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Original Article

Effects of curcumin on anion/cation transporters and multidrug response proteins in cisplatin induced nephrotoxicity

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Abstract: Nephrotoxicity is the major side effect that restricts cisplatin usage in solid tumor therapy and oxidative stress has a prominent role in the pathophysiology of cisplatin nephrotoxicity. Curcumin is an effective antioxidant on oxygen metabolites. Organic anion and cation proteins (OATs, OCTs) serve for the transport of the chemicals into kidney cells and excretion to the urine of these molecules become via multi drug resistance associated proteins (MRPs). We investigated the effects of curcumin on OATs, OCTs and MRPs in rats with cisplatin induced nephrotoxicity. Wistar albino rats (6 weeks old, n=28) were assigned to four groups as; (i): control; (ii): curcumin (100 mg/kg/day); (iii): cisplatin (7 mg/kg - single dose, i.p); (iv): curcumin + cisplatin (100 mg/kg/day + 7 mg/kg - single dose, i.p). Nephrotoxicity was associated with elevations in serum urea and creatinine concentrations and oxidative stress as reflected by elevated renal malondialdehyde (MDA). Rat kidney with cisplatin induced nephrotoxicity had decreased the OAT-1, OAT-3, OCT-1 and OCT-2 levels and increased MRP2 and MRP4 levels as compared to healthy rat kidneys. However, administration of resveratrol (100 mg/kg/day) partially alleviated the cisplatin-induced nephrotoxicity as reflected suppressed the expression oxidative stress parameters and OATs and OCTs levels and enhanced the MRPs levels in the kidney of cisplatin treated rats. Furthermore, the histopathological changes due to cisplatin were ameliorated by curcumin treatment. In conclusion, curcumin has the potential of preventing the cisplatin induced nephrotoxicity via the modulation OATs, OCTs and MRPs expressions in the kidney.

Keywords: Cisplatin nephrotoxicity, curcumin, anionic and cationic transporters, renal prevention, multi drug resistance associated proteins

Introduction

Cisplatin indicates a high antitumor activity and has a wide application area as an antineoplastic agent. Despite this agent is highly effective, usage of this drug is limited in 25% of patients in a dose-dependent manner due to resultant nephrotoxicity [1, 2]. Nephrotoxicity is most common and dose-limiting side effect of the cisplatin therapy [3]. Kidney cells are quite sensitive to toxic injuries because of encountering with a high blood flow, having specific transporters in tubular epithelium and their abilities to concentrate toxins in medullary interstitium [4]. Cisplatin is a fairly strong tubular toxin and its toxicity is more evident in particularly lower chlorine content environments [5]. Cisplatin has been reported to make renal oxidative damage due to its excessive free radical production [6]. Oxidative damage is an important mechanism in the pathogenesis of cisplatin nephrotoxicity [7]. Administration of cisplatin increases the amount of intracellular calcium in the renal tubular cells and this causes damage to the mitochondria and induces the production of free oxygen radicals which activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system [8]. Superoxide anion, hydrogen peroxide and hydroxyl radicals increase in the kidneys after the administration of cisplatin, and these free radicals cause cellular damage by peroxidation of cell membrane

lipids and disrupting the structural proteins [9]. It has been shown that cisplatin reduced the cellular antioxidant activity, superoxide dismutase, glutathione peroxidase and catalase enzyme activities in the kidneys and therefore preventive roles of different antioxidant agents have been studied in experimental animals of cisplatin-induced nephrotoxicity [10, 11]. The renal symptoms of cisplatin nephrotoxicity include hypomagnesaemia, salt loss, Fanconi-like syndrome and anemia. As a result of all these symptoms by cisplatin nephrotoxicity, kidneys lose the normal functions and acute kidney injury occurs [12, 13]. Cellular uptake of cisplatin mainly happens by organic transport mediated system [14]. Human and animal studies of cisplatin indicated that the organic cation transporter (OCT) protein has critical role in cisplatin uptake to the proximal tubules [13]. The highest proportion of cisplatin is accumulated in S3 segment of proximal tubules. Even the proximal tubule cell concentration may reach up to five times more of serum concentrations [15]. It has been demonstrated in a study that the transition of cisplatin to proximal tubule cells after administration, was through OCT2 proteins [12]. Cisplatin accumulates in the kidneys much more than the other organs because it is excreted through the kidneys [16]. Proximal tubule is the main nephron segment, which the renal organic cation secretion takes its place [17]. OCT1 and OCT2 have been shown to be the most abundantly expressed organic cation transporters in the kidneys [18]. OCT2 is an important organic transport mechanism for the transition of cisplatin in to the cells. The other cisplatin analogues carboplatin and oxaliplatin are not transported by OCT2 and that can elucidate these agents to be less nephrotoxic [12]. Interestingly, a single toxic dose of cisplatin to rats could reduce OCT2 mRNA levels in seven days and re-application of cisplatin creates a defense against uptake into the cells [19].

Renal secretion of organic anions, primarily occurs in the proximal tubules [17, 20]. In the kidney, the Organic Anion Transporter protein 1 (OAT1) was found to be less expressed than OAT3 [21]. Human studies also have shown a reduction in the expression of OAT1 in renal diseases [22]. Resistance to anticancer agents, characterized in many tumors and this kind of resistance occurs by the expression of MRP, which belongs to ATP Binding Casette Superfamily (ABC) Transporters [23]. The ABC subfamily MRPs are membrane glycoproteins and mediate the ATP-dependent export of organic anions from cells [24]. MRP2 and MRP4 are more often expressed in the kidneys [25].

Curcuma longa (turmeric), a member of *Zingiberaceae* family, is a perennial plant and its native land is South Asia [26]. Curcumin is the major component of turmeric and in addition to its anti-inflammatory and anti-oxidant effects; it has chemopreventive properties [27]. Beside antioxidant and cytoprotective activity of curcumin that has been shown in many studies, it also has the protective effects on ischemia reperfusion injury [28]. It is indicated that the antioxidant capacity of curcumin could be related by its activity of inhibiting xanthine dehydrogenase conversion into xanthine oxidase [29], inhibiting the lipid peroxidation [30], increasing the level of intracellular glutathione [31] and increasing the iron ion binding request [32]. Curcumin decreases the peroxidation of lipids in the cell membrane by increasing the catalase, superoxide dismutase and glutathione peroxidase enzyme activities [33]. It was observed in the kidneys that curcumin inhibited the oxidative cellular damage against the lipid peroxidation [34]. However, there are no current data available concerning OATs, OCTs and MRPs changes by kidney exposure to cisplatin and curcumin. Therefore, the purpose of this study is investigating the protective effects of curcumin on oxidative stress, histopathological effects and the expressions of OATs, OCTs and MRPs in the kidneys of cisplatin-treated rats.

Materials and methods

Animals

Twenty eight Wistar albino rats (6 weeks old) weighing 190-205 g were obtained from Firat University Research Center (Elazig, Turkey). The rats were housed in a ventilated room under a 12-h light/dark cycle at 25±5°C. The animals were acclimatized for 1 week before the study and had free access to standard laboratory feed and water *ad libitum* during the study. This study was reviewed by the Committee for Ethics in Animal Experiments of the Firat University and NIH Guidelines for the Care and Use of Laboratory Animals.

Table 1. The effect of curcumin on urea-n, creatinine and mda levels in the kidneys of experimental rats

Item	*Groups*				
Parameter	*Control*	*Curcumin*	*Cisplatin*	*Curcumin+Cisplatin*	*–P-*
Urea-N (mg/dl)	57.71+4.61[c]	46.29+1.63[c]	253.43+28.42[a]	123.68+17.63[b]	< 0.001
Creatinine (mg/dl)	0.49+0.02[b]	0.46+0.01[b]	2.16+0.40[a]	1.01+0.17[b]	< 0.001
MDA (μmol/L)	0.51+0.05[c]	0.45+0.05[c]	1.97+0.26[a]	1.12+0.07[b]	< 0.001

Values are mean ± SE of 7 rats from each group. a-c: means in the same line not sharing a common superscript are significantly different between groups ($P < 0.05$).

Experimental design

Cisplatin (Ebewe Pharma Ges. m.b.H. Nfg. KG Unterach, Austria) was given (7 mg/kg body weight) by a single dose of intraperitoneal (i.p.) injection in 0.9% saline (1 ml/100 g b.w.) [35, 36]. Curcumin administration was carried out directly into the stomach via a gavage needle. Rats were divided into four groups: (i) Control rats, receiving a single injection of saline and 10 daily administrations of saline by oral gavage (1 mL/rat/d); (ii) Curcumin group, receiving a single injection of saline and 10 daily administrations of curcumin (100 mg/kg/day) diluted in saline by oral gavage; (iii) Cisplatin group, injected with 7 mg/kg cisplatin alone and 10 daily administrations of saline by oral gavage (1 mL/kg bw/d); (iv) Curcumin + Cisplatin group, injected with 7 mg/kg cisplatin and 10 daily administrations of curcumin (100 mg/kg/day) diluted in saline by oral gavage. 10 days after cisplatin administration, rats were decapitated under anesthesia. Kidneys of the animals were removed for histopathological and western blot analysis by a perfusion through the aorta with a phosphate-buffered solution (PBS; 0.15 M NaCl and 0.01 M sodium phosphate buffer, pH 7.4). Blood samples were also collected for the measurement of serum urea-nitrogen (urea-N), creatinine and MDA levels.

Laboratory analyses

Blood samples were centrifuged at 3,000 *g* for 10 minutes, and serum collected. Serum urea-N and creatinine were measured by a biochemical analyzer (Olympus AU-660, Osaka, Japan). MDA is the major product of membrane lipid peroxidation and measured by the method of Karatepe [37] with a slight modification. MDA concentration of the sera samples were measured by a high performance liquid chromatography (HPLC; Shimadzu, Tokyo, Japan) using Shimadzu UV-vis SPD-10 AVP detector and C_{18}-ODS-3, 5 *μ*m, 4, 6 × 250 mm column. The mobile phase was 30 mM KH2PO4-Methanol (82.5 + 17.5, v/v %, pH 3.6) and the flow rate was 1.2 mL min^{-1}. Chromatograms were monitored at 250 *n*m and injection volume was 20 *μ*L.

Western blot analysis

Protein extraction was performed by homogenizing the rat kidney tissues in 1 ml ice-cold hypotonic buffer A, containing 10 mM 2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES; pH 7.8), 10 mM KCl, 2 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 0.1 mM ethylenediaminetetraacetic acid (EDTA), and 0.1 mM phenylmethylsulfonyl-fluoride (PMSF). 80 μl of 10% Nonidet P-40 (NP-40) solution was added to the homogenates and the mixture was centrifuged for 2 min at 14,000 × g. The supernatant was collected to analyze the protein levels [38]. Equal amounts of each protein were electrophoresed and subsequently transferred to nitrocellulose membrane (Schleicher and Schuell Inc., Keene, NH, USA). Primary antibodies (anti-OCT1, anti-OCT2, anti-OAT1, anti-OAT3, anti-MRP2, anti-MRP4; Abcam, Cambridge, USA) were diluted (1:500 or 1:1000) in the same buffer containing 0.05% Tween-20. Protein loading was controlled using a polyclonal rat antibody against *β*-actin. Bands were analyzed densitometrically using an image analysis system (Image J; National Institute of Health, Bethesda, USA).

Histopathological analysis

The left kidneys of the rats were immediately fixed in 20% neutral buffered formalin solution for histopathology. Kidneys were gradually dehydrated, embedded in paraffin, cut into 5-*μ*m sections, and stained with hematoxylin

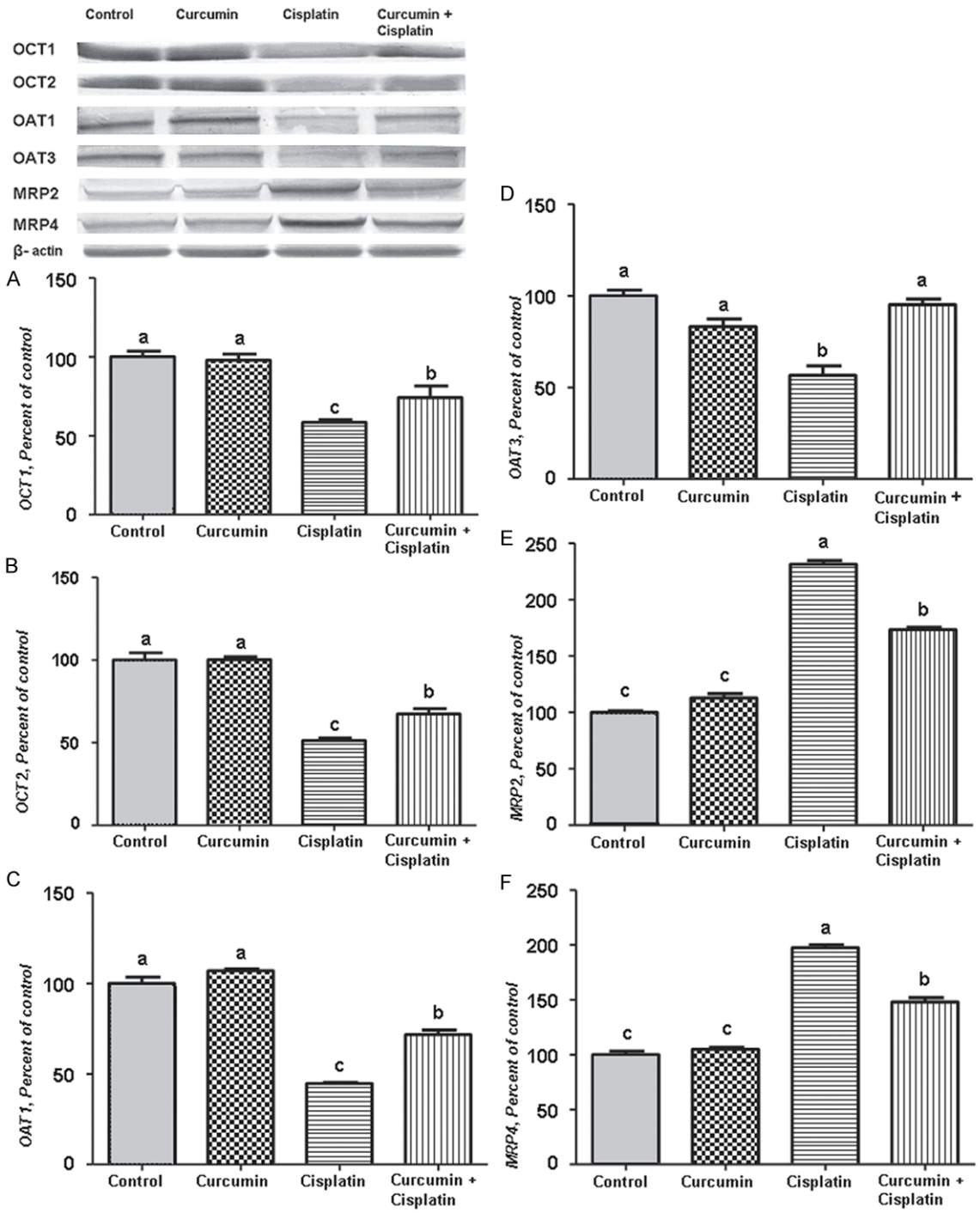

Figure 1. The intensity of the bands was quantified by the densitometric analysis. Western blot using the OCT1 (A), OCT2 (B), OAT1 (C), OAT3 (D), MRP2 (E) and MRP4 (F) revealed specific bands. β-actin levels were monitored to ensure equal protein loading (top panel). Western blot analysis of OCT1, OCT2, OAT1, OAT3, MRP2, and MRP4 in kidney cells in control, curcumin, cisplatin and cisplatin + curcumin treated rats. Data are percent of the control. The bar represents the standard error of the mean. Blots were repeated at least three times (n=3) and a representative blot is shown. A-C. Means in the same line without a common superscript differ significantly ($P<0.05$).

and eosin for histological examination according to the standard procedure [39]. Histological changes were evaluated semi-quantitatively by a pathologist who was unaware of the type of

Table 2. The effect of curcumın on morphologıcal changes ın rat kıdney tıssue

Morphologicalfindings	Groups			
	Control	Curcumin	Cisplatin	Curcumin+Cisplatin
Tubularvacuolization	-	-	+	-
Interstitialedema	+/-	+/-	++	+
Tubularnecrosis	-	-	++	+/-
Tubularatrophy	-	-	+	-
Interstitialinflammation	-	-	+	+/-
Tubulardilatation	-	-	+/++	+

–: none, +: mild (<% 25), ++: moderate (% 25 - % 50), +++: severe (>% 50).

treatment. A minimum of 10 fields for each kidney slide was examined in terms of tubular vacuolization, interstitial edema, tubular necrosis, tubular atrophy, interstitial inflammation and tubular dilation. Severity of changes was assigned using the following scale: -, none; +, mild damage (below 20%); ++, moderate damage (20%-50%); and +++, severe damage (above 50%).

Statistical analysis

The data were analyzed using the GLM procedure of SAS [40]. A sample size of seven per treatment was calculated. The treatments were compared using ANOVA and Student's unpaired *t* test. $P<0.05$ value or less were considered as statistically significant. The differences in latencies between groups were determined by the analysis of variance for repeated measurements (ANOVAR) and followed by Fisher's post-hoc test for all groups. Data are expressed as means ± SE.

Results

Effect of curcumin on urea-N, creatinine and MDA levels

Serum urea-N, creatinine and MDA are shown in **Table 1**. Curcumin administration did not affect serum urea-N and creatinine concentrations in the healthy rats ($P>0.05$). However, cisplatin treated rats had higher serum urea-N (253.43 mg/ld.) and creatinine (2.16 mg/ld.) concentrations than the control rats (57.71 mg/ld. for urea and 0.49 mg/ld. for creatinine ($P<0.0001$). The curcumin treatment caused a reduction serum urea and creatinine concentration in the cisplatin treated rats ($P<0.0001$), which was similar to the control group ($P>0.05$).

Upon the cisplatin induction serum MDA concentration increased from 0.51 moll/L (control) to 1.97 µmoll/L (cisplatin group) ($P<0.001$). The curcumin treatment caused a reduction serum MDA concentration in the cisplatin treated rats ($P<0.001$), which was similar to the control group ($P>0.05$).

Effect of curcumin on OATs, OCTs and MRPs levels

Western blots analysis of OATs, OCTs and MRPs was administered to determine the effects of curcumin on organic anion and cation transporter protein levels. **Figure 1** shows the representative blots. Densitometry analysis evidently indicated that cisplatin administration decreased the levels of OCT1, OCT2, OAT1 and OAT3 in the kidney (**Figure 1**; $P<0.001$). However, curcumin reversed this effect and the levels of OCT1 (Panel A), OCT2 (Panel B), OAT1 (Panel C) and OAT3 (Panel D) were increased in kidneys of animals treated with cisplatin and curcumin ($P<0.05$). Cisplatin treatment increased the renal protein levels of the efflux transporter proteins MRP2 (Panel E) and MRP4 (Panel F). Curcumin treatment reduced the renal levels of MRP2 and MRP4 in cisplatin and curcumin treated rats. There was no significant change observed between normal control rats and curcumin alone treated rats (**Figure 1**; $P>0.05$).

Renal histopathology

There were no pathological changes observed in the kidneys of control and curcumin group of rats (**Table 2**). In contrast, there were mild interstitial inflammation, tubular vacuolization and atrophy in the cortex and outer medulla and moderate interstitial edema, tubular necrosis and dilatation were observed in the group treated with cisplatin alone (**Figure 2**, panel A and **Table 2**). Cisplatin-induced histopathological changes (especially interstitial edema, tubular vacuolization, necrosis and atrophy) were found to be considerably less in the group treated with cisplatin and curcumin (**Figure 2**, Panel B and **Table 2**).

Discussion

The present study demonstrates that the administration of curcumin exerts a renal protective effect in a rat model of nephrotoxicity

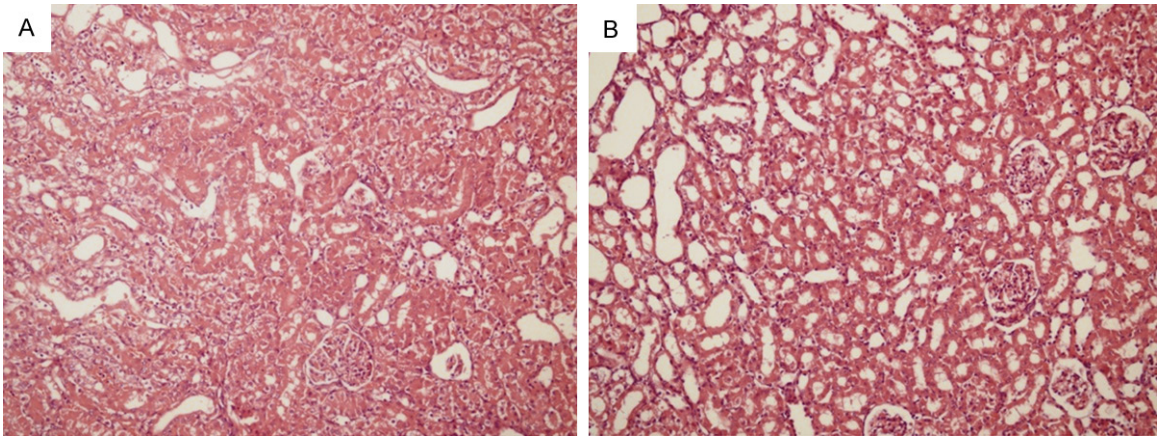

Figure 2. Histopathology of kidney using hematoxylin and eosin (H&E) in cisplatin (A) and curcumin + cisplatin (B) group of rats.

induced by cisplatin. This study builds a positive case for the use of curcumin as a protective agent in cisplatin-based chemotherapy cures. Cisplatin is a broad spectrum of antineoplastic drug used in solid tumors like ovarian, testicular, bladder, head and neck cancers as well as small cell lung tumors and hematologic malignancies such as refractory lymphoma [41]. Several strategies against nephrotoxicity mechanisms have been developed with the purpose of reducing this important side effect of cisplatin. Recent studies proved that the oxidative damage has a major role in the pathogenesis of nephrotoxicity and the preventive antioxidant agent studies have been increased [42, 43]. Antunes *et al.,* [44] administered two doses of 8 mg/kg curcumin (diferuloylmethane) in Wistar rats before 24 hours and 10 minutes of the cisplatin application to prevent cisplatin-induced nephrotoxicity and reported that this dose of curcumin could not prevent renal GSH loss and failed to protect kidneys from secondary developing cisplatin nephrotoxicity. These negative results reported with curcumin in the prevention of secondary developing cisplatin nephrotoxicity are thought to be linked the use of inadequate doses.

Curcumin have been shown to reduce tissue damage and oxidative stress in the ischemia and reperfusion injury of the kidney, heart, liver and brain tissues by its antioxidant effect [28]. The antioxidant effectiveness of curcumin are identified to be caused by the inhibition of xanthine dehydrogenase into xanthine oxidase, [29] inhibiting the lipid peroxidation, [45] the intracellular glutathione level enhancement [31] and increasing the iron ion binding request [32]. Curcumin also reduces the peroxidation of lipids in the cell membrane by increasing the catalase, superoxide dismutase and glutathione peroxidase enzyme activities [33]. Curcumin has been observed to inhibit the lipid peroxidation against the oxidative cell damage in kidneys [34]. There were improvements recorded in kidney by the treatment with curcumin and apoptotic gene expressions inhibited by the effect of curcumin [46]. The protective effect of curcumin has found in cholesterol-fed rabbits and also hydrogen peroxide-treated human renal epithelial cells [47]. Curcumin has been shown to reduce myocardial damage resulting from ischemia in mouse, cat and rabbit models [48, 49].

Increased glucose concentration with oxidative stress enhances lipid peroxidation and glycosylation of the proteins. This excessive oxygen radical production by auto oxidation of glucose is determined by the excessive production of NADPH in glucose metabolism by glycosylated proteins and cytochrome P450-like activity [50, 51]. Curcumin decreased free radical formation in diabetic mice and glycosylation of proteins and lipid peroxidation in erythrocytes [52]. Curcumin is reported to be a potent stimulator in stress induced Hsp70 expression and has protective effect against heat or toxic stress [53]. Both Ghoneim *et al.,* [54] and Madan *et al.,* [55] reported that curcumin increases the expression and activity of antioxidant enzymes in the tissue and inhibits neutrophil infiltration. This situation suggests cellular protective effects in different stress factors.

A dose-dependent increase could be observed in parallel with enzyme activities when the antioxidant effects of curcumin on enzyme activities are analyzed. Piper *et al.,* [56], have determined significant changes in enzyme levels with curcumin doses ranging 1 mg/kg and 500 mg/kg. However, the maximum increase was determined at 75 to 500 mg/kg doses. Safirstein *et al.,* [57], reported that cisplatin increased levels of plasma creatinine after administration of 5 mg/kg dose. Mansour *et al.,* [58], reported increasing serum urea and creatinine levels between 2.5 and 3.3 times, respectively, in their 7.5 mg/kg of cisplatin nephrotoxicity group compared to the control group. Antunes *et al.* [44], investigated the possible protective role of curcumin or selenium before the treatment with cisplatin against cisplatin nephrotoxicity in rats and found out that; cisplatin group provides 166% increase in creatinine compared to the control group and neither curcumin nor selenium administration ensured any reduction in these values.

In the present study, significant increase was determined in serum urea and creatinine levels, which indicates renal dysfunction, in cisplatin group compared to control and curcumin group. In group treated with cisplatin evidence of nephrotoxicity has been found; average serum urea and creatinine levels found to be about four times higher in cisplatin group compared to control group. In group treated with curcumin + cisplatin, average serum urea and creatinine levels also found to be about two times higher when compared with control group. In cisplatin group, serum urea and creatinine values found to be approximately two-fold higher compared to curcumin + cisplatin group (**Table 1**). These results indicates that curcumin improves renal function, to be more spesific it prevents damage that caused by cisplatin.

In cisplatin nephrotoxicity, MDA has been shown to be increased in kidney tissue as a result of lipid peroxidation [59, 60]. In a rat experiment which concluded by Bolaman *et al.,* [61] to investigate role of alpha-tocopherol to prevent cisplatin-induced lipid peroxidation; renal tissue MDA levels, indicator of lipid peroxidation in cisplatin group, was found to be significantly higher compared to control group. In the same study; in the group treated with alpha-tocopherol, renal tissue MDA levels was found to be significantly lower compared to cisplatin group and the researchers asserted that alpha-tocopherol could be used to prevent ciplastin-induced lipid peroxidation. Husain *et al.,* [62] have investigated the effectiveness of ebselen on cisplatin-induced lipid peroxidation and they have reported 74% increase at MDA levels in group treated with cisplatin compared to control group. Kuhad *et al.,* [63] have found that in mice treated with cisplatin, curcumin significantly and dose-dependently reduced lipid peroxidation.

MDA is one of the most reliable and acceptable *in vivo* indicator of lipid peroxidation [64]. In the present study, significant increases in MDA levels were detected with administration of cisplatin. In curcumin + cisplatin group, significant decrease in serum MDA levels was obtained. Serum MDA levels found to be about four-fold higher in cisplatin group compared to control group. In group treated with curcumin + cisplatin, serum MDA levels also found to be about two-fold higher when compared with control group. In cisplatin group, serum MDA levels found to be approximately two-fold higher compared to curcumin + cisplatin group. Curcumin addition to cisplatin provided a decline in serum MDA levels (**Table 1**). These results indicate that curcumin usage improves antioxidant defense system in serum. In addition, these results are consistent with results of many studies using variety of antioxidants which we mentioned earlier.

According to results of OCT1 and OCT2, tubular transport systems, when comparing cisplatin-treated group with control group; 42% and 49% decrease in OCT1 and OCT2 levels were obtained respectively and these results are consistent with similar studies in literature on this subject [12, 19]. With curcumin addition to cisplatin, these values were reduced to 26% in OCT1 and 33% in OCT2 levels. In various studies, ischemia-reperfusion damage has been reported to cause a decrease in OAT1 and OAT3 expression levels [65, 66]. In our study, administration of cisplatin has been found to cause a reduction in OAT1 and OAT3 levels (55% and 43%), and an increase in MRP2 and MRP4 (131% and 98%). Curcumin addition to cisplatin has provided increase in OAT1 and OAT3 levels (28% and 5%), and reduction in MRP2 and MRP4 (73% and 48%).

In a study conducted by Kuhad *et al.,* [63] while there were no morphological changes in control

group; in mice treated with cisplatin, histological changes of renal cortex and medulla were observed. Also, in renal tubular epithelial cells of mice treated with cisplatin, characteristic morphological changes such as necrosis, desquamation, tubular atrophy and dilatation, intersitial nephritis and hyaline cylinders were monitered and most of these specified morphological changes were stated to seen at outer cortex and medulla of the kidney. In group treated with curcumin, curcumin has been found to provide distinct morphological protection and prevent structural changes.

In our study, histopathological changes in cisplatin nephrotoxicity and effects of curcumin on these changes were also investigated (**Table 2**). No pathology was observed in kidneys of control and curcumin group rats however intersitial inflammation, tubuler vacuolization and atrophy on mild level; intersitial edema, tubuler necrosis and dilatation on moderate level was observed in cortex and outer medulla of the group treated with cisplatin. These histopathological changes were consistent with similar studies in the literature [42, 67]. In curcumin + cisplatin group treated with curcumin, cisplatin-induced histopathological changes (especially intersitial edema; tubuler vacuolation, necrosis and atrophy) were found to be considerably less. All these findings indicate that curcumin is also effective in reducing cisplatin-induced renal damage on histopathological levels.

In conclusion, curcumin increased the levels of OCT1, OCT2, OAT1, and OAT3 and reduced the levels of MRP2, MRP4 in the kidney of rats. These results demonstrated that curcumin could protect against cisplatin nephrotoxicity. There is a need of further experimental and clinical studies whether cisplatin lead to toxicity via transport proteins that are reaching high concentrations on renal tubular cells.

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Disclosure of conflict of interest

None.

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