Original Research

The effect of thymoquinone treatment on the combined renal and pulmonary toxicity of cisplatin and diesel exhaust particles

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Abstract

Particulate air pollution (PAP) exposure is associated with increased morbidity and mortality, particularly in patients with renal disease. However, there are only a few studies on the interaction between PAP and renal injury, and none on agents that may ameliorate it. We studied the interaction between cisplatin (CP) nephrotoxicity and a single exposure to diesel exhaust particle (DEP) in rats 24 h before sacrifice, and assessed the effect of co-treatment with the active ingredient in Nigella Sativa seed oil, thymoquinone (TQ) thereon. Rats were injected intraperitoneally with CP (6 mg/kg) and four days later, they were exposed intratracheally to DEP (0.5 mg/kg), and were sacrificed 24 h later. Oral TQ (20 mg/kg) was given daily throughout the experimental period. CP alone caused several physiological, biochemical, and histopathological changes that included reduced growth and creatinine clearance, and raised plasma neutrophil gelatinase-associated lipocalin (NGAL), interleukin 6 (IL-6) and C-reactive protein (CRP), creatinine and urea concentrations, and urinary *N*-acetyl-b-D-glucosaminidase (NAG) activities. It adversely affected several indices of oxidative damage in the kidneys, and induced renal tubular necrosis. Most of these actions were significantly potentiated in rats given both CP and DEP. TQ significantly abrogated many of the effects of CP and DEP, given alone and in combination. These results provide experimental evidence that subjects with renal diseases can be at higher risk from PAP, and that TQ, pending further pharmacological and toxicological studies, can be considered a useful agent in patients with renal diseases and exposed to PAP.

Keywords: Cisplatin, diesel exhaust particle, nephrotoxicity, thymoquinone, rats

Experimental Biology and Medicine **2015; 240: 1698–1707. DOI: 10.1177/1535370215579013**

Introduction

Cisplatin (*cis*-diamminedichloroplatinum II, CP), first discovered in 1965, is still one of the most effective and commonly used chemotherapeutic agents in the treatment of variety of solid tumors including those of the head, neck, testis, ovary, breast, and penis,[1,2] mainly because of its high efficacy and low cost. The drug's efficacy increases with dose, but high doses are associated with many severe adverse effects, particularly nephrotoxicity which is often the main dose-limiting factor in therapy with CP.[3–5] Despite many studies, the mechanism underlying the side effects induced by CP is not fully understood, but is hypothesized to be multi-factorial in nature.[5–9] These possible mechanisms include the generation of reactive oxygen species (ROS) and inflammation. The former could interfere with the antioxidant defense system and result in oxidative damage in different tissues, and reaction with thiols in protein and glutathione, which could cause cell dysfunction.[10,11] The latter (inflammation) could be provoked by damage to the epithelial cells of the kidneys and may amplify renal injury and dysfunction *in vivo*.[12] Mediated by glutathione-S-transferase, CP is known to be transformed *in vivo* into a more toxic compound in the kidneys.[13]

Various strategies and drugs have been attempted to ameliorate or prevent CP nephrotoxicity via simultaneous supplementation of preventive agents. These include antioxidants, modulators of nitric oxide, diuretics, cytoprotective, and anti-apoptotic agents and others.[11,14–17] However, none of these have been tested clinically in humans.

Particulate air pollution (PAP), especially with particle diameter less than 2.5 mm such as diesel exhaust particle (DEP) is established to induce pulmonary and extrapulmonary morbidity and mortality on vital systems that include the cardiovascular, respiratory and renal systems, especially in at-risk populations such as the elderly and those with diabetes or hypertension.[18,19]

Acute renal failure (ARF, or acute kidney injury) is becoming increasingly prevalent in both the developed and the developing world, ensuing in severe morbidity and mortality,[20] and is on the rise due to ageing of the population.[21] About 20% of ARF cases among hospitalized patients are caused by CP nephrotoxicity, and more than a third of the patients develop renal injury within 10 days following a single dose of CP.[22]

The number of subjects affected by PAP is extremely large, and as a result, estimates of its public health burden are substantial.[23] A meta-analysis of 110 peer-reviewed time series studies have shown that exposure to air pollution can cause several health adverse conditions, and significantly increases the risk of morbidity and mortality.[24] Lung and kidney functions are closely related in both healthy and diseased subjects. Several studies have reported consistent association between ARF and pulmonary dysfunction.[25,26] Lung injury is established to aggravate ARF and vice versa.[27] When ARF and acute lung injury are combined, the mortality rate exceeds 80%.[28] Experimentally, ARF has been reported to induce caspase-dependent pulmonary apoptosis.[29] Patients with various renal diseases are particularly vulnerable to the adverse effects of PAP.[30]

Thymoquinone (TQ; 2-isopropyl-5-methyl-1, 4-benzoquinone) is the main active component of *Nigella sativa* seed oil. TQ is known to possess strong antioxidant and anti-inflammatory properties.[31–33] As TQ has been previously found to be beneficial in several experimental models in which oxidative and inflammatory tissue injuries are the basis of toxicity,[19,25,34,35] and as it is present in the oil of the commonly available black seed, a safe natural product, it was therefore of interest to test here if this compound has a possible ameliorative action against the combined toxicity of CP and DEP. As far as we are aware, this has not been reported before.

Materials and methods

Animals

Forty-eight male Wistar rats, aged 7–8 weeks and initially weighing about 200 g, were obtained from the Small Animal House of Sultan Qaboos University (SQU) and provided with standard laboratory diet and water ad libitum. They were randomly divided into eight equal groups and housed in plastic cages, at a temperature of $23 \pm 2^{\circ}C$, relative humidity of 50–60% and a 12-h dark–light cycle. An acclimatization period of four days was allowed for the rats before any experimentation. The rats were weighed at the beginning of the experiment and just before sacrifice. Rats were cared for under a protocol approved by the Animal Research Ethics Committee of our institution, and according to the NIH Guide for the Care and Use of Laboratory Animals, NIH publication no. 85-23, 1985.

Particles

DEP (SRM 2975) was obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD), and was suspended in sterile normal saline (NaCl 0.9%) containing Tween 80 (0.01%). To prevent aggregation of its particles, it was sonicated for 15 min and vortexed before dilution and intratracheal (i.t.) administration. Control rats were given the vehicle Tween 80 (0.01%). The size of the DEP used in this study has been analyzed before by transmission electron microscopy.[18]

Treatments

A summary of the treatment of the eight groups is given in Table 1. The groups were treated as follows:

Group 1. Corn oil (control, 0.5 mL/rat) given orally for 11 consecutive days, and on day 7 also given a single intraperitoneal injection of saline (0.5 mL per rat), and also subjected to a single i.t. administration of Tween 80 (0.01%) (as a vehicle of DEP) 24 h before sacrifice.

Group 2. Corn oil (control, 0.5 mL/rat) given orally for 11 consecutive days, and on day 7 also given a single intraperitoneal injection of CP (6 mg/kg), at a concentration of 1 mg/mL, and also subjected to a single i.t. administration of Tween 80 (0.01%) 24 h before sacrifice.

Group 3. Corn oil (control, 0.5 mL/rat) given orally for 11 consecutive days, and on day 7 also given a single intraperitoneal injection of saline (0.5 mL per rat), and also subjected to a single i.t. administration of DEP (0.5 mg/kg) 24 h before sacrifice.

Group 4. Corn oil (control, 0.5 mL/rat) given orally for 11 consecutive days, and on day 7 also given a single intraperitoneal injection of CP (6 mg/kg), at a concentration of 1 mg/mL, and also normal saline (control, 0.5 mL/rat) given orally for seven consecutive days, and subjected to a single i.t. administration of DEP (0.5 mg/kg).

Groups 5, 6, 7, and 8. These groups were treated in an identical manner to groups 1, 2, 3, and 4, respectively, except that all rats in groups 5–8 were also given TQ

Table 1 Treatments with intratracheal instillation of Saline (Sa) or diesel exhaust particles (DEP) with or without cisplatin (CP) administration (given intraperitoneally) in rats

Groups/treatment	CO	TQ	Saline	CP	Sa	DEP
Control	+	−	+	−	+	−
CP	+	−	−	+	+	−
DEP	+	−	−	−	−	+
CP + DEP	+	−	−	+	+	+
TQ	+	+	+	−	−	−
CP + TQ	+	+	−	+	+	−
DEP + TQ	+	+	−	−	−	+
CP + DEP + TQ	+	+	−	+	+	+

Note: Thymoquinone, TQ (20 mg/kg) in corn oil (CO) was given orally continuously for 11 days, and CP (6 mg/kg) was injected intraperitoneally on the sixth day of treatment. DEP was given by instillation on the 11th day. All rats were killed on the 12th day.

(20 mg/kg) orally by gavage from day 1 until 24 h before sacrifice.

One day before the rats were sacrificed, urine of each rat was collected over a 24-h period, and its volume measured. After 24-h of DEP or vehicle administration, rats were anesthetized with a combination of ketamine (60 mg/kg) and xylazine (5 mg/kg) given intraperitoneally. Blood was collected from the inferior vena cava in heparinized tubes and centrifuged at 900 g for 15 min at 5°C, to separate plasma. The plasma harvested was stored frozen at −80°C to await biochemical analyses, and the rats were killed by an overdose of anesthesia. The kidneys and lungs were removed from the rats, washed with ice-cold saline, blotted with a piece of filter paper and weighed. A small piece from the left kidney and lung were fixed in 10% buffered formalin for subsequent histopathological processing, and the rest of the renal tissue was immediately deep frozen at −80°C for measuring platinum concentration. The right kidney was also stored immediately deep frozen at −80°C and was thawed (within less than a week) and homogenized in ice-cold saline to produce 10% (w/v) tissue homogenate for the biochemical assays.

Biochemical methods

Plasma creatinine and urea and urinary creatinine concentrations, as well as plasma L-c-glutamyltransferase (GGT), aspartate transaminase (AST), alanine transaminase (ALT) activities, and sodium (Na), potassium (K), and chloride (Cl) concentrations were measured by an automated analyzer, as described before.[19,36] *N*-acetyl-D-glucosaminidase (NAG) activity in urine was measured using kits purchased from Diazyme, General Atomics (San Diego, CA, USA). Plasma neutrophil gelatinase-associated lipocalin (NGAL) was measured with a sandwich enzyme linked immunosorbent assay (BioPorto Diagnostics, Hellerup, Denmark).

Interleukin-6 (IL-6) and C-reactive protein (CRP) were measured by ELISA kits from R & D systems (Minneapolis, MN, USA). Kidney reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) and total antioxidant capacity (TAC) were measured as described before.[16]

Histopathology

The formalin-fixed lungs and kidneys were dehydrated in increasing concentrations of ethanol, cleared with xylene and embedded in paraffin. Five-micrometer sections were prepared from the tissue blocks and stained with hematoxylin and eosin. The microscopic examination of the tissue sections was carried out in a blinded fashion by a histopathologist who was unaware of the treatment each donor animal had received.

Measurement of renal platinum concentration

The CP concentration (as platinum, Pt) in renal tissue was measured and validated by inductively coupled plasma atomic emission spectrometry (Perkin Elmer, Shelton, CT, USA) as described previously.[37]

Drugs and chemicals. CP used was from Pharma GES (Unterach, Austria) and platinum standard solution and thymoquinone were from Sigma (St. Louis, MO, USA). The rest of the chemicals were of the highest purity grade available.

Statistical analysis. All values are presented as means ± S.E.M. The data were analyzed by analysis of variance (ANOVA), followed by Tukey–Kramer multiple comparison test. Analysis of the histology scores was by a two-sided Mann–Whitney test. A value of $p < 0.05$ was selected as the criterion for statistical significance. All statistical analyses were performed with GraphPad Prism version 4.03 (GraphPad Software Inc, San Diego, CA, USA).

Results

To induce ARF, rats were treated with a single CP injection at a dose of 6 mg/kg and this resulted in ARF, similar to previously reported studies.[16,26]

As shown in Table 2, rats given saline or TQ alone gained an increase in their body weight of about 16.5% and 18.6%, respectively ($p < 0.05$), while those receiving CP only lost 15.3%, and those given DEP only gained about 8.7% ($p < 0.05$, when compared to control). However, rats treated with CP together with TQ lost 11.5% of their bodyweight, while rats treated with DEP together with TQ gained only 0.6% of their body weight. When the three agents (CP, DEP, and TQ) were given concomitantly, the treated rats lost 12.0% of their body weight. The kidney weights were not significantly changed in rats treated with CP, DEP, or TQ (given either singly or all together) when compared to saline-treated rats. However, treatment with CP + DEP significantly increased relative kidney weight ($p < 0.05$).

Water intake was insignificantly increased by treatment of CP or CP + TQ (by about 34% and 37%, respectively). Urine output was significantly increased in rats treated with CP, CP + DEP, and CP + TQ ($p < 0.05$). Rats treated with CP + DEP and TQ urinated significantly less than rats treated with CP + DEP ($p < 0.05$). Neither food intake, nor fecal outputs were significantly affected by any of the agents used.

Figure 1 illustrates the effects of saline, CP, DEP, TQ, each one alone or in combination, on some kidney function tests in plasma and urine. CP alone induced significant rises in the concentrations of urea, NGAL, and in NAG activity, and a significant reduction in creatinine clearance ($p < 0.001$). Treatment of DEP alone did not significantly affect any of the indices measured. Treatment of rats with CP + DEP did not significantly affect the actions of CP mentioned above. In CP and CP + DEP-treated rats, however, the concentration of urea were significantly higher than the corresponding values obtained from control rat ($p < 0.05$) or rats treated with CP + DEP + TQ. The values for the other renal function tests in plasma and urine were insignificantly abated in rats treated with CP + DEP + TQ, when compared with values obtained from rats treated with CP alone, or CP + DEP. Creatinine clearance was significantly diminished in groups treated with CP, CP + DEP, and CP + TQ

Table 2 The effect of treatment of rats with saline (CON), cisplatin (CP), diesel exhaust particle (DEP), thymoquinone (TQ) singly or in combination, on biological characteristics

	CON	CP	DEP	CP + DEP	TQ	CP + TQ	DEP + TQ	CP + DEP + TQ
Initial Body wt. (g)	274.0 ± 7.6	279.3 ± 3.7	275.0 ± 6.8	279.2 ± 8.3	279.2 ± 5.2	274.1 ± 5.9	274.7 ± 13.5	276.7 ± 8.2
Final Body wt. (g)	290.2 ± 7.1	264.6 ± 5.5	283.8 ± 6.6	$240.3 \pm 11.8^{*,\ddagger}$	$297.6 \pm 6.6^{\S\S}$	262.6 ± 9.5	275.3 ± 15.8	264.3 ± 6.5
Change in body wt. (%)	6.2 ± 1.9	$-5.5 \pm 0.7^{**}$	$3.4 \pm 2.8^{\dagger}$	$-11.8 \pm 2.1^{***,\ddagger\ddagger\ddagger}$	$6.6 \pm 1.3^{\dagger\dagger,\S\S\S}$	$-4.3 \pm 1.7^{**,\P\P}$	$0.3 \pm 2.7^{\S\S}$	$-4.4 \pm 0.7^{**,\P\P}$
Kidney wt. (g)	2.0 ± 0.1	2.5 ± 0.1	2.0 ± 0.1	$2.7 \pm 0.2^{**,\ddagger\ddagger}$	$2.1 \pm 0.1^{\S}$	2.4 ± 0.1	$2.0 \pm 0.1^{\S\S}$	2.2 ± 0.1
Kidney wt. (% final body wt.)	0.7 ± 0.03	0.9 ± 0.05	0.7 ± 0.03	$1.1 \pm 0.06^{***,\ddagger\ddagger\ddagger}$	$0.7 \pm 0.06^{\S\S\S}$	0.9 ± 0.03	$0.7 \pm 0.01^{\S\S\S}$	$0.8 \pm 0.06^{\S\S\S}$
Water intake (ml)	21.3 ± 3.6	28.5 ± 3.3	22.3 ± 1.5	$35.2 \pm 2.3^{*,\ddagger}$	$19.0 \pm 1.9^{\S\S}$	$33.3 \pm 3.6^{\P}$	$19.7 \pm 2.5^{\S\S,\$}$	28.3 ± 2.9
Urine output (ml)	7.6 ± 0.8	$19.5 \pm 1.1^{***}$	$11.8 \pm 1.6^{\dagger\dagger}$	$20.6 \pm 1.5^{***,\ddagger\ddagger\ddagger}$	$6.2 \pm 0.7^{\dagger\dagger\dagger,\ddagger,\S\S\S}$	$18.3 \pm 1.9^{***,\ddagger,\P\P\P}$	$5.6 \pm 0.6^{***,\dagger,\ddagger\ddagger\ddagger,\S\S\S,\$\$\$}$	$13.3 \pm 0.9^{*,\dagger,\S\S,\P\P,\#\#}$
Food intake (g)	16.1 ± 1.4	15.2 ± 1.1	11.0 ± 1.4	$9.5 \pm 1.0^{**,\dagger}$	12.4 ± 1.7	12.2 ± 1.0	11.4 ± 0.7	12.2 ± 1.0
Feces wt. (g)	5.6 ± 0.4	5.2 ± 0.5	3.9 ± 1.0	3.2 ± 0.8	3.9 ± 0.6	5.1 ± 0.5	5.0 ± 0.4	4.5 ± 0.7

Note: Values in the table are means ± SEM ($n = 6$). TQ (20 mg/kg) in corn oil was given orally continuously for 11 days, and CP (6 mg/kg) was injected intraperitoneally on the 6th day of treatment. DEP was given by inhalation on the 11th day. All rats were killed on the 12th day.
$^{*}p < 0.05$, $^{**}p < 0.001$, $^{***}p < 0.0001$ vs. CONTROL.
$^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.001$, $^{\dagger\dagger\dagger}p < 0.0001$ vs. CP.
$^{\ddagger}p < 0.05$, $^{\ddagger\ddagger}p < 0.001$, $^{\ddagger\ddagger\ddagger}p < 0.0001$ vs. DEP.
$^{\S}p < 0.05$, $^{\S\S}p < 0.001$, $^{\S\S\S}p < 0.0001$ vs. CP + DEP.
$^{\P}p < 0.05$, $^{\P\P}p < 0.001$, $^{\P\P\P}p < 0.0001$ vs. TQ.
$^{\$}p < 0.05$, $^{\$\$}p < 0.001$, $^{\$\$\$}p < 0.0001$ vs. CP + TQ.
$^{\#}p < 0.05$, $^{\#\#}p < 0.001$, $^{\#\#\#}p < 0.0001$ vs. DEP + TQ.

when compared ($p < 0.05$), with that in saline-treated control ($p < 0.0001$).

As shown in Table 3, CP significantly increased the plasma activities of ALT and AST ($p < 0.05$) and insignificantly increased the activity of GGT. These actions were potentiated by concomitant DEP treatment. Adding TQ to either CP or DEP alone or in combination abrogated these effects.

Treatment with CP alone significantly increased the concentrations of the two pro-inflammatory cytokines IL-6 and CRP ($p < 0.0001$). DEP alone significantly increased the concentration of IL-6 but not CRP. Combination of CP + DEP did not significantly increased the concentrations of the two pro-inflammatory cytokines -IL-6 and CRP beyond that caused by CP alone. Adding TQ to CP and DEP treatments insignificantly reduced the rise in the concentrations of the two pro-inflammatory cytokines (Figure 2).

As shown in Table 4 treatment with CP, alone or together with DEP, caused significant decreases in GSH, SOD, CAT, and TAC ($p < 0.05$). Treatment with TQ in rats treated with CP and DEP caused insignificant improvement in the values of these antioxidant indices.

As shown in Figure 3, the concentration of Pt in the renal tissue of rats given CP alone (5.65 ± 0.5 ppm) was insignificantly higher than that in rats treated with CP + TQ (4.34 ± 0.2 ppm). However, the concentration of Pt in the renal tissue of rats given CP + DEP (7.00 ± 0.80 ppm) was significantly higher than in rats given CP + DEP + TQ (5.00 ± 0.30 ppm).

The effects of the agents used in this work on the histopathological appearance of the kidneys are shown in Figure 4. The renal section of the rats treated with saline (A), DEP (D), TQ (E) and TQ+DEP (G) showed normal glomeruli and tubules (A). Sections from the CP-treated rats showed acute tubular necrosis with tubular dilatation, interstitial edema and congestion (B). These changes were aggravated when CP and DEP were given together, with more foci of acute tubular necrosis and dilatation with some apoptotic cells in the tubules, interstitial edema and congestion (C). TQ treatment lessened the combined effect of CP + DEP (H).

The effects of the agents used in this work on the histopathological appearance of the lungs are shown in Figure 5. Lung sections from rats treated with saline (A), CP (B), and TQ (G) showed only mild congestion or no apparent damage. Section in the lung from DEP-treated rats showed increased interstitial cellularity with widening of the inter-alveolar spaces with congested inter-alveolar capillaries and foci of intra-alveolar edema (C). TQ treatment did not seem to affect the combined action of CP + DEP (H).

Discussion

We have previously shown, and for the first time, that the air pollutant DEP (deposited in the lungs) aggravates experimental ARF induced by CP.[19] In this study, we presented further experimental evidence that exposure of rats to DEP can potentiate CP nephrotoxicity, and that treatment

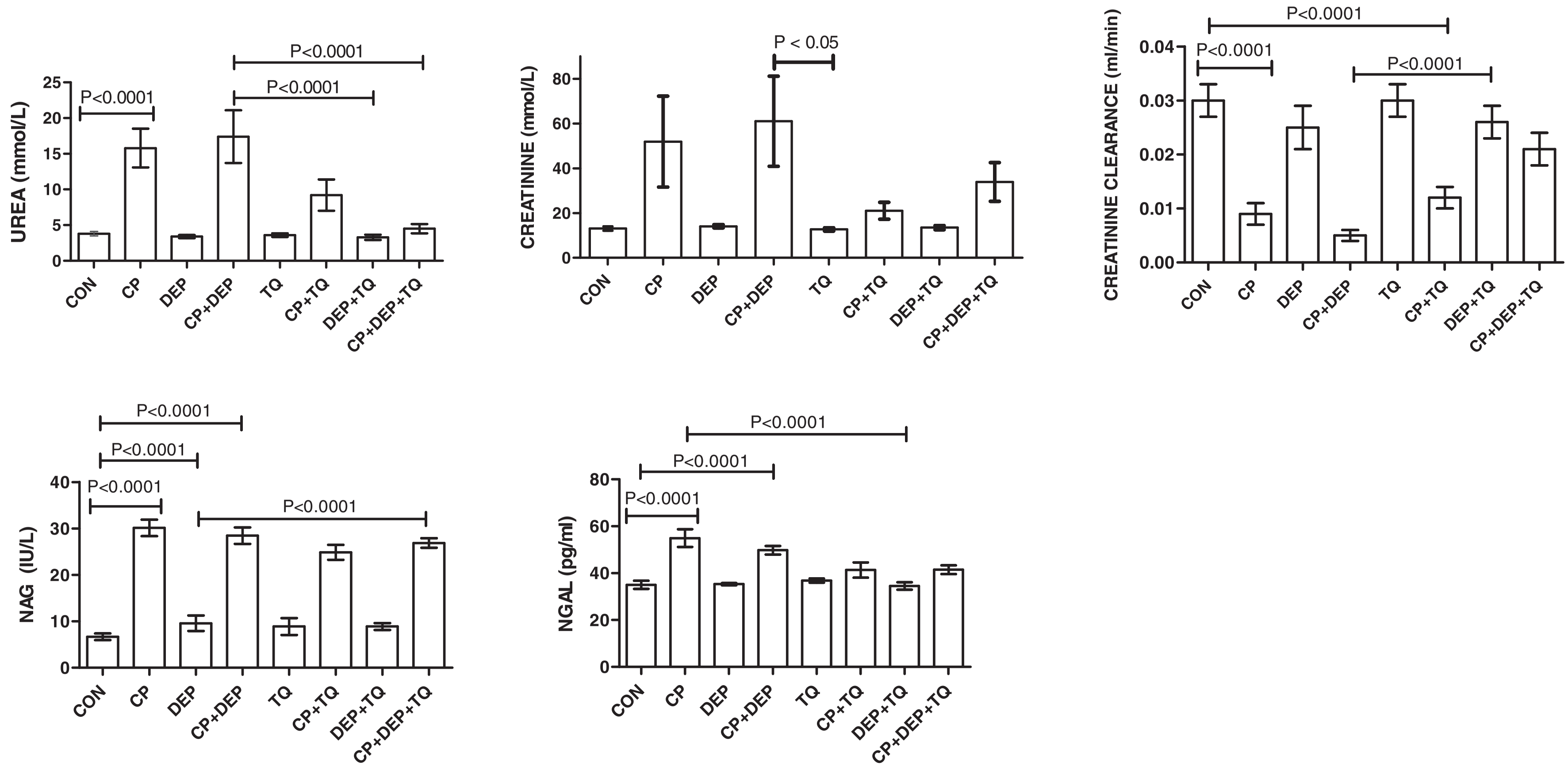

Figure 1 Plasma urea, creatinine and NGAL concentrations, and urine NAG activity, as well as creatinine clearance in rats treated with saline (control, CON), cisplatin (CP) 6 mg/kg, diesel exhaust particles (DEP) 0.5 mg/kg, CP + DEP, thymoquinone (TQ) 20 mg/kg, CP + TQ, DEP + TQ, and CP + DEP + TQ. Each column represents mean ± SE ($n = 6$). Statistical analysis by Tukey–Kramer multiple comparison test

with TQ can mitigate some of the effects of CP and DEP, when each is given alone or in combination.

Treatment with CP caused a significant loss in body weight in rats (amounting to about 5% of their initial body weight, compared to a gain in their control counterparts of about 17%). The causes of this action could not be related to anorexia, as the feed intake of these rats was not significantly reduced but may be due to the cytotoxic effect of CP, or to renal tubular injury influencing the reabsorption of water and causing dehydration and/or due to gastrointestinal disturbance and inflammation.[12,15] Renal damage was also marked by elevated relative kidney weight which has been ascribed to the increase in glomerular volume and other cellular changes, and retention of water and solutes in renal tissues or changes induced by CP in renal blood flow.[38,39]

Table 3 The effect of treatment of rats with cisplatin (CP),diesel exhaust particle(DEP), thymoquinone (TQ) given singly or in combination, on some biochemical parameters in plasma

Groups	ALT (IU/L)	AST (IU/L)	GGT (IU/L)
Control	27.3 ± 1.5	54.2 ± 1.7	3.6 ± 0.4
CP	47.3 ± 1.6**	76.2 ± 8.9*	4.8 ± 0.7
DEP	38.0 ± 2.4	70.6 ± 4.9	3.5 ± 0.4
CP + DEP	52.6 ± 6.5***	77.5 ± 8.8***	8.3 ± 2.4*,‡
TQ	25.2 ± 2.0†††,§§§	62.2 ± 2.7†,§§§	3.6 ± 0.4§
CP + TQ	38.3 ± 4.5	65.0 ± 3.7§	4.2 ± 0.5
DEP + TQ	29.1 ± 2.3†,§§§	58.2 ± 3.1§§	3.3 ± 0.6§
CP + DEP + TQ	38.6 ± 3.0	75.8 ± 15.5§§	5.0 ± 0.1

Note: Values in the table are mean ± SEM ($n = 6$). TQ (20 mg/kg) in corn oil (CO) was given orally continuously for 11 days, and CP (6 mg/kg) was injected intraperitoneally on the 6th day of treatment. DEP was given by inhalation on the 11th day. All rats were killed on the 12th day to collect blood.
Values with different superscript are statistically different
*$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$ vs CONTROL.
†$p < 0.05$, ††$p < 0.001$, †††$p < 0.0001$ vs CP.
‡$p < 0.05$ vs DEP.
§$p < 0.05$, §§$p < 0.001$, §§§$p < 0.0001$ vs CP + DEP.

CP treatment in this work induced, as has consistently been shown before, nephrotoxicity which was evident by several biochemical alterations. Plasma creatinine and urea levels were elevated because of decreased glomerular filtration, or secondarily, due to increased generation of free radicals, which is known to induce mesangial cells contraction, changing the filtration surface area and/or modifying the ultrafiltration factors that can all lead to decreased glomerular filtration rate.[6] CP treatment also caused polyuria, probably due to reduced glomerular filtration or to disturbance in the water permeability regulation in the collecting ducts that is associated with a decreased expression of renal aquaporins in the upper medulla.[40]

In the present study, we showed that a single exposure to DEP exacerbates some signs of CP-induced ARF, an action which was similar to the effect of repeated DEP exposure, albeit with severer tubular necrosis in the renal tissues of rats treated with CP + DEP compared with those from rats treated with CP + saline.[41] This seems to suggest that a single exposure to DEP is still a risk factor in subjects with ARF, as it is a risk in healthy, hypertensive, or diabetic animals.[18]

In this work, and as shown in Table 3, the plasma activities of ALT, AST, and GGT (all markers of tissue damage) have been measured 24 h after the end of the experiments. TQ treatment did not significantly affect the activity of any of these enzymes, confirming the safety of this compound. Treatment with either CP or DEP alone significantly increased the activities of the three enzymes. Earlier, we have histologically shown that DEP exposure causes lung inflammation,[41] and this may be the cause of the elevation of the activities of these plasma enzymes. CP treatment, because of hepatic and nephrotoxicity also increased the activities of these enzymes. However, treatment with a combination of CP and DEP potentiated GGT activity but not the two other enzymes. TQ completely prevented the rise of the three enzymes measured seen after DEP, and significantly abated the elevations of the three enzymes measured seen after CP. TQ, however, was less effective in

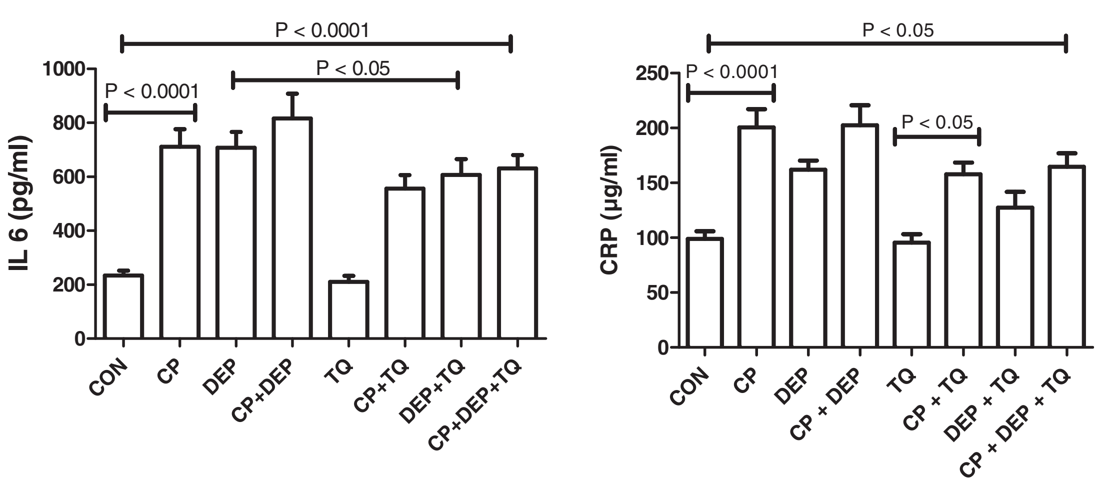

Figure 2 Plasma interleukin-6 (IL-6) and C-reactive protein (CRP) in rats treated with saline (control, CON), cisplatin (CP) 6 mg/kg, diesel exhaust particles (DEP) 0.5 mg/kg, CP + DEP, thymoquinone (TQ) 20 mg/kg, CP + TQ, DEP + TQ and CP + DEP + TQ. Each column represents mean ± SE ($n = 6$). Statistical analysis by Tukey–Kramer multiple comparison test

Table 4 The effect of treatment of rats with cisplatin (CP), diesel exhaust particle (DEP), thymoquinone (TQ) singly or in combination on the renal concentration of some oxidative stress parameters

GROUP	GSH (nmol/mg protein)	SOD (μmol/min/mg/protein)	CAT (μmol/min/mg/protein)	TAC (nmol/mg protein)
CONTROL (C)	35.5 ± 1.6	49.4 ± 1.9	64.0 ± 3.4	70.3 ± 5.4
CP	11.7 ± 1.0***	26.0 ± 1.7**	36.1 ± 2.2***	37.8 ± 2.4***
DEP	31.4 ± 2.2†††	45.2 ± 1.1†	51.9 ± 3.3†	54.9 ± 1.7*,†
CP + DEP	12.3 ± 1.2***,‡‡‡,§§§	27.4 ± 5.9**,‡	35.6 ± 2.7***,‡‡	36.2 ± 2.8***
TQ	32.2 ± 1.5†††	47.4 ± 4.9††,§	55.1 ± 4.9††,§	61.1 ± 2.6†,§
CP + TQ	16.1 ± 1.6***,‡‡‡,¶¶¶	28.2 ± 0.5**,$	38.3 ± 3.9***,¶	42.2 ± 3.0***,¶
DEP + TQ	27.0 ± 1.9*,†††,§§§,$$$	46.2 ± 3.3†	52.9 ± 3.3†,§	55.6 ± 3.5*,†,§
CP + DEP + TQ	17.2 ± 1.4***,‡‡‡,¶¶¶,$$,###	28.2 ± 6.0$	37.5 ± 2.2***,¶,#	41.0 ± 2.9***,¶,#

Values in the table are mean ± SEM ($n = 6$). TQ (20mg/kg) in corn oil (CO) was given orally continuously for 11 days, and CP (6mg/Kg) was injected intraperitoneally on the 6th day of the treatment. DEP was given by inhalation on the 11th day. All the rats were killed on the 12th day and the kidneys removed to measure the above indices of oxidative stress.
GSH = reduced glutathione; SOD = superoxide dismutase; CAT = catalase; and TAC = total antioxidant capacity.
*$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$ vs CONTROL.
†$p < 0.05$, ††$p < 0.001$, †††$p < 0.0001$ vs CP.
‡$p < 0.05$, ‡‡$p < 0.001$, ‡‡‡$p < 0.0001$ vs DEP.
§$p < 0.05$, §§$p < 0.001$, §§§$p < 0.0001$ vs CP + DEP.
¶$p < 0.05$, ¶¶$p < 0.001$, ¶¶¶$p < 0.0001$ vs TQ.
$$p < 0.05$, $$$p < 0.001$, $$$$p < 0.0001$ vs CP + TQ.
#$p < 0.05$, ##$p < 0.001$, ###$p < 0.0001$ vs DEP + TQ.

antagonizing the rises in the activities of the three enzymes in rats treated with both CP and DEP.

CP nephrotoxicity and DEP exposure are both known to be associated with increased production of inflammatory mediators, and this has been shown in the current study by significant elevations in the pro-inflammatory cytokines CRP and IL-6. TQ anti-inflammatory action was evident in significant reduction in the concentration of both cytokines in plasma.

In the present study, we showed that a single exposure to DEP exacerbate some signs of CP-induced ARF, an action which was similar to the effect of repeated DEP exposure, albeit with severer tubular necrosis in the renal tissues of rats treated with CP + DEP compared with those from rats treated with CP + saline.[41] This seems to suggest that a single exposure to DEP is still a risk factor in subjects with ARF, as it is a risk in healthy, hypertensive, or diabetic animals.[18]

In this work, and as shown in Table 3, the plasma activities of ALT, AST, and GGT (all markers of tissue damage) have been measured 24 after the end of the experiments. TQ treatment did not significantly affect the activity of any of these enzymes, confirming the safety of this compound. Treatment with either CP or DEP alone significantly increased the activities of the three enzymes. Earlier, we have histologically shown that DEP exposure causes lung inflammation,[41] and this may be the cause of the elevation of the activities of these plasma enzymes. CP treatment, because of hepatic and nephrotoxicity also increased the activities of these enzymes. However, treatment with a combination of CP and DEP potentiated GGT activity but not the two other enzymes. TQ completely prevented the rise of the three enzymes measured seen after DEP, and significantly abated the elevations of the three enzymes measured seen after CP. TQ, however, was less effective in

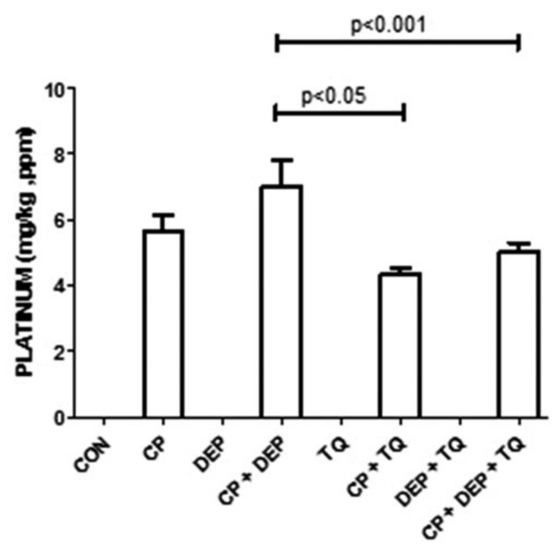

Figure 3 Platinum concentration in rats treated with saline (control, CON), cisplatin (CP) 6 mg/kg, diesel exhaust particles (DEP) 0.5 mg/kg, CP + DEP, thymoquinone (TQ) 20 mg/kg, CP + TQ, DEP + TQ and CP + DEP + TQ. Each column represents mean ± SE ($n = 6$). Statistical analysis by Tukey–Kramer multiple comparison test

antagonizing the rises in the activities of the three enzymes in rats treated with both CP and DEP.

TQ is the bioactive constituent of the volatile oil of black seed (*Nigella sativa)* with relatively strong anti-inflammatory, anti-oxidant, and anti-tumor activity.[32] As the toxicity of both CP and DEP involve inflammation and generation of free radicals, it was logical to test whether TQ would ameliorate the single and combined toxicity of CP and DEP. TQ treatment was effective in mitigating some of the their toxic effects.

The functional alterations induced by CP treatment, was broadly in close agreement with the histopathological data.

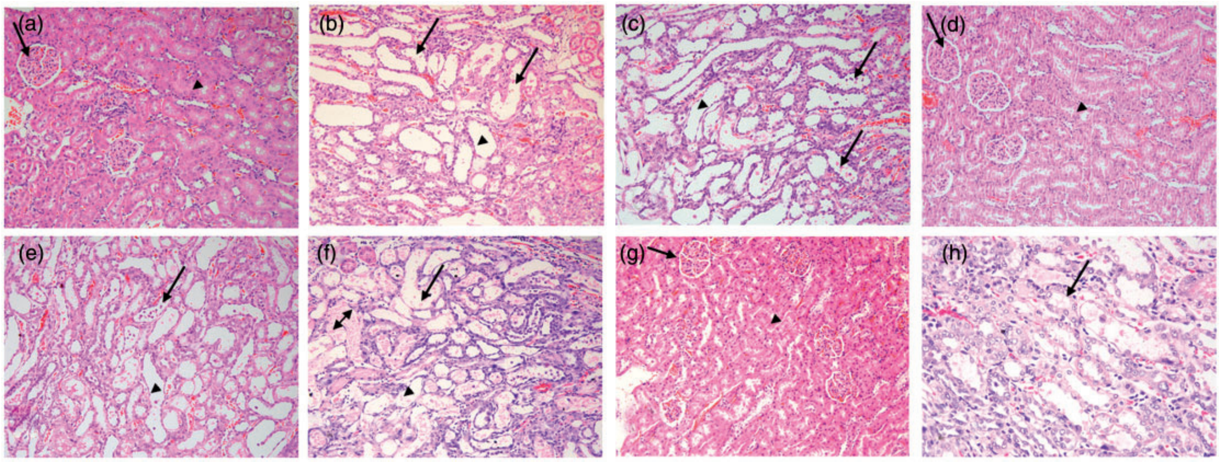

Figure 4 Representative light microscopy sections of renal tissue of rats treated with: saline (control, CON) (a), cisplatin (CP) 6 mg/kg (b), diesel exhaust particles (DEP) 0.5 mg/kg (c), thymoquinone (TQ) 20 mg/kg (d), CP + DEP (e), CP + TQ (f), DEP + TQ (g) and CP + DEP + TQ (H).The kidney architecture was not affected in groups (a), (d), and (g). The renal glomeruli (arrows) and renal tubules (arrow heads) indicate normal appearance. The micrographs in groups (B), (C), (E), and (F) showed acute tubular necrosis with eosinophilic material within the lumen (double headed arrow), shedding of epithelial cells within lumen (arrows) and flattened epithelial cells of the tubules with dilated lumens (arrow heads). (A color version of this figure is available in the online journal.)

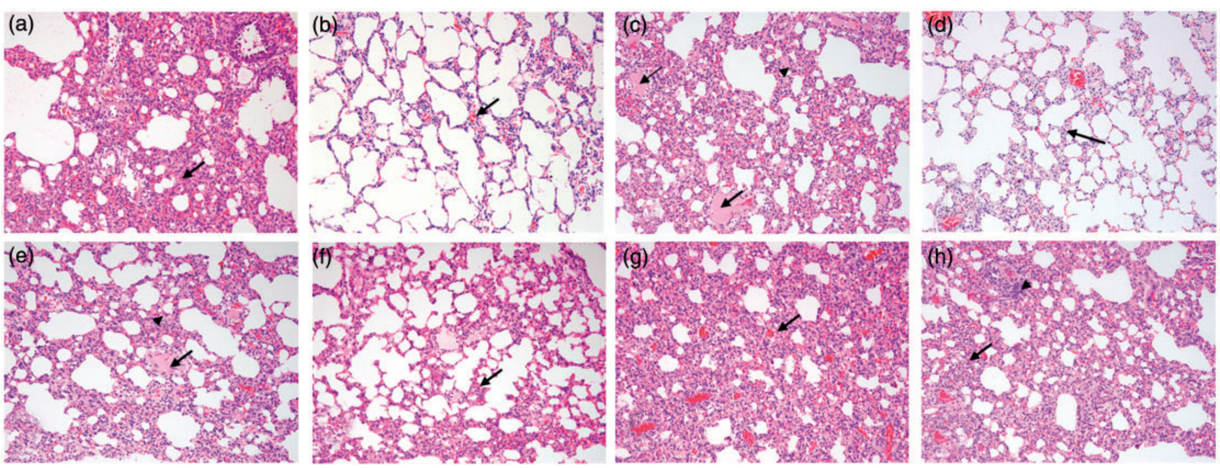

Figure 5 Representative light microscopy sections of lung tissues of rats given: saline (control, CON) (a), cisplatin (CP) 6 mg/kg (b), diesel exhaust particles (DEP) 0.5 mg/kg (c), thymoquinone (TQ) 20 mg/kg (d), CP + DEP (e), CP + TQ (f), DEP + TQ (g) and CP + DEP + TQ (h). The lung tissue showed only mild congestion (arrows) in groups (a), (b), (f), and (g), and increased interstitial cellularity with widening of the inter-alveolar spaces with congested inter-alveolar capillaries (arrow heads) and foci of intra-alveolar edema (arrows) in the groups (c), (e), and (h). (A color version of this figure is available in the online journal.)

The effect of TQ on lung function in the rats treated with CP, DEP or their combination has not been investigated here. However, it was shown histopathologically that TQ was effective in lessening the effect of CP and CP + DEP on the kidneys, with no apparent effect on lung histology.

The Pt renal concentration in CP-treated rats was slightly and insignificantly decreased by TQ treatment. However, the concentration of Pt in the renal tissue of rats given CP + DEP was significantly higher than in rats given CP + DEP + TQ. We have not studied here the mechanism(s) by which TQ caused this small reduction in Pt renal concentration in the latter group, its possible therapeutic implications, if any, or whether the TQ-induced amelioration in the signs of CP + DEP toxicity could be ascribed with certainty to that reduction in renal Pt accumulation. Accumulation of Pt in renal tubular cells is one of the reasons for CP nephrotoxicity.[42] Future *in vitro* experiments using renal cells and *in vivo* experiments in animals with tumor xenografts could provide an explanation for the Pt reduction in renal tissues after TQ, and whether it would affect the anti-tumor action of CP.

The molecular mechanism by which TQ has produced its salutary action on the combined toxicity of CP and DEP is not fully known. However, it is known that an early response to CP treatment may include an elevated nuclear factor kappa-B (NF-κB) signaling, associated with increases in TNFα and other molecules (such as intercellular adhesive molecule-1).[43] TQ has also been shown to cause its ameliorative action on CP toxicity via NF-κB,[44] and this may be a plausible molecular mechanism for its beneficial action on the toxicity induced in this work by CP and DEP. At the cellular level TQ is known to possess strong antiapoptotic, anti-oxidant, and anti-inflammatory actions, and with such properties, it is conceivable that it will be successful in mitigating the actions of CP and DEP, both of which are known to increase apoptosis, systemic inflammation, and generation of free radicals.[31–33,45]

In conclusion, this work has shown that a single exposure to DEP potentiated the nephrotoxicity of CP. TQ abrogated the toxicities of CP and DEP, when given singly or in combination, probably through its antioxidant and anti-inflammatory actions.

Authors' contributions: Conceived and designed the experiments: BHA. Performed the experiments: MZ, ES, PM, MIW, JY, AN, MF. Analyzed the data: BHA, MZ, AN. Wrote the MS: BHA. Revised the MS: MZ, AN. All authors read and approved the submission.

ACKNOWLEDGMENTS

We thank SQU and UAEU for financial support of this work, and the staff in the Small Animal House of SQU for looking after the rats. This work was supported by a grant from the SQU–UAEU Joint Research Program to BH Ali (Oman) and A Nemmar (UAE).

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(Received July 25, 2014, Accepted January 7, 2015)