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Original Research

Potentiation of cisplatin-induced nephrotoxicity by repeated exposure to diesel exhaust particles: An experimental study in rats

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Abstract

Several epidemiological and clinical studies have shown that exposure to particulate air pollution is associated with increases in morbidity and mortality, and this is more evident in patients with renal diseases. However, the basis of the possible exacerbating effect of particulate air pollution on animal model of renal injury has received scant attention. Here, we assessed the effect of repeated exposure to diesel exhaust particles (DEP) on cisplatin (CP)-induced nephrotoxicity in rats. DEP (0.5 m/kg) was intratracheally (i.t.) instilled every second day for eight days (a total of five exposures). CP, 6 mg/kg was given 1 h before the third exposure to DEP. Two days following the last exposure to either DEP or saline (control), various renal endpoints were measured. Water intake, urine volume, and relative kidney weight were significantly increased in CP + DEP versus DEP and CP + saline versus saline. Plasma creatinine increased and creatinine clearance decreased in CP + DEP versus DEP and CP + saline versus saline. Interestingly, blood urea nitrogen, albumin concentrations, and gamma-glutamyl transpeptidase (GGT) activity in urine were significantly increased in DEP + CP compared with either DEP or saline + CP. The combination of DEP and CP enhanced kidney injury molecule-1, neutrophil gelatinase-associated lipocalin, 8-isoprostane and total nitric oxide in the kidney compared with either saline + CP or DEP. Similarly, systolic blood pressure was increased in CP + DEP versus CP + saline or DEP. The renal tubular necrosis observed in kidneys of CP-treated rats was aggravated by the combination of CP + DEP. We conclude that repeated exposure to DEP potentiated CP-induced nephrotoxicity. Our data provide experimental evidence that patients with kidney injury could be at higher risk than the general population.

Keywords: Air pollution, diesel exhaust particles, cisplatin, nephrotoxicity

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Introduction

Particulate air pollution with particle diameter less than 2.5 μm ($PM_{2.5}$) contributes to pulmonary and extrapulmonary morbidity and mortality.[1] Diesel exhaust particles (DEP), which are the main contributors to $PM_{2.5}$ and ultrafine (nano-sized) particles (diameter ≤ 0.1 μm) in cities, have been identified in a number of studies to cause adverse health effects, including cardiorespiratory diseases.[2,3]

It has been reported that exposure to particulate air pollution plays some role in a variety of human diseases involving the cardiovascular, nervous, and urinary systems.[4] This increased risk is particularly apparent in at-risk populations such as the elderly and those with diabetes or hypertension.[5–11] All of these diseases and conditions are known to be associated with a high risk of developing complications, such as acute renal failure.[12] Studies have suggested that this risk occurs within few hours to days of exposure to high levels of $PM_{2.5}$.[1,9,10] Recently, an association between ambient air pollution and cardiovascular disease in kidney transplant recipients has been reported,[13] and kidney transplant recipients have been identified as a novel subpopulation susceptible to ambient air pollution.[13]

Attention into the extrapulmonary influence of particulate air pollutants has been growing since the establishment that ultrafine (nano-sized) particles are able to translocate directly from the lungs into systemic circulation and gain access to extra-pulmonary tissues.[14–17] Also, inhaled particles were reported to cause the release of soluble inflammatory mediators from the lungs into the blood which can

have impact on various organs, such as the liver, kidney, the heart, and the brain.[14–17]

Although the interest in the effects of particulate air pollution in animal model of enhanced susceptibility is increasing, the possible aggravating effect of particulate air pollution on animal model of renal injury has received only scant attention. We have recently reported the first *in vivo* experimental evidence that single dose exposure to DEP in the lung aggravated the renal, pulmonary, and systemic effects of cisplatin (CP)-induced acute renal failure in rats.[18] In that study, acute renal failure was first induced by CP administration and then the rats were exposed to a single dose of DEP.[18] Subsequently, we also showed that DEP aggravated the CP-induced decreased cell viability and oxidative stress in human embryonic kidney (HEK-293) cells, and that curcumin pretreatment significantly suppressed the observed DEP and CP-induced cellular insults.[19]

Since many humans are exposed to DEP on a continual basis, we intended, in the present study, to equate the real world situation by repeatedly administering DEP prior and after to inducing acute renal failure. Therefore, the aim of this study was to investigate the possible exacerbating effect of repeated pulmonary exposure to DEP on CP-induced nephrotoxicity by examining a comprehensive set of renal endpoints including renal histopathology and several renal, serum, and urine biochemical and physiological indices.

Material and methods

Animals

Male Wistar rats (Taconic Farms Inc., Germantown, New York, USA), aged 10–12 weeks and initially weighing 280 ± 18 g, were given a standard laboratory chow and water ad libitum. They were randomly divided into four groups ($n = 8$–10) and individually housed in metabolic cages, to facilitate urine collection, at a temperature of 23 ± 2°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 college, and according to the NIH Guide for the Care and Use of Laboratory Animals, NIH publication no. 85-23, 1985.

Intratracheal (i.t.) instillation

We used DEP (SRM 2975) obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). We have recently analysed the size of DEP used in the present study by transmission electron microscopy and found a substantial amount of ultrafine (nano) sized particle aggregates and larger particle aggregates (<1 μm in largest diameter).[20] DEP were suspended in sterile normal saline (NaCl 0.9 %) containing Tween 80 (0.01 %). To minimize aggregation, particle suspensions were always sonicated (Clifton Ultrasonic Bath, Clifton, NJ, USA) for 15 min and vortexed before their dilution and prior to i.t. administration. Control animals received normal saline containing Tween 80 (0.01%).

Treatments

The animals were anesthetized with i.p. injection of ketamine (75 mg/kg) and xylazine (10 mg/kg), and placed supine with extended neck on an angled board. A Becton Dickinson 18 Gauge cannula was inserted via the mouth into the trachea. DEP suspension (0.5 mg/kg in 150 μL) or saline were intratracheally (i.t.) instilled (150 μL) via a sterile syringe and followed by an air bolus of 150 μL. Either DEP or saline was i.t. instilled every second day for eight days (a total of five exposures) (Figure 1). CP, 6mg/kg (David Bull Laboratories, PTY Ltd, Victoria, Australia)[21,22] was given intraperitoneally (i.p.) 1 h before the third exposure to DEP (Figure 1). Control animals received similar volume of normal saline i.p. The urine collection for volume and biochemistry analysis was done one time. On day 9, rats were shifted into individualized metabolic cages, and urine of each rat was collected over a 24-h period (i.e. on day 10) and the volume measured. On day 10 (i.e. two days following the last exposure to DEP), various renal endpoints were measured (Figure 1).

Systolic blood pressure measurement

On day 10, the Systolic BP (SBP) was measured using a computerized noninvasive tail-cuff manometry system (ADInstrument, Colorado Springs, CO, USA). To avoid procedure-induced anxiety, rats were trained for three consecutive days before the experimental procedure.

Blood collection and biochemical analysis

Following SBP measurement, the same animals were sacrificed with an overdose of anesthesia and kidneys were excised, washed with ice-cold saline, blotted with filter

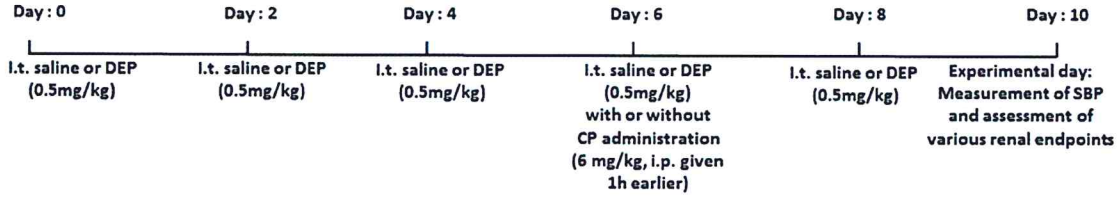

Figure 1 Treatments and endpoints following the repeated intratracheal instillation (i.t.) of saline or diesel exhaust particles (DEP) with or without cisplatin (CP) administration [given intraperitoneally (i.p.)] in rats. SBP: systolic blood pressure

paper and weighed. The cortex of the right kidney was excised away from the medulla, and rapidly homogenized in ice-cold normal saline to produce 10 (w/v) tissue homogenate.

The concentrations of urea and creatinine in serum were spectrophotometrically measured using commercial kits (Roche Diagnostics, Indianapolis, IN, USA). Urine albumin concentration and gamma-glutamyl transpeptidase (GGT) activity were measured spectrophotometrically using kits from Roche (Roche Diagnostics, IN, USA). The concentrations of kidney injury molecule-1 (KIM-1) (R & D systems, MN, USA) and 8-isoprostane (Cayman Chemicals, MI, USA) as well as neutrophil gelatinase-associated lipocalin (NGAL) activity (R & D systems, MN, USA) were measured in renal cortex homogenates. The determination of nitric oxide (NO) concentration in kidney homogenates was performed with a total NO assay kit (R & D systems, MN, USA) which measures the more stable NO metabolites NO_2^- and NO_3^-.[23,24] The concentration of CP (as platinum) in cortical tissue was measured by flameless atomic absorption spectrophotometry (Perkin-Elmer, 3300 DV ICP-OES equipped with a cross-flow nebulizer, in addition to an ultrasonic nebulizer). The procedure involved mineralization of the kidney cortex tissue with a mixture of concentrated HNO_3 and H_2O_2, followed by determination of platinum in the extract, using inductively coupled plasma optical emission spectrometry, at an emission wavelength of 265.945 nm.

Histopathology

From each animal in the experiment, a small piece of the left kidney was fixed in 10% neutral-buffered formalin, dehydrated in increasing concentrations of ethanol, cleared with xylene and embedded in paraffin. Five micrometer sections were prepared from kidney paraffin blocks and stained with hematoxylin and eosin (H & E). The microscopic scoring of the kidney sections was carried out in a blinded fashion by a histopathologist who was unaware of the treatment groups and assigned a score as described earlier,[22] which represents the approximate extent of necrotic area in the cortical proximal tubules on a scale of 0–4 (0, no necrosis; 1, a few focal necrotic spots; 2, necrotic area was about one half; 3, necrotic spots formed about two-thirds; 4, nearly the entire area was necrotic).

Statistics

All data were analyzed with GraphPad Prism Version 4.01 for Windows software (Graphpad Software Inc., San Diego, USA). Data were analyzed for normal distribution using the D'Agostino and Pearson omnibus normality test. Data are expressed as means ± SEM. Comparisons between groups were performed by one way analysis of variance (ANOVA), followed by Newman Keuls test for comparing treated with control data. P values <0.05 are considered significant.

Results

Body weight, relative kidney weight, water intake, and urine volume

Figure 2(a) shows that rats given saline gained about 1, while those receiving DEP gained about 3% of their initial body weight. No significant difference was found between saline and DEP groups. However, rats treated concurrently

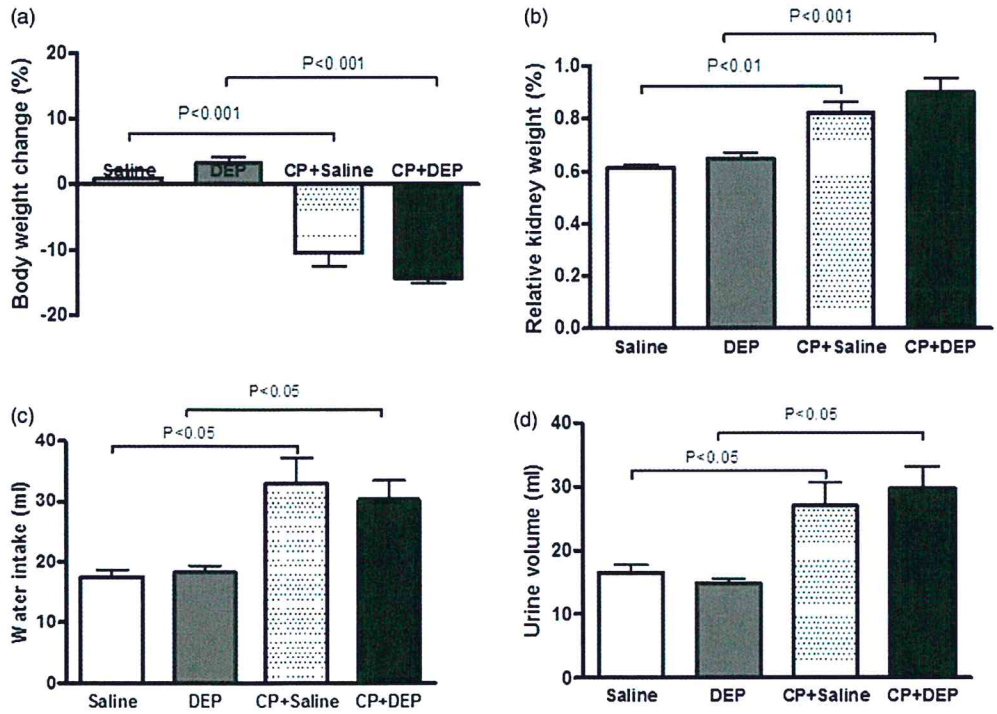

Figure 2 Body weight change (a), relative kidney weight (b), water intake (c), and urine volume (d) in rats repeatedly treated with either saline (control) or diesel exhaust particles (DEP, 0.5 mg/kg) with or without cisplatin (CP, 6 mg/kg) administration ($n = 8$–10). Mean ± SEM. Statistical analysis by ANOVA 1 followed by Newman–Keuls test

with CP and saline lost about 10% ($P < 0.001$ versus saline group), whereas those administered with CP and DEP lost about 14% ($P < 0.001$ versus DEP group), respectively. Figure 2(b) illustrates that the relative kidney weights were significantly increased in rats given CP + saline compared with those given saline only ($P < 0.01$), and in rats given CP + DEP compared with those given DEP only ($P < 0.001$). Likewise, the water intake (Figure 2c) and urine volume (Figure 2d) were significantly increased in the CP+saline group compared with the saline group ($P < 0.05$), and in the CP + DEP group compared with the DEP group ($P < 0.05$).

Renal platinum concentration

Measurement of CP (as platinum) in renal tissues showed similar values in rats treated with saline+CP (6.2 ± 0.8 ppm) and rats treated with DEP + CP (6.4 ± 1.0 ppm)-treated rats. No platinum has been found in the kidneys of rats treated with saline or DEP.

Plasma creatinine and urea concentration and creatinine clearance

Figure 3 illustrates the effects saline and CP with or without DEP, on the plasma concentrations of creatinine and urea and creatinine clearance. DEP did not significantly affect the concentrations of urea compared with the saline-treated group. However, treatment with CP+saline significantly increased the concentration of urea compared with the saline-treated rats. Interestingly, CP+DEP treatment significantly increased the concentration of urea more than in rats treated with DEP alone or CP + saline (Figure 3a). Creatinine in plasma significantly increased in the CP + saline group compared with the saline group ($P < 0.05$), and the DEP + CP group compared with the DEP-treated group ($P < 0.01$). A statistically insignificant increase in plasma creatinine was observed in CP + DEP versus CP + saline (Figure 3b). Creatinine clearance was significantly reduced in rats treated with CP+saline compared with rats treated with saline ($P < 0.05$), and in rats treated with DEP + CP compared with in rats treated with DEP ($P < 0.05$). The decrease in creatinine clearance in CP + DEP-treated rats compared with CP + saline-treated rats was not statistically significant (Figure 3c).

Albumin concentration and GGT activity in urine

Urinary albumin concentration was significantly increased in CP+saline compared with saline-treated controls ($P < 0.001$), and in rats treated with CP + DEP compared to the DEP-treated group ($P < 0.001$). Interestingly, the combination of CP + DEP significantly enhanced ($P < 0.01$) the urinary albumin concentration compared with CP + saline (Figure 4a). Urinary GGT activity was significantly increased in CP + DEP compared with CP+saline or DEP alone (Figure 4b).

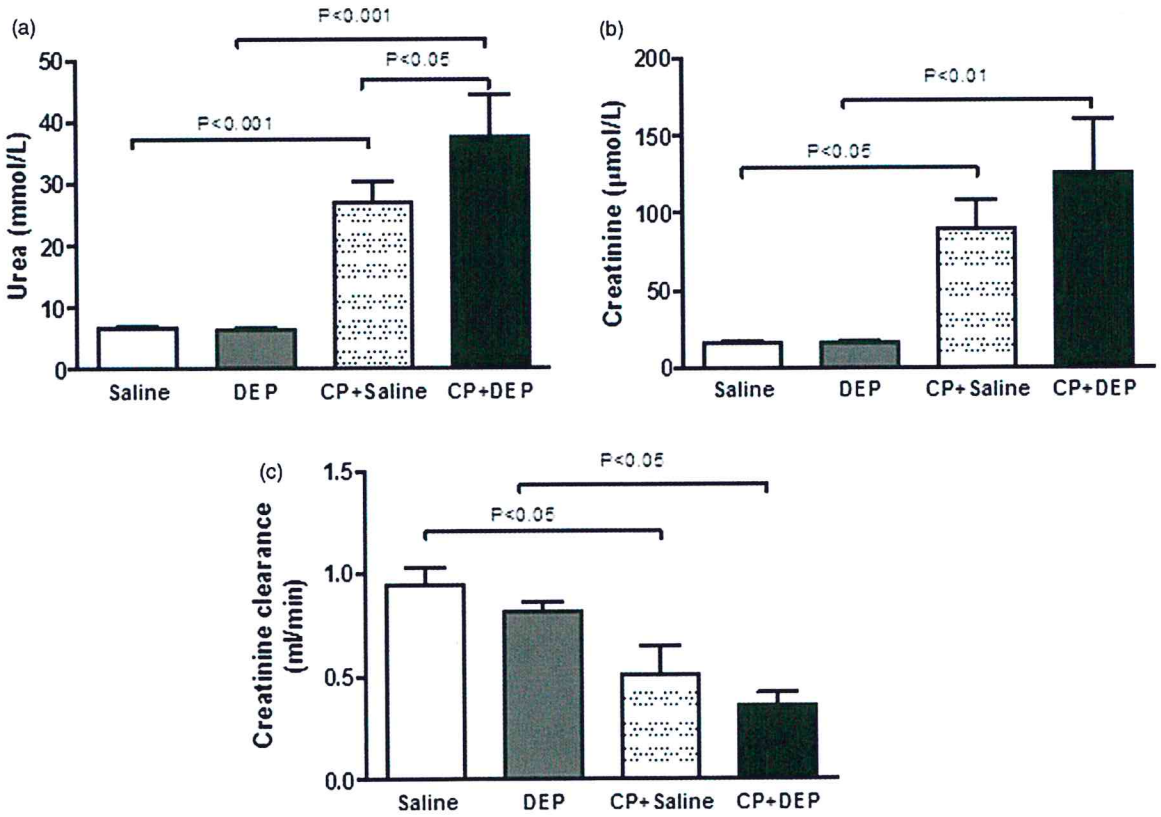

Figure 3 Plasma levels of urea (a) and creatinine (b), and creatinine clearance (c) in rats repeatedly treated with either saline (control) or diesel exhaust particles (DEP, 0.5 mg/kg) with or without cisplatin (CP, 6 mg/kg) administration ($n = 8$–10). Mean ± SEM. Statistical analysis by ANOVA 1 followed by Newman–Keuls test

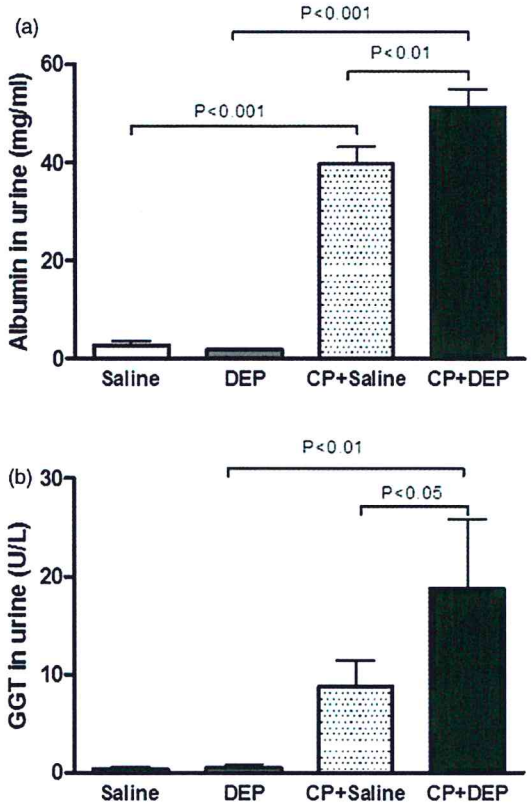

Figure 4 Concentration of albumin (a) and activity of gamma-glutamyl transpeptidase (GGT, b) in urine of rats repeatedly treated with either saline (control) or diesel exhaust particles (DEP, 0.5 mg/kg) with or without cisplatin (CP, 6 mg/kg) administration ($n = 8$–10). Mean ± SEM. Statistical analysis by ANOVA 1 followed by Newman–Keuls test

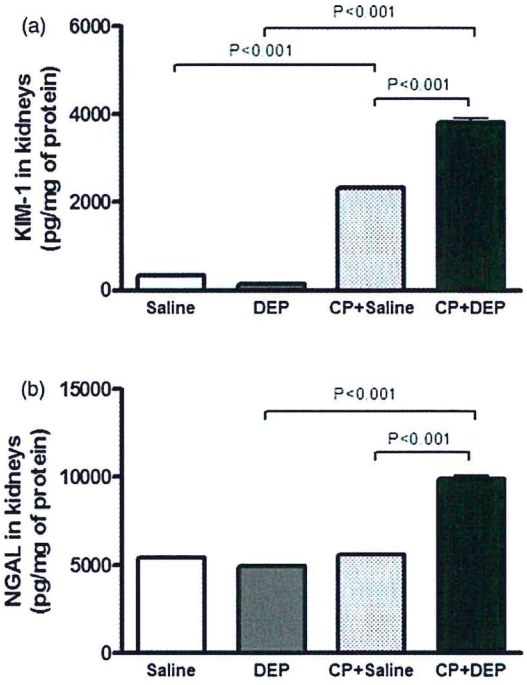

Figure 5 Renal levels of kidney injury molecule-1 (KIM-1, a) and neutrophil gelatinase-associated lipocalin (NGAL, b) in rats repeatedly treated with either saline (control) or diesel exhaust particles (DEP, 0.5 mg/kg) with or without cisplatin (CP, 6 mg/kg) administration ($n = 8$–10). Mean ± SEM. Statistical analysis by ANOVA 1 followed by Newman–Keuls test

KIM-1 and NGAL concentrations in kidney homogenates

Figure 5(a) illustrates that treatment with CP+saline significantly enhanced the concentration of KIM-1 compared with that in the saline-treated group. Concomitant treatment with CP+DEP significantly increased the concentration of KIM-1 in kidney homogenates when compared with the values obtained from rats treated with CP+saline or DEP. Likewise, NGAL renal concentration was significantly increased by the combination of CP+DEP versus CP+saline or DEP alone (Figure 5b).

8-isoprostane concentration and NO activity in kidney homogenates

A significant increase in markers of oxidative stress including 8-isoprostane and total NO has been found following the treatment of rats with CP+DEP compared with treatment with CP+saline or DEP alone (Figure 6).

Histopathology

Figure 7 depicts representative micrographs of renal cortex from the four studied groups. The kidney architecture was not affected by saline (Figure 7a) or DEP (Figure 7b) treatments. In rats treated with CP+saline (Figure 7c) and those treated with CP+saline (Figure 7d), renal cortex showed the

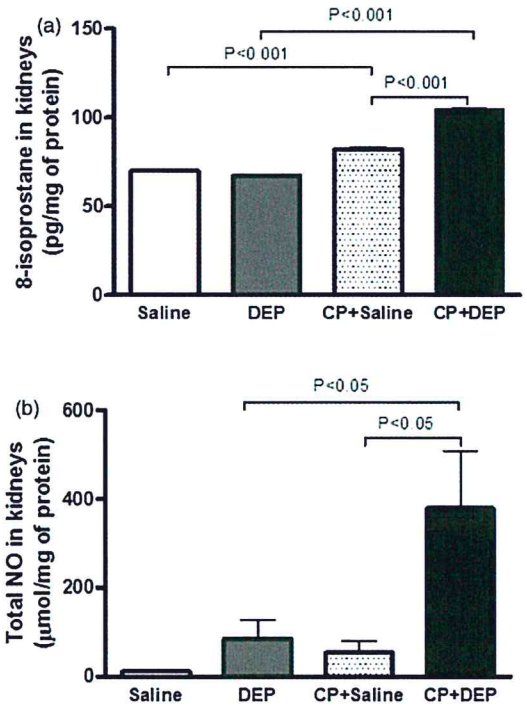

Figure 6 Renal levels of 8-isoprostane (a) and total nitric oxide (NO, b) in rats repeatedly treated with either saline (control) or diesel exhaust particles (DEP, 0.5 mg/kg) with or without cisplatin (CP, 6 mg/kg) administration ($n = 8$–10). Mean ± SEM. Statistical analysis by ANOVA 1 followed by Newman–Keuls test

presence of acute tubular necrosis, tubular distension with eosinophilic material, interstitial edema, and congestion. In the CP+saline group (Figure 7c), the acute tubular necrosis involved about one half of the examined tissue and was given a score of 2, whereas in the CP +DEP group (Figure 7d) acute tubular necrosis involved about two-thirds of the examined tissue and was given a score of 3.

Systolic blood pressure

DEP slightly but insignificantly increased the SBP compared with saline group. CP+saline significantly increased SBP compared to saline group. Interestingly, CP+DEP significantly enhanced SBP compared with CP+saline and DEP (Figure 8).

Discussion

In the present study, we showed that repeated exposure to DEP exacerbate CP-induced acute renal failure. We found that compared with treatment with CP+saline or DEP alone, the combination of CP and DEP aggravated plasma urea concentration, urinary GGT activity, and albumin concentration, and renal concentration of KIM-1, total NO and 8-isoprostane, and NGAL activity. Histological analysis revealed the presence of a severer tubular necrosis in the kidneys of rats treated with CP+DEP compared with those from rats treated with CP+saline. Moreover, as a consequence of aggravation renal failure, the SBP was potentiated by CP+DEP compared with CP+saline or DEP treatments.

CP [cis-diaminedichloroplatinum(II)], an anticancer drug, is commonly used for the therapy of solid cancers such as ovarian, head and neck carcinomas, and germ cell tumors.[25] The drug is thought to be bioactivated to a nephrotoxicant, and it damages cell mitochondria, arrests cell cycle in the G2 phase, inhibits ATPase activity, alters the cellular transport system and eventually causes apoptosis, inflammation, necrosis and death in proximal tubules and collecting ducts.[25] Nephrotoxicity is frequent and is the major limitation in CP-based chemotherapy and has been ascribed to several mechanisms that include oxidative and

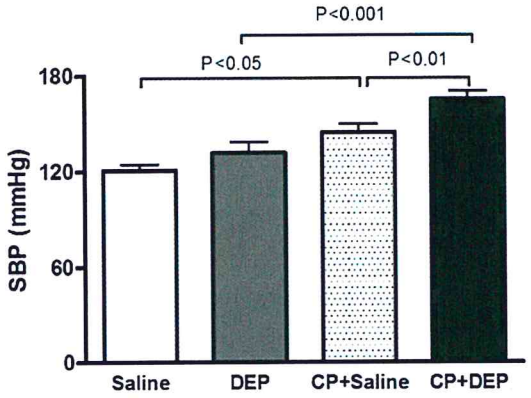

Figure 8 Systolic blood pressure (SBP) in rats repeatedly treated with either saline (control) or diesel exhaust particles (DEP, 0.5 mg/kg) with or without cisplatin (CP, 6 mg/kg) administration ($n = 8$–10). Mean ± SEM. Statistical analysis by ANOVA 1 followed by Newman–Keuls test

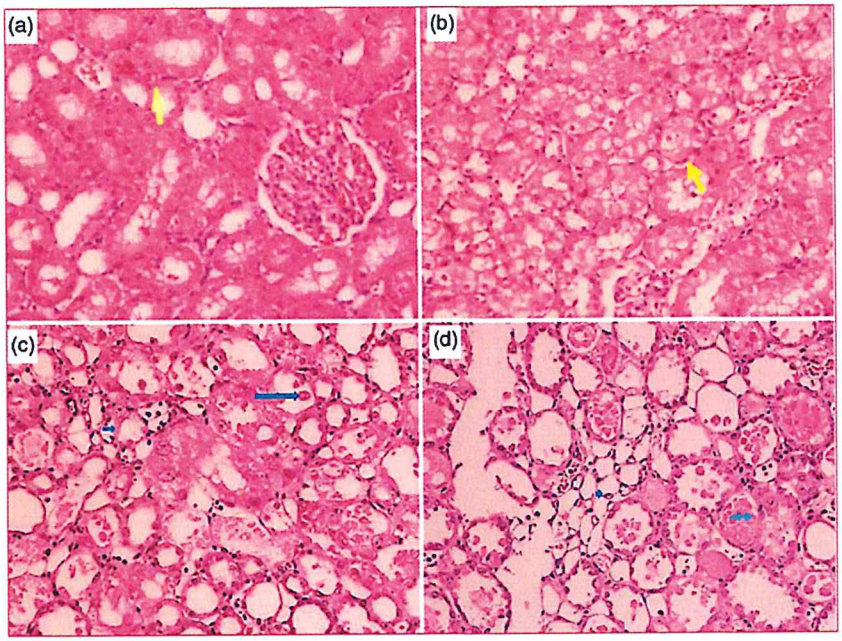

Figure 7 Representative light microscopy sections of renal tissue of rats repeatedly given saline (control, a), diesel exhaust particles (DEP 0.5 mg/kg, b), cisplatin (CP, 6 mg/kg)+saline (c), CP+DEP (0.5 mg/kg, d). The kidney architecture was not affected in groups (a) and (b) treatments. The renal tubules have normal appearance (yellow arrow). The micrographs in groups (c) and (d) showing acute tubular necrosis with eosinophilic material within the lumen (double arrow), interstitial oedema and congestion (arrow head) and shedding of epithelial cells within lumen (long arrow) and flattened epithelial cells of the tubules (short arrow). In CP+saline group (c), the acute tubular necrosis involved about one half of the examined tissue and was given a score of 2, whereas in CP +DEP (d) acute tubular necrosis involved about two-thirds of the examined tissue and was given a score of 3. (A color version of this figure is available in the online journal.)

nitrosative stress.[26] Several reports have documented the development of renal failure with a single i.p. dose of 6 mg/kg in rats.[18,21,27]

It is well-established that patients with kidney disease, including those requiring dialysis, develop states of increased inflammation and oxidative stress. It is plausible that those states make patients with kidney disease vulnerable to air pollution.[28] In the present study, we assessed the effect of repeated exposure to DEP on CP-induced nephrotoxicity. This approach is more relevant to human exposure scenarios than single dose exposure. We have recently assessed the effect of single dose exposure to DEP (0.5 and 1 mg/kg) on renal, pulmonary, and systemic parameters in CP-induced acute renal failure in rats.[18] In the present study, since we wanted to investigate the effect of repeated exposure of DEP on CP-induced nephrotoxicity, we have purposefully selected the lower dose of DEP, i.e. 0.5 mg/kg that we previously tested.[18] Moreover, the dose of particles we tested here (0.5 mg/kg every second day) is comparable to several dosage regimens previously reported.[29,30] Rats were exposed to DEP by i.t. instillation because it provides more accurate dosing, given that rats are obligate nose breathers that filter most inhaled particles.[31]

Our data show that compared to saline or DEP alone, the treatment of animals with either CP+DEP or CP+saline decreased body weight and increased relative kidney weight, water intake, and urine volume. However, no aggravating effect was observed in rats given CP+DEP when compared to those that have been given CP + saline.

Creatinine in plasma significantly increased in CP+saline compared with saline, and DEP + CP compared with DEP. The increase in CP + DEP versus CP+saline failed to reach statistical significance. Interestingly, CP + DEP treatment consistently increased the concentration of urea more than in rats treated with either CP+saline or DEP. Such effect was not previously observed following single-dose exposure to DEP in rats with acute renal failure.[18] Creatinine clearance was significantly reduced in CP+saline compared with saline, and DEP + CP compared with DEP. The decrease in creatinine clearance in CP + DEP compared with CP+saline did not achieve statistical significance.

We found that the combination of CP + DEP aggravated the urinary excretion of albumin compared with either CP+saline or DEP. Albumin is a major serum protein and is often the most abundant protein found in urine during renal injury.[32] The quantity of albumin appearing in urine is very important to distinguish the etiology of renal disease.[32] Albuminuria may occur in renal tubular disease, such as early stage diabetes, or in drug-induced human acute kidney injury caused by several chemotherapeutics such as CP and by gentamicin antibiotics known to cause direct tubular toxicity.[33] This probably indicates that the sieving properties of the glomerular filtration barrier are aggravated by the combination of CP + DEP and/or the reduction in the tubular reabsorption of this protein.[33] GGT is brush border enzymes located in the proximal renal tubule, and is normally present in urine as a consequence of tubular cell shedding. Its activity becomes increased in cases of acute tubular necrosis due to excess cellular loss, and elevated urinary enzyme activity has been used as a biomarker of proximal tubular injury.[34] Our data is in line with an aggravation proximal tubular injury since we found an increase in urinary activity of GGT in CP + DEP versus CP + DEP or DEP.

KIM-1 and NGAL are relatively new and very sensitive biomarkers of early renal injury expressed by renal tubular epithelium.[34] Our findings confirm that administration of CP + DEP exacerbate the kidney injury evidenced by marked increase in both KIM-1 and NGAL compared with either CP + saline or DEP.

We have previously shown that pulmonary exposure to DEP cause oxidative stress and pretreatment with antioxidant such as thymoquinone, curcumin or 2-oxothiazolidine-4-carboxylate prevents DEP-induced cardiorespiratory toxicity.[30,35,36] On the other hand, oxidative stress in kidney plays an important role in CP-induced renal damage, and several antioxidants and thiol compounds have been shown to protect against CP-induced nephrotoxicity.[21,37,38] In the present study, as a marker for oxidative stress, we selected to measure the kidney levels of NO and 8-isoprostane. Isoprostanes are a family of eicosanoids of nonenzymatic origin, produced by the random oxidation of tissue phospholipids by oxygen radicals. Elevated levels of isoprostanes have been found in plasma, urine and lung tissue of mice exposed to carbon nanoparticles[39] and plasma of diabetic mice exposed to DEP.[40] We found a significant increase of 8-isoprostane and NO in CP + DEP group compared with either CP+saline or DEP alone, suggesting that the aggravating effect of CP and DEP is mediated, at least partly, through the oxidative stress. We have previously demonstrated that single dose exposure to DEP in rat model of acute renal failure exacerbates the decrease of reduced glutathione compared with DEP or CP + saline.[18] Waly et al.[19] reported that DEP augmented the CP-induced cytotoxicity and oxidative stress in cultured human kidney (HEK 293), and that the pretreatment with curcumin protected against DEP and CP-induced cytotoxicity.

In line with the aggravation of the biochemical parameters by the combination of CP and DEP, the histological findings showed the presence of acute tubular necrosis, tubular distention with eosinophilic material, interstitial edema, and congestion. Interestingly, in CP +DEP group, acute tubular necrosis involved about two-thirds of the examined tissue, whereas in CP+saline acute tubular necrosis involved about one half of the examined tissue. Such effect was not previously observed following single dose exposure to DEP in rats with acute renal failure.[18] Indeed, in our previous study, the degree of acute tubular necrosis observed in CP + DEP was similar to that of CP+saline.[18]

The kidneys are the major site for CP accumulation, and this results in necrosis of the terminal portion of the proximal renal tubules and apoptosis in the distal nephron.[21] The concentrations of CP in the renal cortex in rats treated with CP + DEP were similar to those treated with CP + saline. Thus, our findings suggest that DEP does not affect the accumulation of CP, and, therefore, the

potentiating effect in nephrotoxicity that we have seen does not result from the renal accumulation of CP.

To investigate the possible pathophysiological consequence of the aggravation of nephrotoxicity in CP + DEP, we assessed the SBP in the four studied group. Our data show that the combination of CP and DEP augmented the SBP compared with either CP+saline or DEP. Although several factors clearly contribute to the pathogenesis and maintenance of blood pressure elevation, it is well established that renal mechanisms play a primary role.[41] Our finding suggests that acute renal failure has a significant effect on extrarenal organs including the cardiovascular or respiratory systems.[18] Studies in humans and in animal models have demonstrated that acute renal failure has a significant effect on the function of extrarenal organs.[42–44] Inflammation and oxidative stress are major components of the initiation and exacerbation of kidney injury, and local inflammation of kidney tissues could be a source of the development of inflammation and injury in extrarenal organs.[42–44]

We conclude that repeated exposure to DEP potentiate CP-induced nephrotoxicity. Our data provide experimental evidence that patients with kidney injury are at higher risk than the general population.

Author contributions: All authors have read and approved the manuscript. AN designed, planned, supervised the experiments and wrote the article. SB, PY, and JY performed the experiments. MF performed the measurement of platinum and contributed in the design of the study. EEK contributed in the design of the study and critically reviewed the manuscript. IA performed the histological parts of the study, and contributed in the writing of the article BHA contributed in the design of the study and wrote the article.

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REFERENCES

1. Brook RD, Rajagopalan S, Pope CA, Brook JR, Bhatnagar A, Diez-Roux AV, Holguin F, Hong YL, Luepker RV, Mittleman MA, Peters A, Siscovick D, Smith SC, Whitsel L, Kaufman JD. Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. *Circulation* 2010;**121**:2331–78
2. Atkinson RW, Anderson HR, Sunyer J, Ayres J, Baccini M, Vonk JM, Boumghar A, Forastiere F, Forsberg B, Touloumi G, Schwartz J, Katsouyanni K. Acute effects of particulate air pollution on respiratory admissions: Results from APHEA 2 project. Air pollution and health: A European approach. *Am J Respir Crit Care Med* 2001;**164**:1860–6
3. Pope CA III, Schwartz J, Ransom MR. Daily mortality and PM10 pollution in Utah Valley. *Arch Environ Health* 1992;**47**:211–7
4. Brook RD. Potential health risks of air pollution beyond triggering acute cardiopulmonary events. *JAMA* 2008;**299**:2194–6
5. O'Neill MS, Veves A, Zanobetti A, Sarnat JA, Gold DR, Economides PA, Horton ES, Schwartz J. Diabetes enhances vulnerability to particulate air pollution – Associated impairment in vascular reactivity and endothelial function. *Circulation* 2005;**111**:2913–20
6. Bateson TF, Schwartz J. Who is sensitive to the effecs of particulate air pollution on mortality? A case-crossover analysis of effect modifiers. *Epidemiology* 2004;**15**:143–9
7. Pope CA, Burnett RT, Thurston GD, Thun MJ, Calle EE, Krewski D, Godleski JJ. Cardiovascular mortality and long-term exposure to particulate air pollution – Epidemiological evidence of general pathophysiological pathways of disease. *Circulation* 2004;**109**:71–7
8. Kunzli N, Jerrett M, Mack WJ, Beckerman B, LaBree L, Gilliland F, Thomas D, Peters J, Hodis HN. Ambient air pollution and atherosclerosis in Los Angeles. *Environ Health Perspect* 2005;**113**:201–6
9. Peters A, Dockery DW, Muller JE, Mittleman MA. Increased particulate air pollution and the triggering of myocardial infarction. *Circulation* 2001;**103**:2810–15
10. Peters A, von Klot S, Heier M, Trentinaglia I, Hormann A, Wichmann HE, Lowel H. Exposure to traffic and the onset of myocardial infarction. *N Engl J Med* 2004;**351**:1721–30
11. Brook RD, Franklin B, Cascio W, Hong YL, Howard G, Lipsett M, Luepker R, Mittleman M, Samet J, Smith SC, Tager I. Air pollution and cardiovascular disease – A statement for healthcare professionals from the expert panel on population and prevention science of the American Heart Association. *Circulation* 2004;**109**:2655–71
12. Leblanc M, Kellum JA, Gibney RT, Lieberthal W, Tumlin J, Mehta R. Risk factors for acute renal failure: inherent and modifiable risks. *Curr Opin Crit Care* 2005;**11**:533–6
13. Spencer-Hwang R, Knutsen SF, Soret S, Ghamsary M, Beeson WL, Oda K, Shavlik D, Jaipaul N. Ambient air pollutants and risk of fatal coronary heart disease among kidney transplant recipients. *Am J Kidney Dis* 2011;**58**:608–16
14. Nemmar A, Hoylaerts MF, Hoet PH, Nemery B. Possible mechanisms of the cardiovascular effects of inhaled particles: systemic translocation and prothrombotic effects. *Toxicol Lett* 2004;**149**:243–53
15. Vermylen J, Nemmar A, Nemery B, Hoylaerts MF. Ambient air pollution and acute myocardial infarction. *J Thromb Haemost* 2005;**3**:1955–61
16. Peters A, Veronesi B, Calderon-Garciduenas L, Gehr P, Chen LC, Geiser M, Reed W, Rothen-Rutishauser B, Schurch S, Schulz H. Translocation and potential neurological effects of fine and ultrafine particles a critical update. *Part Fibre Toxicol* 2006;**3**:13
17. Oberdorster G, Oberdorster E, Oberdorster J. Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. *Environ Health Perspect* 2005;**113**:823–39
18. Nemmar A, Al Salam S, Zia S, Yasin J, Al HI, Ali BH. Diesel exhaust particles in the lung aggravate experimental acute renal failure. *Toxicol Sci* 2010;**113**:267–77
19. Waly MI, Ali BH, Nemmar A. Acute effects of diesel exhaust particles and cisplatin on oxidative stress in cultured human kidney (HEK 293) cells, and the influence of curcumin thereon. *Toxicol In Vitro* 2013;**27**:2299–304
20. Nemmar A, Al Maskari S, Ali BH, Al Amri IS. Cardiovascular and lung inflammatory effects induced by systemically administered diesel exhaust particles in rats. *Am J Physiol Lung Cell Mol Physiol* 2007;**292**:L664–70
21. Ali BH, Al Moundhri MS, Tag EM, Nemmar A, Tanira MO. The ameliorative effect of cysteine prodrug L-2-oxothiazolidine-4-carboxylic acid on cisplatin-induced nephrotoxicity in rats. *Fundam Clin Pharmacol* 2007;**21**:547–53
22. Ali BH, Al Moundhri M, Eldin MT, Nemmar A, Al Siyabi S, Annamalai K. Amelioration of cisplatin-induced nephrotoxicity in rats by tetramethylpyrazine, a major constituent of the Chinese herb Ligusticum wallichi. *Exp Biol Med (Maywood)* 2008;**233**:891–6
23. Wennmalm A, Benthin G, Edlund A, Jungersten L, Kieler-Jensen N, Lundin S, Westfelt UN, Petersson AS, Waagstein F. Metabolism and excretion of nitric oxide in humans. An experimental and clinical study. *Circ Res* 1993;**73**:1121–7
24. Nemmar A, Raza H, Subramaniyan D, Yasin J, John A, Ali BH, Kazzam EE. Short-term systemic effects of nose-only cigarette smoke exposure in mice: role of oxidative stress. *Cell Physiol Biochem* 2013;**31**:15–24
25. Yao X, Panichpisal K, Kurtzman N, Nugent K. Cisplatin nephrotoxicity: a review. *Am J Med Sci* 2007;**334**:115–24

26. Chirino YI, Pedraza-Chaverri J. Role of oxidative and nitrosative stress in cisplatin-induced nephrotoxicity. *Experimen Toxicol Pathol* 2009;**61**:223–42
27. Mohan IK, Khan M, Shobha JC, Naidu MU, Prayag A, Kuppusamy P, Kutala VK. Protection against cisplatin-induced nephrotoxicity by Spirulina in rats. *Cancer Chemother Pharmacol* 2006;**58**:802–8
28. Laden F, Winkelmayer WC. Air pollution and coronary risk in kidney transplant recipients. *Am J Kidney Dis* 2011;**58**:506–7
29. Mutlu GM, Green D, Bellmeyer A, Baker CM, Burgess Z, Rajamannan N, Christman JW, Foiles N, Kamp DW, Ghio AJ, Chandel NS, Dean DA, Sznajder JI, Budinger GR. Ambient particulate matter accelerates coagulation via an IL-6-dependent pathway. *J Clin Invest* 2007;**117**:2952–61
30. Nemmar A, Subramaniyan D, Ali BH. Protective effect of curcumin on pulmonary and cardiovascular effects induced by repeated exposure to diesel exhaust particles in mice. *PLoS One* 2012;**7**:e39554
31. Driscoll KE, Costa DL, Hatch G, Henderson R, Oberdorster G, Salem H, Schlesinger RB. Intratracheal instillation as an exposure technique for the evaluation of respiratory tract toxicity: uses and limitations. *Toxicol Sci* 2000;**55**:24–35
32. Tojo A, Kinugasa S. Mechanisms of glomerular albumin filtration and tubular reabsorption. *Int J Nephrol* 2012
33. Quesada A, Vargas F, Montoro-Molina S, O'Valle F, Rodriguez-Martinez MD, Osuna A, Prieto I, Ramirez M, Wangensteen R. Urinary aminopeptidase activities as early and predictive biomarkers of renal dysfunction in cisplatin-treated rats. *PLoS One* 2012;**7**:e40402
34. Waring WS, Moonie A. Earlier recognition of nephrotoxicity using novel biomarkers of acute kidney injury. *Clin Toxicol (Phila)* 2011;**49**:720–8
35. Nemmar A, Al Salam S, Dhanasekaran S, Sudhadevi M, Ali BH. Pulmonary exposure to diesel exhaust particles promotes cerebral microvessel thrombosis: protective effect of a cysteine prodrug l-2-oxothiazolidine-4-carboxylic acid. *Toxicology* 2009;**263**:84–92
36. Nemmar A, Al-Salam S, Zia S, Marzouqi F, Al-Dhaheri A, Subramaniyan D, Dhanasekaran S, Yasin J, Ali BH, Kazzam EE. Contrasting actions of diesel exhaust particles on the pulmonary and cardiovascular systems and the effects of thymoquinone. *Br J Pharmacol* 2011;**164**:1871–82
37. Ali BH, Al-Salam S, Al Husseini IS, Al-Lawati I, Waly M, Yasin J, Fahim M, Nemmar A. Abrogation of cisplatin-induced nephrotoxicity by emodin in rats. *Fundam Clin Pharmacol* 2013;**27**:192–200
38. Waly MI, Ali BH, Al-Lawati I, Nemmar A. Protective effects of emodin against cisplatin-induced oxidative stress in cultured human kidney (HEK 293) cells. *J Appl Toxicol* 2013;**33**:626–30
39. Alessandrini F, Beck-Speier I, Krappmann D, Weichenmeier I, Takenaka S, Karg E, Kloo B, Schulz H, Jakob T, Mempel M, Behrendt H. Role of oxidative stress in ultrafine particle-induced exacerbation of allergic lung inflammation. *Am J Respir Crit Care Med* 2009;**179**:984–91
40. Nemmar A, Subramaniyan D, Yasin J, Ali BH. Impact of experimental type 1 diabetes mellitus on systemic and coagulation vulnerability in mice acutely exposed to diesel exhaust particles. *Part Fibre Toxicol* 2013;**10**:14
41. Oparil S, Zaman MA, Calhoun DA. Pathogenesis of hypertension. *Ann Intern Med* 2003;**139**:761–76
42. Hoke TS, Douglas IS, Klein CL, He Z, Fang W, Thurman JM, Tao Y, Dursun B, Voelkel NF, Edelstein CL, Faubel S. Acute renal failure after bilateral nephrectomy is associated with cytokine-mediated pulmonary injury. *J Am Soc Nephrol* 2007;**18**:155–64
43. Seujange Y, Leelahavanichkul A, Yisarakun W, Khawsuk W, Meepool A, Phamonleatmongkol P, Saechau W, Onlamul W, Tantiwarattanatikul P, Oonsook W, Eiam-Ong S, Eiam-Ong S. Hibiscus sabdariffa Linnaeus aqueous extracts attenuate the progression of renal injury in 5/6 nephrectomy rats. *Renal Fail* 2013;**35**:118–25
44. Vaziri ND. Causal link between oxidative stress, inflammation, and hypertension. *Iran J Kidney Dis* 2008;**2**:1–10

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