GSH was expressed as percentages of the respective control values.

2.8 Determination of protein content in kidney homogenate

Protein concentration was assayed by the method of Itzhaki and Gill (1964).

2.9 Statistical analysis

All experimental data were presented as mean ± standard error of mean (S.E.M.). One-way analysis of variance (ANOVA) was used to evaluate the differences between the subgroups of the same experimental days. Multiple comparisons after the analyses of variances were investigated by Student-Newman-Keuls post-hoc test. Statistical significance of the mean differences was accepted when the p value was <0.05.

3. Results

3.1 Survival rate and body weight change

The percentages of survival rates of all groups during the 14 days of the experiment are shown in Figure 1. All rats in the control groups showed 100% survival. After a single i.p. injection of cisplatin (7.5 mg/kg), the survival rates of the groups of experimental animals on day 3, day 7, and day 14 were 100%, 59%, and 50%, respectively. Quercetin treatment significantly improved the survival rate of the day 7 group from 59% to 75% and the day 14 group from 50% to 75%.

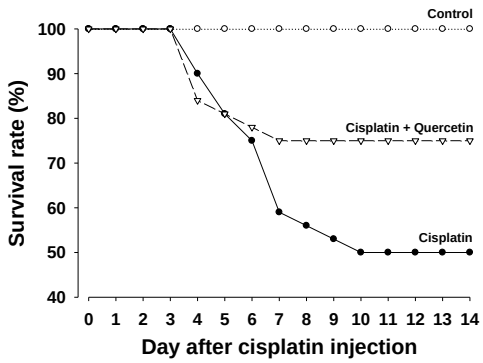

Figure 1. Effect of quercetin treatment on survival rate of cisplatin-induced nephrotoxic rats. The numbers of rats at the beginning of the experiment in the control group, cisplatin, and cisplatin + quercetin group were 18, 32, and 32, respectively.

The pre-body weight, post-body weight, and body weight change are shown in Table 1. In the cisplatin-treated groups, the reductions in body weight of the day 3 group (-36.0 ± 3.2 g), day 7 group (-66.2 ± 12.0 g), and day 14 group (-32.2 ± 10.6 g) were significantly different from their respective vehicle control groups: +6.7 ± 3.2 g, +23.6 ± 3.6 g, and +42.3 ± 7.3g, respectively. Quercetin treatment significantly protected the reduction in body weight observed on day 7 (-12.2 ± 15.8 g) and day 14 (+10.7 ± 14.9 g) but not on day 3 (-33.6 ± 2.6 g).

Table 1. Effect of oral administration of quercetin (50 mg/kg, p.o.) on pre-body weight, post-body weight and body weight change (ΔBW) in cisplatin (7.5 mg/kg, i.p.) treated rats. Cis = cisplatin.
+, increase and -, decrease.

Treatment	Pre-body weight (g)	Post-body weight (g)	ΔBW (g)
Day 3			
Control (n=6)	252.5 ± 5.4	259.2 ± 5.3	+6.7 ± 3.2
Cisplatin (n=6)	265.8 ± 4.7	229.8 ± 3.0*	-36.0 ± 3.2*
Cis+Quercetin (n=7)	246.1 ± 1.8	212.6 ± 2.3*†	-33.6 ± 2.6*
Day 7			
Control (n=6)	251.5 ± 7.1	275.2 ± 9.8	+23.6 ± 3.6
Cisplatin (n=6)	256.5 ± 2.7	190.3 ± 11.3*	-66.2 ± 12.0*
Cis+Quercetin (n=11)	247.5 ± 4.3	235.4 ± 15.0*†	-12.2 ± 15.8*†
Day 14			
Control (n=6)	241.7 ± 9.9	284.0 ± 13.7	+42.3 ± 7.3
Cisplatin (n=4)	247.0 ± 12.0	214.8 ± 17.6*	-32.2 ± 10.6*
Cis+Quercetin (n=6)	241.2 ± 6.1	251.8 ± 13.2	+10.7 ± 14.9†

Data are mean ± S.E.M. *, † P <0.05 compared with control and cisplatin group, respectively.

3.2 Renal clearance study

During the clearance studies of all experimental groups, MABP levels were maintained between 110-140 mm Hg, hematocrit values were 41-54%, urine flow rates were 12.5-36.0 µl/min/g kidney weight (KW), and plasma sodium and potassium concentrations were 138-154 and 3.7-5.2 mmol/L, respectively (Table 2).

Table 2. Effects of oral administration of quercetin (50 mg/kg) on mean arterial blood pressure (MABP), renal blood flow (RBF), renal vascular resistance (RVR), urine flow rate ($\dot{V}$), hematocrit (Hct), plasma sodium (P_{Na}) and potassium (P_K) concentration and kidney weight (KW) in cisplatin (7.5 mg/kg, i.p.) treated rats.
Cis = cisplatin.

Treatment	MABP (mm Hg)	Hct (%)	RBF (ml/min/gKW)	RVR (mm Hg/ml/min/gKW)	$\dot{V}$ (µl/min/gKW)	P_{Na} (mmol/L)	P_K (mmol/L)	KW (g)
Day 3								
Control (n=6)	116 ± 4	44.9 ± 0.3	7.84 ± 0.46	15.5 ± 1.2	20.2 ± 3.3	154 ± 5	4.4 ± 0.1	1.477 ± 0.066
Cisplatin (n=6)	119 ± 4	53.5 ± 1.1*	0.53 ± 0.14*	294.2 ± 48.3*	36.1± 3.9*	147 ± 7	4.1 ± 0.1	1.742 ± 0.041
Cis+Quercetin (n=7)	113 ± 5	51.4 ± 0.6	0.39 ± 0.12*	436.3 ± 92.7*	27.6 ± 2.1	149 ± 5	4.1 ± 0.1	1.640 ± 0.051
Day 7								
Control (n=6)	109 ± 5	45.5 ± 1.3	7.76 ± 0.67	14.7 ± 1.3	19.4 ± 5.0	138 ± 3	4.4 ± 0.1	1.630 ± 0.058
Cisplatin (n=6)	112 ± 7	48.9 ± 1.8	0.56 ± 0.27*	593.6 ± 285.2*	12.8 ± 1.4	145 ± 2	5.2 ± 0.7	2.230 ± 0.121*
Cis+Quercetin (n=11)	115 ± 8	43.8 ± 2.4	5.42 ± 1.19†	115.6 ± 48.4†	28.6 ± 4.3†	145± 2	4.8 ± 0.6	1.846 ± 0.136†
Day 14								
Control (n=6)	117 ± 3	47.2 ± 0.8	8.28 ± 0.36	14.6 ± 0.9	17.0 ± 3.2	145 ± 4	3.7 ± 0.1	1.709 ± 0.063
Cisplatin (n=4)	139 ± 3*	41.3 ± 0.3*	1.97 ± 0.46*	81.2 ± 14.3*	13.2 ± 2.8	146 ± 3	4.1 ± 0.2	2.684 ± 0.144*
Cis+Quercetin (n=6)	122 ± 6†	44.5 ± 1.4†	5.57 ± 1.14*†	32.0 ± 9.5†	12.5 ± 1.5	145 ± 3	3.9 ± 0.1	1.767 ± 0.101†

Data are mean ± S.E.M. *, † P <0.05 compared with vehicle control and cisplatin group, respectively.

On days 3, 7, and 14, cisplatin injection caused a significant decrease in RPF levels compared with their respective control groups from 3.92 ± 0.25 to 0.22 ± 0.06, from 3.74 ± 0.36 to 0.28 ± 0.14, and from 3.96 ± 0.14 to 1.05 ± 0.24 ml/min/g KW, respectively. When quercetin was given along with cisplatin, the RPF levels for days 3, 7, and 14 were 0.17 ± 0.05 (not significant), 2.62 ± 0.60, and 2.75 ± 0.53 ml/min/g KW compared to cisplatin injection alone (P <0.05). The calculated RVR of cisplatin treated groups on days 3, 7, and 14 were 20-, 40-, and 5.5-fold of the control values (P <0.05) (Table 2). Quercetin treatment did not significantly alter the RVR on day 3 (28-fold of control) but lower values were observed on day 7 (8-fold) and day 14 (2.2-fold) compared with the control values.

A significant decrease in GFR was observed in cisplatin treated rats on days 3, 7, and 14 from 1.29 ± 0.04 to 0.24 ± 0.04, from 1.34 ± 0.72 to 0.11 ± 0.38, and from 1.38 ± 0.06 to 0.37 ± 0.06 ml/min/g KW, respectively, compared with the respective control (Figure 2B). The GFR in cisplatin + quercetin groups observed on days 3, 7, and 14 were 0.19 ± 0.04 (not significant), 0.81 ± 0.19, and 0.89 ± 0.18 ml/min/g KW, respectively, compared with the cisplatin-treated groups (P <0.05).

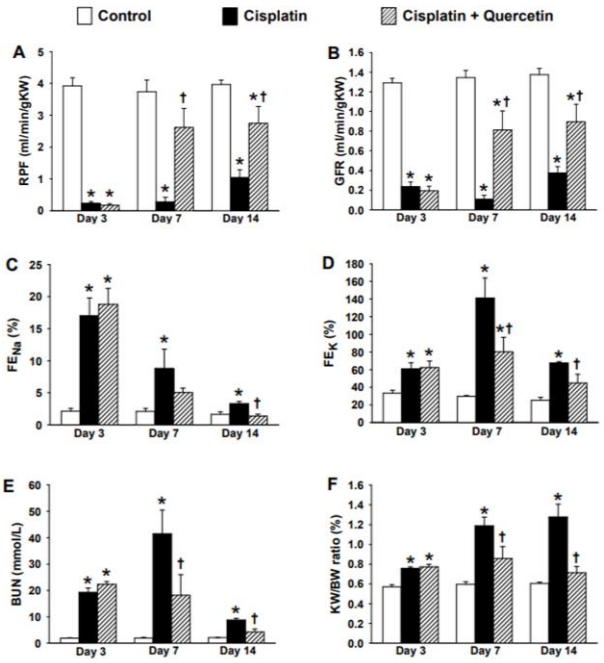

Figure 2. Effect of quercetin on renal plasma flow (RPF) glomerular filtration rate (GFR), fractional excretion of sodium (FE_{Na}) and potassium (FE_K), blood urea nitrogen (BUN) and kidney weight/body weight (KW/BW) ratio in cisplatin-induced nephrotoxic rats. *, † P <0.05 compared to vehicle control and cisplatin group, respectively (one-way ANOVA with multiple comparison using Student-Newman Keuls post hoc test).

On days 3, 7, and 14, the FE_{Na} of the cisplatin injection groups increased significantly from the respective control groups from 2.1 ± 0.4 to 17.0 ± 2.8, from 2.1 ± 0.5 to 8.8 ± 3.0, and from 1.6 ± 0.4 to 3.3 ± 0.3 %, respectively, (Figure 2C). The quercetin treatment did not significantly subside these high excretion rates on either day 3 (18.8 ± 5.5%) or day 7 (5.0 ± 0.7%) but did on day 14 (1.4 ± 0.3%).

The FE_K significantly increased after cisplatin injection on days 3, 7, and 14 compared with the corresponding control from 33.3 ± 3.4 to 60.8 ± 7.0, from 29.4 ± 1.2 to 141.2 ± 22.8, and from 25.1 ± 3.4 to 67.5 ± 1.4 %, respectively (Figure 2D). The quercetin treatment partially reduced the high potassium excretion on days 7 and 14 to 80.2 ± 16.4 and 44.7 ± 10.0, respectively, compared to the cisplatin groups (P <0.05).

In the cisplatin-treated rats on days 3, 7, and 14, the BUN increased 10-, 20-, and 4-fold compared to their respective control. Quercetin treatment did not lower these values in the day 3 group but significantly lowered the BUN observed on days 7 and 14 to 9-fold and 2-fold of controls, respectively (Figure 2E).

Either KW or the kidney weight per body weight (KW/BW) ratio of cisplatin-treated rats on days 3, 7, and 14 increased significantly compared with the respective controls (Table 2 and Figure 2F). Quercetin treatment did not affect the increased KW/BW ratio observed on day 3 but significantly suppressed these increased values observed on days 7 and 14.

3.3 Renal histopathological examination

The three vehicle control groups showed mostly normal structures of glomeruli and tubules (Figures 3, 4, and 5). Seven days after cisplatin injection, glomerular atrophy with dilatation of Bowman's capsule were observed and the score was 2 in 34% of the total number of animals. Quercetin treatment abolished the score of 2 and glomerular atrophy was observed in 9%.

The structural alterations of the renal tubules appeared in both the cortex and medulla in the cisplatin treated rats observed on days 3, 7, and 14 (Figure 3). However, in the cisplatin treated groups, the score for intratubular hyaline cast formation dramatically increased to a score of 3 on day 7 which was observed in 17% of all rats. Quercetin completely reduced the percentage of intratubular hyaline cast formation to 0%. Likewise, in the cisplatin groups, the score for renal tubular dilatation increased to 3 which included 66% on day 7. The dilatation of the renal tubules in the OSOM area on day 7 was more severe than in the cortex (Figure 4). Quercetin treatment lowered the occurrence of tubular dilatation to 18% (Figure 3). Advanced renal tubular nuclear swelling, pyknosis, and karyorrhexis, which were observed in day 3 after cisplatin administration, were partially reduced by the quercetin treatment (Figure 3).

Light microscopy examination indicated renal tubular nuclear pyknosis and karyorrhexis after cisplatin injection on day 3 (Figure 4). Both the cortical and OSOM areas were markedly damaged. Quercetin treatment reduced the amount of damage and was easily observed from day 7 onwards.

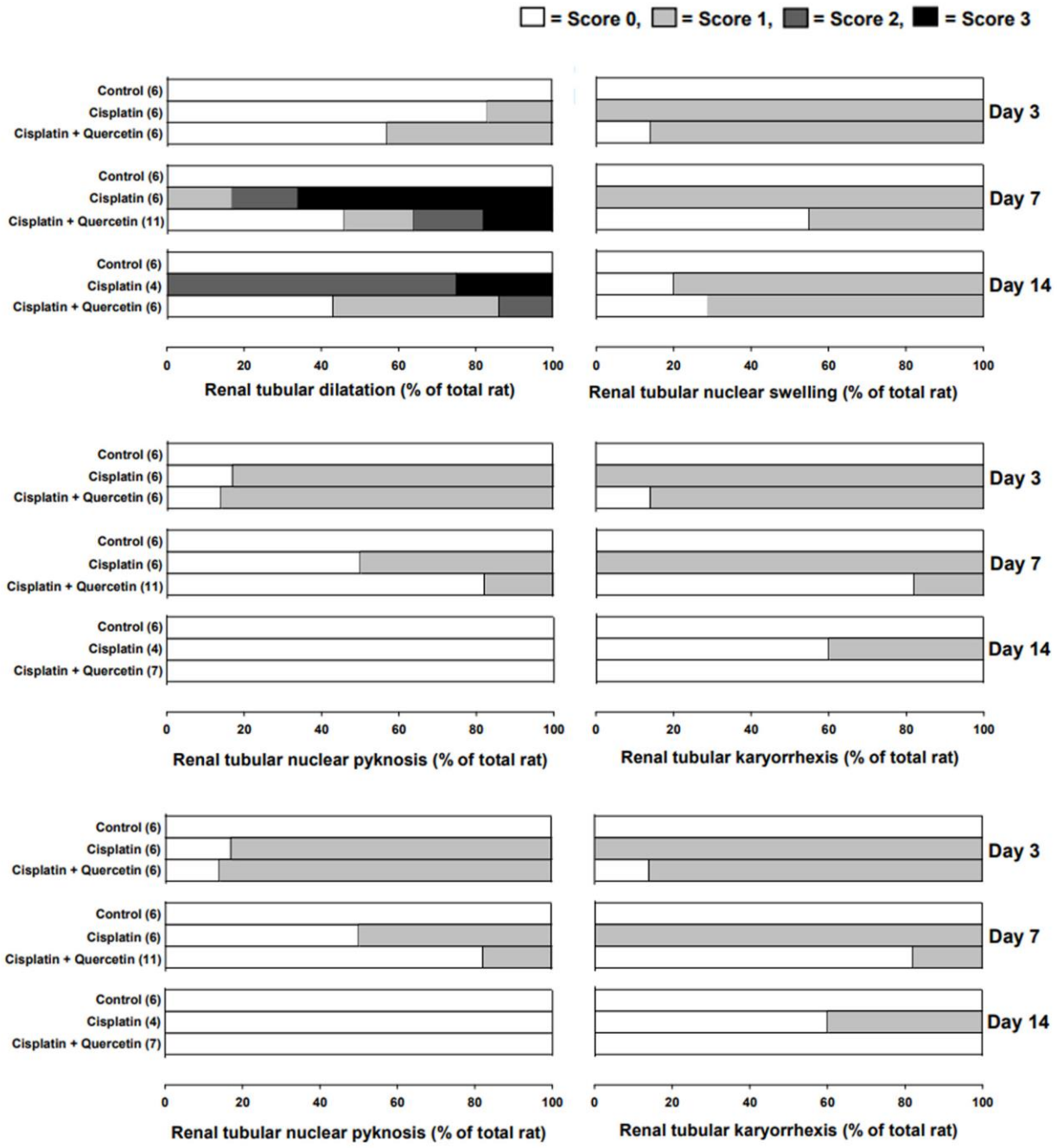

Figure 3. Histopathological scoring of glomerular atrophy, intratubular hyaline cast formation, renal tubular dilatation, renal tubular nuclear swelling, pyknosis and karyorrhexis in control, cisplatin and cisplatin+quercetin treated-rats.

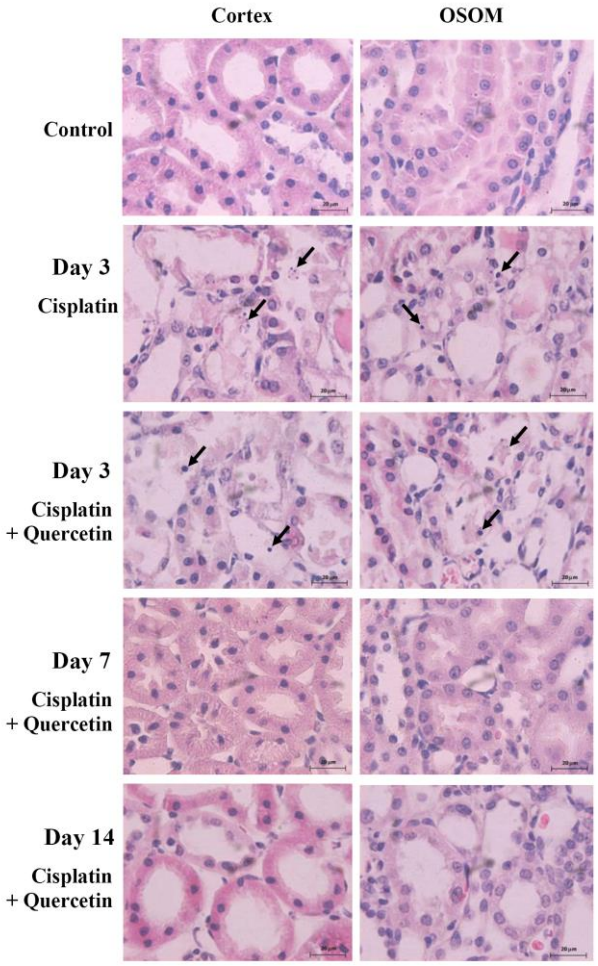

Figure 4. Light micrograph of cortex (left panels) and outer stripe of the outer medulla (OSOM) (right panels) showing normal renal tubules in control group and renal tubular cell death (as indicated by nuclear pyknosis and karyorrhexis) in cisplatin or cisplatin + quercetin groups. Arrow showed renal tubular nuclear pyknosis or karyorrhexis in cisplatin and cisplatin + quercetin groups). Hematoxylin & eosin stain. Scale bar = 20 µm.

Intratubular hyaline cast formation and tubular dilatation in both the cortex and medulla were markedly present in the cisplatin-treated groups on day 3 and day 7 (Figure 5). Quercetin treatment lowered the injuries seen clearly from day 7 onwards. In addition, tubular epithelial cell desquamation, which denuded the basement membrane, was observed on day 7 after cisplatin injection. This tubular damage was also attenuated by quercetin treatment (Figure 5).

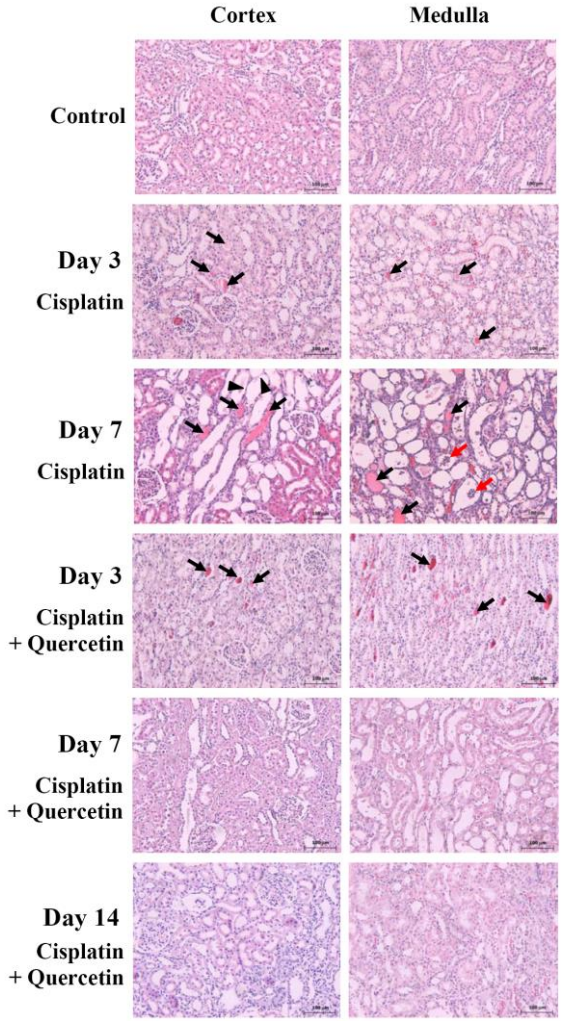

Figure 5. Light micrograph of renal tubules in cortex (left panels) and medulla (right panels) in control, cisplatin and cisplatin + quercetin groups. In cisplatin-treated groups, on day 3, intratubular hyaline cast formations (black arrow) were observed in both cortex and medulla. On day 7, in both cortical and medullary tubules appeared intratubular hyaline cast accompanied by markedly tubular dilatation. Quercetin treatment did not lower these damages on day 3 but it did on day 7 and 14. Desquamation of renal epithelial cells (red arrow) was also observed on day 7 after cisplatin injection. This resulted in denudation of the basement membrane (arrowhead). Hematoxylin & eosin stain. Scale bar = 100 µm.

3.4 Renal lipid peroxidation and antioxidants

Only on day 3 after cisplatin injection did the renal MDA level significantly increase to 134 ± 10% of the control group (Figure 6A). Quercetin treatment suppressed this elevated renal MDA level (P <0.05). However, on days 7 and 14, an increase in renal MDA level was not observed.

The renal CAT activity, determined on day 3 after cisplatin injection, significantly decreased to 55 ± 11% of control value. Quercetin treatment did not improve this decreased activity (54 ± 7% of control) (Figure 6B). The renal SOD activity and GSH concentration after either cisplatin alone or cisplatin with quercetin treatment were similar to the respective control values observed on days 3, 7, and 14 (Figure 6C and D).

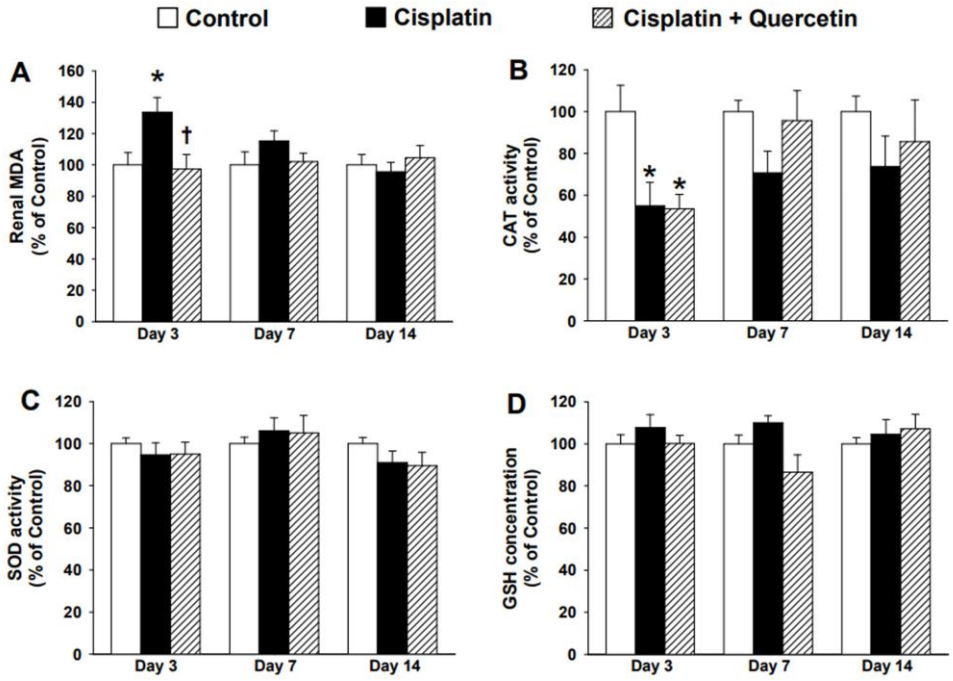

Figure 6. Effect of quercetin on renal malondialdehyde (MDA) level, catalase (CAT), superoxide dismutase (SOD) activities and glutathione (GSH) concentration in cisplatin-induced nephrotoxic rats. *, † $P < 0.05$ compared to vehicle control and cisplatin group, respectively (one-way ANOVA with multiple comparison using Student-Newman-Keuls post-hoc test.

4. Discussion

The present study revealed the significant effect of quercetin in improving the survival rate of rats after a critical dose of cisplatin injection. From day 3 to day 10 after cisplatin treatment, the mortality rate increased to 50% of the total number of rats in the experiment and this percentage was stable until day 14. The death of the experimental animals may be due to gastrointestinal toxicity along with less food and water consumption of the animals. In addition, the individual susceptibility of cisplatin nephrotoxicity may contribute to death. When quercetin was combined with cisplatin, it significantly lowered the mortality rate of the experimental animals from day 6 of the treatment schedule. All survived animals of the quercetin + cisplatin groups showed a lower reduction in their body weight and a lower BUN level compared to their respective cisplatin groups, which indicated better food consumption and increased renal clearance of plasma urea.

Acute renal failure (ARF) after cisplatin injection in this study was likely an intrinsic ARF rather than the pre-renal ARF. The arterial blood pressure, plasma sodium, and potassium remained unaltered while azotemia and the reduction in RPF and GFR were markedly observed. The glomerular damage caused by cisplatin was supported by the pronounced glomerular atrophy. Cisplatin injection was previously reported to damage renal blood vessels (Atessahin *et al.*, 2006; Shirwaikar *et al.*, 2004). Experiments in isolated blood vessels have shown that ROS may participate in an increased RVR (Gao & Lee, 2005; Tang & Vanhoutte, 2009). We postulated that the higher ROS generated in renal tissue after cisplatin injection may be responsible for the renal vascular damage and an increased RVR. As a consequence, RBF and GFR reduced. Post-glomerular injury indicated by intratubular cast formation, tubular dilatation, and a reduction in tubular reabsorption of sodium and potassium may also contribute in this nephrotoxicity caused by cisplatin.

The possible mechanisms underlying the renal protective effects of quercetin in cisplatin-induced acute renal failure rats were investigated in this study. Despite the lower cumulative quercetin doses given to the animals in this present study, the effect of quercetin was confirmed to increase RBF and GFR in cisplatin treated rats similar to previous reports (Behling *et al.*, 2006; Sanchez-Gonzalez *et al.*, 2011),. We also found a significant increase in RBF while MABP remained unchanged which suggested the renal vasodilatation effect of quercetin. This was assured by a decrease in the calculated RVR in the quercetin co-treatment groups compared to the cisplatin treatment groups.

It is likely that the ROS may play a role in cisplatin-induced nephrotoxicity. Renal CAT activity in cisplatin treated rats was significantly reduced by 50% of control value observed on day 3 together with an increase in renal MDA. The enzymatic antioxidant function of CAT would generally

catalyze the decomposition of ROS, such as H_2O_2 caused by cisplatin, to O_2 and H_2O. Once the level of this antioxidant is reduced, the ROS scavenging property diminishes, hence, an increase in renal lipid peroxidation appears. However, the changes in renal SOD and GSH could not be detected in this study. The function of SOD is to catalyze the conversion of $O^{\cdot}_2$ production to O_2 and H_2O_2 while GSH is the substrate that glutathione peroxidase uses to catalyze the breakdown of H_2O_2. It is likely that cisplatin injection directly resulted in an accumulation of renal H_2O_2 and a significant reduction in the scavenging enzyme, CAT, which was possibly responsible for the renal damage caused by ROS. Quercetin treatment restored the renal MDA level but did not improve the decrease in renal CAT activity. This suggested that the direct quercetin scavenging ROS property took place rather than the upregulating of CAT.

Quercetin treatment in cisplatin-treated rats was able to suppress the renal ischemic perfusion injury that possibly occurred on days 7 and 14. Cisplatin treatment caused a significant reduction in RBF and hence GFR. This ischemic kidney and the subsequent re-oxygenation could generate ROS at the reperfusion phase that might exacerbate the inflammation, cell death, and acute kidney failure. It is likely that the scavenging effect of quercetin may be responsible for improving blood flow and glomerular filtration. Since GFR improved along with RBF, this may suggest a lower afferent arteriolar resistance than efferent arteriolar resistance. However, other glomerular filtering parameters such as mesangial contraction could not be excluded. Moreover, the improvement of tubular function indicated by reduced fractional electrolyte excretion and a significant reduction in BUN level were also observed on days 7 and 14 in the quercetin treatment group which would further support the ROS scavenging property of quercetin.

Our histological examination revealed a similar degree of general glomerular atrophy in all groups of the experiment. Therefore, the reduction in GFR after cisplatin injection in this study may not be due to the severe glomerular damage. The renal tubular cast formation found in the cisplatin-treated groups may be one of the factors that caused luminal obstruction, reduction in net filtration pressure and hence reduction in GFR. As a result of intratubular cast formation, tubular dilatation and fluid accumulation were formed which might be the cause of the higher KW/BW in the cisplatin-treated groups. Hyaline casts are solidified Tamm-Horsfall glycoprotein excreted from tubular epithelial cells into the urine as a defense mechanism against urinary tract infection caused by uropathogenic bacteria. Vyletal *et al.* (2010) reported that urinary uromodulin excretion tended to increase after cisplatin administration in patients. In this study, it is found that quercetin coadministration can decrease the formation of hyaline casts in rat tubular lumen by unknown mechanisms which reduced tubular dilatation and KW/BW ratio.

Renal tubular cell death, indicated by nuclear pyknosis and karyorrhexis and cast formation in both the cortex and OSOM regions caused by cisplatin, was also possibly involved in natriuresis and kaliuresis noticed on days 3, 7, and 14. Obstruction of tubular fluid flow by the formation of casts along with the inability of electrolyte reabsorption might result in tubular dilatation in the latter stages of the experiment. The scavenging property of quercetin, as we previously hypothesized, may recover both the Na^+ and K^+ transport defect caused by cisplatin.

In this study, renal tubular injury found after cisplatin injection was more severe in the tubules of the OSOM than in the cortical tubules, especially on days 7 and 14 after i.p. injection of 7.5 mg/kg cisplatin. This was similar to a previous study which showed that on day 3 and day 5 after i.p. injection of 6 mg/kg cisplatin, the S3 segment of the proximal tubules located in the OSOM was the area damaged the most compared to the rest of the nephron (Dobyan *et al.*, 1980).

We conclude that oral administration of quercetin (50 mg/kg) at 24 h and 10 min before a single i.p. injection of cisplatin (7.5 mg/kg) significantly decreased the mortality rates on days 7 and 14. Cisplatin injection resulted in the reduction in body weight, RBF, and GFR while it caused an increase in BUN concentration, FE_{Na}, FE_K, kidney weight, renal MDA level, and tubular damage observed within 14 days. On day 3, quercetin treatment did not prevent these alterations despite the suppression of renal MDA elevation. On days 7 and 14, Quercetin treatment partially improved both glomerular and tubular functions. The likely mechanism of quercetin action was its ROS scavenging property. Therefore, quercetin would be able to permit the use of higher doses of cisplatin without causing a high mortality rate, renal dysfunction or severe renal tubular injury.

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