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Effect of Curcumin on Inflammation and Oxidative Stress in Cisplatin-Induced Experimental Nephrotoxicity

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Nephrotoxicity is a major complication and a dose limiting factor for cisplatin therapy. Recent evidence suggests that inflammation and oxidative stress may contribute to the pathogenesis of cisplatin-induced acute renal failure. Curcumin is claimed to be a potent anti-inflammatory and antioxidant agent. The present study was performed to explore the effect of curcumin against cisplatin-induced experimental nephrotoxicity. Curcumin in the dosages of 15, 30, and 60 mg kg^{-1} was administered 2 days before and 3 days after cisplatin administration. Renal injury was assessed by measuring serum creatinine, blood urea nitrogen, creatinine, urea clearance, and serum nitrite levels. Renal oxidative stress was assessed by determining renal malondialdehyde levels, reduced glutathione levels and enzymatic activities of superoxide dismutase and catalase. Systemic inflammation was assessed by tumor necrosis factor-alpha (TNF-α) levels. A single dose of cisplatin resulted in marked inflammation (486% rise in TNF-α level) and oxidative stress and significantly deranged renal functions as well as renal morphology. The serum TNF-α level was markedly reduced in curcumin-treated rats. Curcumin treatment significantly and dose-dependently restored renal function, reduced lipid peroxidation, and enhanced the levels of reduced glutathione and activities of superoxide dismutase and catalase. The present study demonstrates that curcumin has a protective effect on cisplatin-induced experimental nephrotoxicity, and this effect is attributed to its direct anti-inflammatory and strong antioxidant profile. Hence, curcumin has a strong potential to be used as a therapeutic adjuvant in cisplatin nephrotoxicity.

KEYWORDS: Cisplatin; curcumin; inflammation; nephrotoxicity; oxidative stress; TNF-α

INTRODUCTION

Cisplatin and other platinum derivatives are among the most effective chemotherapeutic agents against solid tumors, including ovarian, head, and neck carcinomas and germ cell tumors. Dose-dependent cumulative nephrotoxicity is the major limitation of this compound, sometimes requiring a reduction in dose or discontinuation of treatment (*1*). Approximately 25–35% of patients develop evidence of nephrotoxicity following a single dose of cisplatin (*2*).

Inflammation and oxidative stress play a key role in cisplatin-induced renal dysfunction (*2*, *3*). Cisplatin has been reported to enhance tumor necrosis factor-alpha (TNF-α) levels (*3*), superoxide anions (*4*), peroxynitrite anions (*5*), hydrogen peroxide (*6*), and hydroxyl radicals via mobilization of iron from renal cortical mitochondria (*7*) and decrease the antioxidant enzymes in renal tissue (*8*). Various studies demonstrated the protective effect of anti-inflammatory agents and antioxidants against cisplatin-induced inflammation and oxidative stress in experimental nephrotoxicity (*9*, *10*).

Renewed interest has been observed in recent years on the multiple activities of natural molecules. Curcumin, a yellow pigment present in the rhizome of turmeric (*Curcuma longa* L. Zingiberaceae), has a wide array of pharmacological and biological activities (*11*). Curcumin has been claimed to be a potential anti-inflammatory agent (*12*, *13*) and antioxidant (*14*, *15*) with phytonutrient and bioprotective properties. The anti-inflammatory and antioxidant effects of curcumin have been assessed in various *in vitro* systems and in experimental animal systems (*16*, *17*). Curcumin exerts anti-inflammatory and growth inhibitory effects in TNF-alpha-treated HaCaT cells through inhibition of NF-$\kappa\beta$ and MAP kinase pathways (*18*). The chemoprotective properties of curcumin have also been extensively investigated and are linked to its anti-inflammatory (*19*) and antioxidant activities (*20*). Curcumin also has the potential to inhibit the expression of inducible nitric oxide synthase (iNOS), cyclo-oxygenase-2 (COX-2), and matrix metalloproteinase-9 (MMP-9) (*21*, *22*). Hence, the present study was

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Table 1. Effect of Different Doses of Curcumin on Cisplatin-Induced Nephrotoxicity[a]

parameter	control	CPT (5)	CMN (60)	CPT + CMN (15 mg/kg)	CPT + CMN (30 mg/kg)	CPT + CMN (60 mg/kg)
BUN (mg/dL)	12.90 ± 0.77	87.44 ± 3.12[b]	12.94 ± 0.65[c]	27.38 ± 1.40[b,c]	19.22 ± 0.97[b,c]	13.36 ± 0.67[b]
serum creatinine (mg/dL)	0.20 ± 0.13	3.48 ± 0.17[b]	0.21 ± 0.02[c]	2.13 ± 0.13[b,c]	1.26 ± 0.16[b,c]	0.22 ± 0.21[c]
creatinine clearance (mL/min)	0.95 ± 0.06	0.06 ± 0.13[b]	0.99 ± 0.15[c]	0.56 ± 0.27[b,c]	0.77 ± 0.13[b,c]	0.93 ± 0.25[c]
urea clearance (mL/min)	0.69 ± 0.15	0.03 ± 0.14[b]	0.65 ± 0.03[c]	0.41 ± 0.18[b,c]	0.53 ± 0.23[b,c]	0.67 ± 0.12[c]

[a] Values are expressed mean ± SEM. CMN = curcumin, CPT = cisplatin. [b] Statistically significant at $P < 0.05$ as compared to control. [c] Statistically significant at $P < 0.05$ as compared to cisplatin.

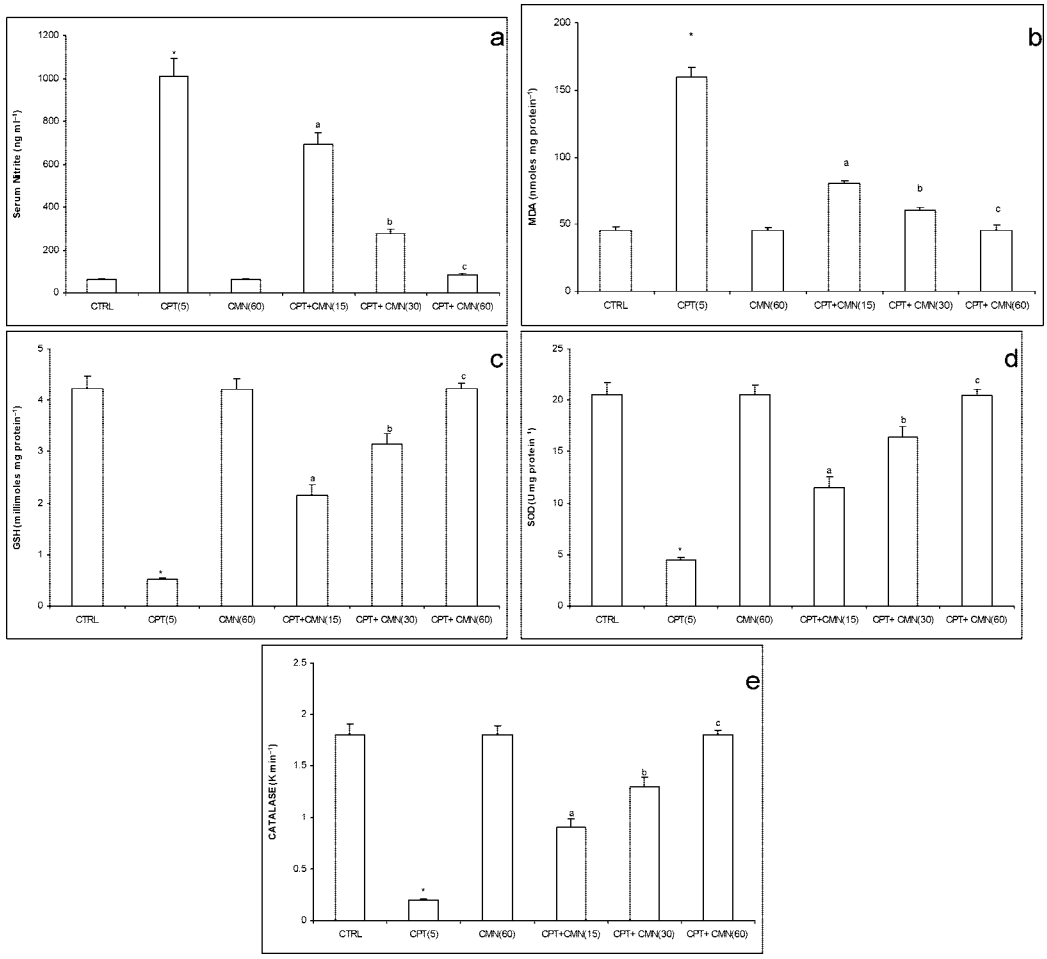

Figure 1. Effect of curcumin (CMN) (15, 30, and 60 mg kg^{-1}, p.o.) on serum nitrite (**a**), lipid peroxidation (**b**), reduced glutathione (**c**), superoxide dismutase (**d**), and catalase (**e**) in CPT (5 mg kg^{-1}, i.p.)-treated rats. *$P < 0.05$ as compared to the control group (CTRL). [a,b,c]$P < 0.05$ as compared to the CPT group and with one another.

designed to investigate the effect of curcumin against cisplatin-induced experimental nephrotoxicity.

MATERIALS AND METHODS

Animals. Male Wistar rats (200–250 g), bred in the Central Animal House Facility of Panjab University, Chandigarh (India), were used. The animals were housed under standard laboratory conditions, were maintained on a 12-h light and dark cycle, and had free access to food (Hindustan Lever Products, Kolkata, India) and water. The experimental protocols were approved by the Institutional Animal Ethics Committee of Panjab University, Chandigarh, and were conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

Drugs. Cisplatin and curcumin (80%) were purchased from Sigma. Cisplatin was dissolved in normal saline (0.9%) and administered intraperitoneally. Curcumin was suspended in 0.25% carboxymethylcellulose (CMC) and administered orally.

Experimental Protocol. Animals were distributed into six groups, with each group comprising 10–12 animals. Group I comprised control animals that received an equivalent volume of 0.25% CMC for 5 days. Group II animals received a single dose of cisplatin (CPT) (5 mg kg^{-1} i.p.). Group III animals received curcumin (60 mg kg^{-1} for 5 days p.o.) *per se*. Group IV, V, and VI animals received curcumin, 15, 30, and 60 mg kg^{-1}, respectively, 2 days before and 3 days after CPT injection. Preliminary dose range (10–200 mg/kg p.o.) studies of curcumin were carried out in our laboratory. The dose of curcumin (60 mg/kg p.o.) was selected on the basis of our preliminary and published data (*12*). The animals were placed in individual metabolic cages for 24 h after the last dose to measure water intake and urine output. Animals were anaesthetized with thiopentone sodium (25 mg kg^{-1}), and blood was collected by insertion of a cannula into the abdominal aorta. Serum was separated and was used freshly for the assessment of renal function tests. A midline incision was performed, and both the kidneys were isolated; the left kidney was deeply frozen till further enzymatic analysis, whereas the right kidney was stored in 10% formalin for the histological studies.

Assessment of Renal Function. The rats were kept individually in metabolic cages for 24 h to collect urine for estimation of renal function.

Table 2. Effects of Curcumin (60 mg/kg, p.o.) on Serum Tumor Necrosis Factor-alpha (TNF-α) Levels[a]

drug treatment	serum TNF-α levels (pg/mL)
control	33.67 ± 1.07
cisplatin	197.33 ± 1.36[b]
cisplatin + curcumin	57.19 ± 1.41[c]

[a] The values are expressed as mean ± SEM. [b] $P < 0.05$ as compared to control. [c] $P < 0.05$ as compared to the cisplatin-treated group (one-way ANOVA followed by Dunnett's test).

Table 3. Effect of Curcumin (60 mg/kg, p.o.) Treatment on Morphological Changes As Assessed by Histopathological Examination of Kidneys in Cisplatin-Treated Rats[a]

group	tubular necrosis	interstitial nephritis	tubular atrophy	hyaline casts
control	−	−	−	−
cisplatin	+++	+++	+++	+++
CPT + curcumin	+/−	+/−	++/−	+/−
curcumin	−	−	−	−

[a] None (−), mild (+), moderate (++), severe (+++). CPT = cisplatin.

The left kidney was removed as described and kept deep frozen for enzymatic analysis, whereas the right kidney was stored in 10% formalin for the histological studies. Plasma samples were assayed for blood urea nitrogen (BUN), urea clearance, serum creatinine, and creatinine clearance by using standard diagnostic kits (Span Diagnostics, Gujarat, India).

Nitrite Estimation. Serum and tissue nitrite were estimated using the Greiss reagent and served as an indicator of NO production. 500 μL of Greiss reagent (1:1 solution of 1% sulfanilamide in 5% phosphoric acid and 0.1% naphthaylamine diamine dihydrochloric acid in water) was added to a suitably diluted 100 μL of plasma, and the absorbance was measured at 546 nm (*23*). The nitrite concentration was calculated using a standard curve for sodium nitrite. Nitrite levels were expressed as nanograms per milliliter of serum.

Postmitochondrial Supernatant Preparation (PMS). Kidneys were perfused with ice-cold saline (0.9% sodium chloride) and homogenized in chilled potassium chloride (1.17%). The homogenates were centrifuged at 800*g* for 5 min at 4 °C to separate the nuclear debris. The supernatant thus obtained was centrifuged at 10500*g* for 20 min at 4 °C to get the postmitochondrial supernatant.

Estimation of Lipid Peroxidation. The malondialdehyde (MDA) content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid-reactive substances (TBARS) by the method of Ohkawa and co-workers (*24*). Briefly, the reaction mixture consisted of 0.2 mL of 8.1% sodium lauryl sulfate, 1.5 mL of 20% acetic acid adjusted to pH 3.5 with sodium hydroxide, and 1.5 mL of a 0.8% aqueous solution of thiobarbituric acid. This mixture was added to 0.2 mL of 10% (w/v) of postmitochondrial supernatant. The sample was brought up to 4.0 mL with distilled water, heated at 95 °C for 60 min, cooled with tap water, mixed with 1.0 mL of distilled water and 5.0 mL of a mixture of *n*-butanol and pyridine (15:1 v/v), and centrifuged. The absorbance of the organic layer was measured at 532 nm. TBARS were quantified using an extinction coefficient of 1.56×10^5 M^{-1}/cm^{-1} and expressed as nanomoles of MDA per milligram of protein. Tissue protein was estimated using the Biuret method, and the renal MDA content was expressed as nanomoles of malondialdehyde per milligram of protein.

Estimation of Endogenous Antioxidant Levels. The antioxidant enzymes were estimated by the well-established procedures already published elsewhere (*9*). The reduced glutathione (GSH) was assayed by the method of Jollow and co-workers (*25*), and the yellow color developed by the reduction of Ellman's reagent by -SH groups of GSH was read at 412 nm. The catalase activity was measured by the method of Claiborne and co-workers (*26*), and the rate of decomposition of H_2O_2 was followed at 240 nm. The superoxide dismutase (SOD) activity was assayed by the method of Kono and co-workers (*27*). The nitro blue tetrazolium reduction by superoxide anion to blue formazan was followed at 540 nm.

Estimation of Tumor Necrosis Factor-alpha (TNF-α). Tumor necrosis factor-alpha (TNF-α) was estimated using a rat TNF-α kit (RD Systems). It is a solid phase sandwich enzyme linked immunosorbent assay (ELISA) using a microtiter plate reader at 450 nm. Concentrations of TNF-α are calculated from plotted standard curves.

Histopathological Examination. For microscopic evaluation, kidneys were fixed in 10% neutral phosphate buffered formalin solution. Following dehydration in an ascending series of ethanol (70, 80, 96, 100%), tissue samples were cleared in xylene and embedded in paraffin. Tissue sections of 5 μm were stained with hematoxylin-eosin (H-E). A minimum of 10 fields for each kidney slide were examined and assigned for severity of changes by an observer blinded to the treatments of the animals and assigned for severity of changes using scores of none (−), mild (+), moderate (++), and severe (+++).

Statistical Analysis. Results were expressed as mean ± SEM. The intergroup variation was measured by one way analysis of variance (ANOVA) followed by Fischer's LSD test. Statistical significance was considered at $P < 0.05$. The statistical analysis was done using the Jandel Sigma Stat Statistical Software version 2.0.

RESULTS

Effect of Curcumin on Cisplatin-Induced Renal Dysfunction. A single dose of cisplatin significantly increased the serum creatinine and blood urea nitrogen (BUN). Chronic curcumin treatment significantly and dose-dependently prevented this rise in BUN and serum creatinine (**Table 1**). Moreover, the creatinine and urea clearance, which was markedly reduced by cisplatin administration, was significantly and dose-dependently improved by curcumin treatment (**Table 1**). However, curcumin *per se* had no effect on serum creatinine, BUN, or creatinine and urea clearance.

Effect of Curcumin on Cisplatin-Induced Nitrosative Stress. Serum nitrite levels were significantly elevated by cisplatin administration. Curcumin treatment significantly and dose-dependently improved this increase in serum nitrite levels (**Figure 1a**). However, curcumin *per se* had no effect on serum nitrite levels.

Effect of Curcumin on Cisplatin-Induced Lipid Peroxidation. TBARS levels were increased significantly by cisplatin administration as compared to the control group. Treatment with curcumin produced a significant and dose-dependent reduction in TBARS in cisplatin-treated rats; however, curcumin *per se* did not alter TBARS (**Figure 1b**).

Effect of Curcumin on Cisplatin-Induced Changes in the Antioxidant Profile. Cisplatin administration significantly decreases the enzyme activity of reduced glutathione (GSH) (**Figure 1c**), superoxide dismutase (SOD) (**Figure 1d**), and catalase (CAT) (**Figure 1e**). This reduction was significantly and dose-dependently improved by the treatment with curcumin. Curcumin *per se* did not improve these reductions in antioxidant profile.

Effect of Curcumin on Tumor Necrosis Factor-alpha (TNF-α). Serum TNF-α levels were markedly increased (486%) in cisplatin-treated rats as compared to the control. Curcumin resulted in a significant decrease in the serum TNF-α level (**Table 2**).

Effect of Curcumin on Cisplatin-Induced Changes in Renal Morphology. The histopathological changes were graded and are summarized in **Table 3**. The control group did not show any morphological changes. By contrast, the kidneys of rats treated with cisplatin showed marked histological changes in the cortex and outer medulla. The renal sections showed severe tubular necrosis, tubular atrophy, interstitial nephritis, and hyaline casts (**Figure 2**). Treatment with curcumin resulted in

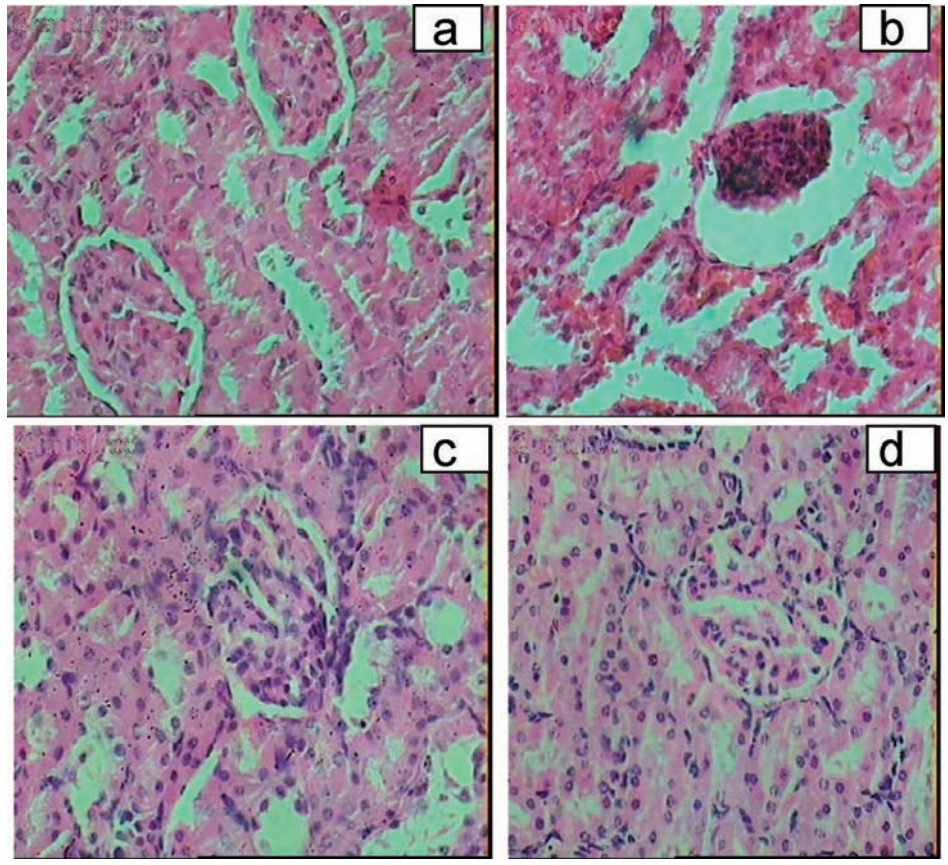

Figure 2. Hemotoxylin- and eosin-stained sections of rat kidney: (**a**) normal kidney section; (**b**) kidney section of a cisplatin (5 mg kg^{-1}, i.p.)-treated rat showing tubular brush-border loss, and necrosis of the epithelium; (**c**) a kidney section of curcumin (60 mg/kg, p.o.) + cisplatin-treated rats showing amelioration of cisplatin-induced alterations; (**d**) a kidney section of curcumin (60 mg/kg, p.o.)-treated rats showing an almost normal morphology.

a marked morphological protection, which was not the case in the curcumin *per se* group.

DISCUSSION

Preclinical studies identified nephrotoxicity as cisplatin's major dose-limiting side effect (*28*), and initial protocols not using aggressive hydration before administration of cisplatin produced severe and frequently irreversible renal failure (*29*). Schrier (*1*) reported that TNF-α could contribute to renal injury and acute renal failure (ARF) after cisplatin administration. Cisplatin may initially reduce the levels and availability of endogenous oxygen radical scavengers such as superoxide dismutase (SOD), much as administration of lipopolysaccharide (LPS) can do (*4*). In addition, reduced glutathione (GSH) and glutathione peroxidase activities in cisplatin-treated tissues may contribute to increased generation of superoxide and other oxygen radicals (*30*), and indeed, cisplatin has been demonstrated to generate superoxide anion in a cell-free system (*31*). The resultant oxidant stress activates the transcription factor NF-κB, which in turn stimulates the production of TNF-α (*32*). In the present study, cisplatin administration markedly increased the serum TNF-α level, which is a potent proinflammatory cytokine (**Table 2**), and curcumin treatment significantly decreased the serum TNF-α level. This effect may be due to the direct anti-inflammatory and strong antioxidant profile of curcumin (*14*, *18*, *19*).

Experimental evidence has suggested that cisplatin (CPT) deteriorates renal function (*33*) and glomerular filtration rate (GFR) in a dose-dependent manner (*34*). The cause of the decrease in glomerular filtration is afferent vasoconstriction and possibly an altered ultrafiltration coefficient, before evidence of tubule obstruction (*35*). Single cisplatin injection causes marked renal dysfunction, as evidenced by creatinine and urea clearance (*9*). In the present study, curcumin significantly and dose-dependently improved creatinine and urea clearance, and decreasing the elevated levels of serum creatinine and BUN provides convincing evidence for participation of reactive oxygen species (ROS) in cisplatin-induced renal dysfunction. It may also be possible that curcumin, due to its potential antioxidant properties, improves renal function via attenuating oxidative stress mediated decline in GFR and renal hemodynamics (*16*, *36*).

Nitrite, the stable end product of nitric oxide metabolism, reacts with superoxide radicals, which finally leads to nitrosative stress. Srivastava and co-workers (*5*) reported that treatment of rats with cisplatin resulted in a significant increase in the activity of calcium-independent nitric oxide synthase (NOS) in kidney and liver, leading to enhanced NO formation. Cisplatin produced nitrosative stress as assessed by serum nitrite levels (*9*). Curcumin administration significantly and dose-dependently attenuated cisplatin-induced nitrosative stress via inhibition of inducible nitric oxide synthase (iNOS) (*21*, *22*). Curcumin inhibits iNOS gene expression in isolated BALB/c mouse peritoneal macrophages and also in the livers of lipopolysaccharide injected mice (*37*). Curcumin and its analogues showed potent peroxynitrite anions scavenging activity *in vitro* using a sodium nitroprusside generating nitric oxide system (*16*).

Malondialdehyde (MDA), a degradation product from lipid hydroperoxide, provides an index of the peroxidation of lipids in biological tissue. It is well documented that cisplatin causes lipid peroxidation in the kidneys via ROS generation (*38*). Earlier we observed an increased production of MDA measured

as TBARS in the kidneys of cisplatin-treated rats (*9*). In this study, curcumin significantly and dose-dependently attenuated lipid peroxidation in cisplatin-treated rats, providing convincing evidence for the involvement of ROS in cisplatin-induced lipid peroxidation. Rukkumani and co-workers (*39*) reported a protective effect of curcumin on circulating lipids and lipid peroxidation. Curcumin attenuates oxidative DNA damage in the mouse epidermis (*40*) and in cultured mouse fibroblast cells (*41*).

It has been suggested that oxidative and nitrosative stresses are the two main cascades involved in cisplatin-induced nephrotoxicity (*6, 7*). Cisplatin administration caused marked deterioration of the endogenous antioxidant profile, as evidenced by decreased SOD and catalase (CAT) activities (*9*). The results of the present study provide convincing evidence that curcumin significantly and dose-dependently ameliorated renal oxidative and nitrosative stress via scavenging ROS. Alasubramanyam and co-workers (*14*) reported curcumin-induced inhibition of cellular ROS generation. Curcumin inhibited hydrogen peroxide-induced cell damage (*17*). Curcumin manganese complex and acetylcurcumin manganese complex showed much greater SOD activity and an inhibitory effect on lipid peroxidation (*42*). Priyadarsini and co-workers (*15*) have shown, by DPPH scavenging *in vitro*, the origin of the antioxidant activity of curcumin to be mainly from the phenolic OH group, although a small fraction may be due to the $>CH_2$ site.

It has been suggested that ROS may lead to tubular damage in cisplatin-treated rats (*6, 9*). The kidneys of cisplatin-treated rats have shown characteristic morphological findings such as necrosis and desquamation of renal tubules lining cells, tubular atrophy and dilation, interstitial nephritis, and hyaline casts. The marked histological changes were prominent in the outer cortex and medullary regions of the kidneys. In our study, curcumin significantly prevented these cisplatin-induced structural changes, suggesting possible involvement of ROS in mediating these histological alterations.

In conclusion, the present study provides convincing evidence for inflammation- and oxidative stress-related renal dysfunction and morphological alterations in this rat model of cisplatin-induced ARF. Moreover, our result clearly indicated the renoprotective potential of curcumin against cisplatin-induced renal dysfunction in rats.

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