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Research Article

Protection of Curcumin and Curcumin Nanoparticles against Cisplatin Induced Nephrotoxicity in Male Rats

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Abstract: Reactive oxygen species and free radicals are involved in the nephrotoxicity induced by a synthetic anticancer drug Cisplatin (CDDP). This paper is based on nonparticipation technique for preparation of Curcumin nanoparticles (CURNPs) and study the effect of Curcumin (CUR) and CURNPs (60, 30 mg/kg b.w) as antioxidants on the nephrotoxicity induced by CDDP. Physicochemical characterization was studied by using Dynamic Light Scattering (DLS) using Zetasizer Nano ZS, FTIR and X-ray diffraction. CUR and CURNPs were evaluated using CDDP (6mg/kg b.wi.p) induced oxidative renal damage in male rats. Urea and creatinine in serum were estimated for the renal function. Also, TBARS was estimated in serum. The results indicated that CUR and CURNPs (60, 30 mg/kg b.w) significantly ($p<0.05$) protected the nephrotoxicity induced by CDDP. The CDDP induced increase of urea and creatinine concentrations were reduced in the CUR (60, 30 mg/kg b.w) + CDDP and CURNPs (60, 30 mg/kg b.w) + CDDP treated groups. On the other hand, CDDP induced rise of serum TBARS. TBARS, was decreased significantly ($p<0.05$) in groups treated by CUR and CURNPs (60, 30 mg/kg b.w) and protected from the increase of CDDP induced lipid peroxidation. This study concluded that CUR and CURNPs (60, 30 mg/kg b.w) are effective to protect against oxidative renal damage. Most importantly, CURNPs was much more effective and better nephroprotective agent than CUR. Hence, CURNPs has a strong potential to be used as a strong antioxidant in CDDP nephrotoxicity.
Keywords: Curcumin (CUR), Curcumin nanoparticles (CURNPs), Cisplatin (CDDP), antioxidant, nephrotoxicity, Oxidative stress, physicochemical properties.

INTRODUCTION

Cisplatin (cis-diammine dichloroplatinum II (CDDP)) is a chemotherapeutic agent that is used for the treatment of a wide variety of cancers, but nephrotoxicity is a major dose-limiting side-effect. Acute kidney injury occurs after high-dose cisplatin chemotherapy in approximately 20% of patients [1]. Acute kidney injury remains a significant cause of increased morbidity and mortality among patients, particularly in critical care units. Although several therapeutic strategies have been suggested to prevent this condition, no specific treatments are currently recommended, except for vigorous hydration with normal saline [2]. Inflammation and oxidative stress play a key role in cisplatin induced renal dysfunction [3]. Cisplatin has been reported to enhance tumor necrosis factor-alpha (TNF-R) levels [4], superoxide anions [5], peroxynitrite anions [6], hydrogen peroxide [7], and hydroxyl radicals via mobilization of iron from renal cortical mitochondria[8, 9]. Various studies demonstrated the protective effect of anti-inflammatory agents and antioxidants against cisplatin-induced inflammation and oxidative stress in experimental nephrotoxicity [10, 11]. Cisplatin-induced nephrotoxicity is closely associated with an increase in lipid peroxidation in the kidney tissues [12-14]. Furthermore, cisplatin induced glutathione depletion is a determinant step in oxidative stress in kidney tissue that leads to nephrotoxicity [15]. Cisplatin chemotherapy induces a fall in plasma antioxidant levels, which may reflect a failure of the antioxidant defense mechanism against oxidative damage induced by commonly used antitumor drugs [16]. Renewed interest has been observed in recent years on the multiple activities of natural molecules. A large number of natural products and dietary components have been evaluated as potential chemoprotective agents.

Curcumin (CUR) is a dietary antioxidant derived from turmeric (*Curcuma longa*, *Zingiberaceae*) and has been known since ancient times to possess therapeutic properties. It has been reported to scavenge oxygen free radicals, to inhibit lipid peroxidation, and has anticarcinogenic activities in experimental models [17-20]. It has been reported that curcumin is a bifunctional antioxidant [21] because of its ability to react directly

with reactive species and to induce an up-regulation of various cytoprotective and antioxidant proteins. Curcumin is able to scavenge superoxide anion ($O_2^{\cdot-}$) [22, 23], hydroxyl radicals ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2) [24, 25], singlet oxygen [25], nitric oxide [26], peroxy nitrite [27] and peroxyl radicals ($ROO^{\cdot}$) [24]. Together, these mechanisms might explain, at least inpart, some of the cytoprotective effects of this compound. As the presence of phenolic groups in the structure of curcumin (Fig.1) explains its ability to react with reactive oxygen species (ROS) and reactive nitrogen species (RNS) and might probably be one of the mechanisms through which curcumin treatment protects the epithelial cells of renal tubules (LLC-PK1) from oxidative damage induced by H_2O_2 [28]. The indirect antioxidant capacity of curcumin is defined by its ability to induce the expression of cytoprotective proteins such as hemeoxygenase1 (HO-1) [28, 29], glutathione-S-transferase (GST), NAD(P)H:quinine oxidoreductase1(NQO1) [30]. It has been well established that oxidative stress is one of the mechanisms involved in cell damage induced by cisplatin. Indeed, a decrease of antioxidant defense is clearly observed in vivo and in vitro experimental models [31, 32] designed a two-day curcumin pretreatment and in parallel treatment of 15, 30 and 60 mg/kg of curcumin in a model of cisplatin-induced nephrotoxicity. The cisplatin-treated group that received 60 mg/kg of curcumin showed normal renal function (evaluated by measuring urea levels and creatinine clearance), which correlated with lipid peroxidation reduction. The study performed by Waly *et al.* [33] showed that oxaliplatin or cisplatin induced oxidative stress in human embryonic kidney cells (HEK293). These cells also showed a decrease in total antioxidant capacity (TAC). Interestingly, curcumin added to these cell cultures significantly restored TAC.

Curcumin also enhanced the antitumor effect of the widely used antitumor agent cisplatin against fibrosarcoma [34].Pre-clinical studies conducted on CUR have demonstrated that it inhibits carcinogenesis in a number of cell lines, including breast, cervical, colon, gastric, hepatic, leukemia, oral epithelial, ovarian, pancreatic, and prostate cancer cell lines [35]. Accordingly, there is interest in the clinical development of this compound as an anti-cancer agent [36]. However, despite its promising anti-cancer properties, the extremely low water solubility of CUR limits its bioavailability and clinical efficacy, for example, its serum concentrations and tissue distributions are low, and furthermore, it is rapidly metabolized and thus has a short half-life [37]. To address these problems, attempts have been made to encapsulate CUR in liposomes [38, 39], polymeric nanoparticles [40], lipid-based nanoparticles [41], biodegradable microspheres [42], cyclodextrin [43], and in hydrogel [44].Therefore, our present study was designed to investigate: 1-Prepare curcumin nanoparticles (CURNPs). 2-Study its physicochemical characterization by using Dynamic Light Scattering (DLS) using Zetasizer Nano ZS, Fourier transform infrared spectroscopy (FTIR), and powder X-ray diffraction (XRD). 3- Study the effect of original curcumin and curcumin nanoparticles on oxidative stress and nephrotoxicity induced by cisplatinin male rats.

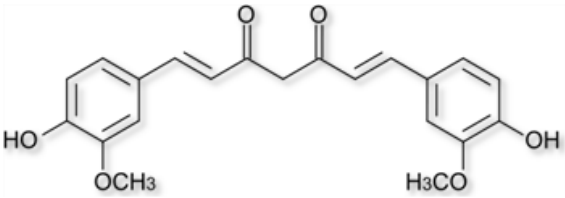

Fig. 1: Chemical structure of Curcumin

MATERIALS AND METHODS

Materials

Poly(D,L-lactic-co-glycolic acid) (PLGA) (Resomer R503H; MW 35–40 kDa),poly(vinyl alcohol) (PVA)(MW 30–70 kDa), Dimethylsulfoxide (DMSO) and ethyl acetatewas purchasedfrom Sigma-Aldrich (Poole, UK),High-performance liquid chromatography-grade ethanol, and distilled waterwere procured from J.T. Baker (now Avantor Performance materials, Phillipsburg, NJ). Cisplatin and curcumin were purchased from Sigma-Aldrich (Poole, UK).

Methods

Preparation of curcumin nanoparticles

Curcumin nanoparticles (CURNPs) were prepared with homogenized for 30 min at 25000 rpm by the nano participation technique [45].Commercial curcumin (7.5 mg) and PLGA (50 mg) were dissolved in2.5 ml of ethyl acetate and stirred at 1,000 rpm for 30 min under room temperature to obtain a homogeneous solution. PVA (50 mg), used as a stabilizer, was dissolved in 5 ml distilled water. The organic phase containing the active ingredient and PLGA was then added in a drop-wise manner to the stabilizer solution during homogenization. After this step, the emulsion was transferred to 20 ml water to facilitate diffusion and was stirred overnight to ensure the complete evaporation of the organic solvent. After the evaporation step was complete, then an particle solution was centrifuged at 15,000×g for 15 min to separate free active ingredient and any unbound stabilizer in the solution. The supernatant was separated and the pellet was redispersed in 20 ml water.

Characterization of curcumin nanoparticles

Particle size analysis

Curcumin nanoparticles were diluted with water to ensure that the signal intensity was suitable for the instrument. Particle size was determined by dynamic light scattering (DLS) using Zetasizer Nano ZS

(Malvern Instruments Ltd., Malvern, UK). Nanoparticles were diluted with water (viscosity (cp) 0.8872) and intensity scattered light was detected at a scattering angle of 173° to an incident beam at a temperature of 25°C. The poly dispersity index range was comprised between 0and1 and the measurements were done in triplicate.

FTIR spectroscopy analysis

The structure was analyzed by FTIR spectra (FTIR-Nicolet 6700**)**. Samples were ground and mixed with Potassium bromide (KBr) to make pellets. It was compressed under high pressure to prepare pellets of 10.0 mm and 1–2 mm thick. The pellets were scanned over a range of 4000 cm^{-1} to 400 cm^{-1}. FTIR spectra in the transmission mode were recorded using a Nicolet Nexus, connected to a PC, in which the number of scans was 128 and the resolution was 4.

X-ray diffraction analysis

The crystalline state of the samples was estimated by an X-ray diffractometer (D/max r-B, Rigaku, Japan). The experiments were performed in symmetrical reflection mode with a Cobalt (Co)line as thesource of radiation. Standard runs using a 40 kV voltage, a 40 mA current and a scanning rate of 0.02° min^{-1} over a 2 range of 5–40° were used.

Biological methods

Male adult rats (66 animals weighing 200-250g) were obtained from the animal house in University of Dammam. Rats were housed in individual cages with screen bottoms and fed on basal diet (corn starch 70%, casein 10%, corn seed oil 10%, cellulose 5%, salt mixture 4% and vitamins mixture 1%)for ten days [46]. Rats were kept under standard conditions of temperature (21±0.5º) and relative humidity (55±5) with 12h light/12h dark cycle. After equilibration, rats were weighted and divided into 11 groups (six animals per each) everyone was assigned to one of the eleven diet groups. Group 1 was used as control (2ml saline orally) once daily for five consecutive days, Group 2 was received DMSO (2ml orally)once daily for five consecutive days, Group 3 was treated with CUR (60 mg/ kg b.w orally) alone. Group 4 was treated with CUR (60 mg/ kg b.w orally) + CDDP(6 mg/ kg b.w i.p), Group 5 was treated with CURNPs (60 mg/ kg b.w orally) alone, Group 6 was treated with CURNPs (60 mg/ kg b.w orally) + CDDP(6 mg/ kg b.w i.p),Group 7 was treated with CUR (30 mg/ kg b.w orally) alone, Group 8 was treated with CUR (30 mg/ kg b.w orally) + CDDP(6 mg/ kg b.w., i.p), Group 9 was treated with CURNPs (30 mg/ kg b.w orally) alone, Group 10 was treated with CURNPs (30 mg/ kg b.w orally) + CDDP(6 mg/ kg b.w i.p), and Group 11 was received CDDP(6 mg/ kg b.w i.p).Groups 4, 6, 8, 10and 11 were similarly treated with CDDP by intraperitoneal injection in the same volumes after 2 days from the experimental initial. Groups 3, 7 and 5, 9 were administrated CUR and CURNPs at 60 and 30 mg/ kg b.w once daily for five consecutive days, respectively. The dose of curcumin (60 mg/kg b.w) was selected on the basis of our preliminary and published data [47].Total feed consumption was weighted, fresh feed was provided every day and total body weight of the animals was recorded at the beginning and during the experimental period. At the end of the experiment, the animals of each group were killed by decapitation. The rats were killed 2 days after cisplatin administration. Blood samples were collected from the orbital plexus by mean of heparinized capillary glass tubes according to Schermer [48]. Each sample was placed into a dry clean centrifuge tube and centrifuged 1500xg for 30 min. at 4˚C to obtain serum.

Biochemical assays

Urea and Creatinine assays

Serum urea was determined according to Fawcett and Soctt [49] and creatinine was determined according to the method of Barthes *et al*. [50].

Thiobarbeturic acid (TBASR)

Malonaldehyde content was measured as described by Ohkawa *et al*. [51]. The mixture consisted of 0.8 ml of sample (1 mg), 0.2 ml of 8.1% SDS, 1.5 ml of 20% glacial acetic acid adjusted to pH 3.5, and 1.5 ml of 0.8%aqueous solution of TBA. The mixture was made up to 4ml with distilled water and heated at 95°C for 60 min using a glass ball as condenser. After cooling with tap water, 1 ml distilled water and 5ml n-butanol and pyridine mixture (15:1) were added and the solution was shaken vigorously. After centrifugation at (2000xg) for 10 min the absorbance of the organic layer was measured at 532 nm. Amount of thiobarbituric reacting substances formed is calculated from standard curve prepared using 1, 10, 3, 30-tetramethoxy propane and the values expressed as nmol per mg protein.

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 AND DISCUSSION

Preparation and characterization of CUR and CURNPs

The preparation based on nano participation technique by Devadasu *et al*. [45]. Fig. 2 and 3 shows the image of original CUR and CURNPs powder and solubility in water, respectively. When resuspended CURNPs in water, the lyophilized powder formed a very fine dispersion and appeared to be soluble, unlike original CUR, which is completely insoluble in water, with un dissolved flakes clearly visible in the suspension (Fig. 3). Corresponding to the Prandtl

equation, Nystrom and Bisrat [52] have shown that, for solids dispersed in a liquid medium under agitation, a decrease in particle size results in a thinner hydrodynamic layer around particles and an increase of the surface specific dissolution rate. Anderberg *et al*. [53] have found a hyperbolic relation between the particle size and the surface specific dissolution rate corrected for solubility. This phenomenon is especially pronounced for materials which have a mean particle size less than 2 mm. At a particle size of 1 mm the intrinsic dissolution rate is very fast, and further decrease in size will lead to no practical advantage in the case of e.g. oral adsorption [54]. The enhanced aqueous solubility of nano-sized curcumin particles could be attributed to their larger surface area, which promotes dissolution [55]. Similar results have been demonstrated in previous studies also, where reduction in the particle size of active ingredients to nanoparticle size has shown improvement in solubility, and bioavailability [56].

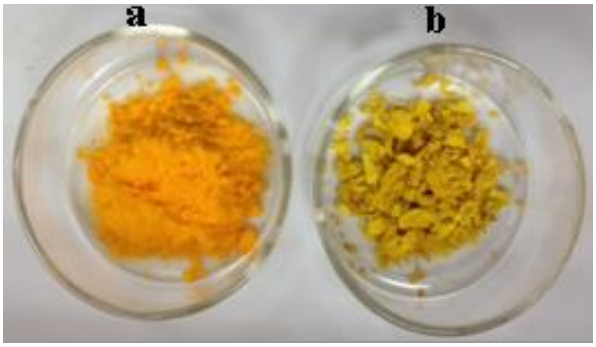

Fig. 2: Image of (a) original Curcumin and (b) Curcumin nanoparticles powder

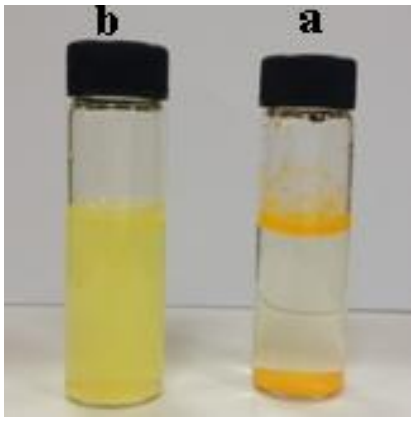

Fig. 3: Solubility of (a) original Curcumin and (b) Curcumin nanoparticles in water

Particle size analysis

The particle size analysis was performed by DLS. DLS of the aqueous dispersion of CURNPs revealed the formation of nanoparticles with an average hydrodynamic diameter of 102.3 nm. Devadasu *et al*. [45] prepared CURNPs and found that the particle Z-average size of curcumin-encapsulated nanoparticles was 237±6 nm and the electron micrographs indicated the spherical shape. The resulting curcumin nanoparticles that were prepared by Kim *et al*. [57] using nanoparticle albumin bound technology had a mean size of 130-150 nm as determined by DLS. Nanocurcumin formulation that were prepared by nano-precipetation technique [58] exhibit smaller particle size 76.2 nm as determined by DLS. The mean particle diameters of Curcumin nanoparticles that were prepared by nano-precipitation technique of Feng-Lin *et al*. [59], showed 142.90±3.12 nm and the poly dispersity index value was 0.19±0.07.

(FT-IR) spectroscopy

FTIR analysis is one of the important tools for the quick and efficient identification of encapsulated chemical molecules. Both spectra of CUR and CURNPs have the same characteristic peaks but with lower intensity (conc) of sample peaks (Fig. 4).CUR displayed the characteristic intensities of O-H str at 3450 cm^{-1}, the aromatic C=C str at 1580 and 1480 cm^{-1}, C=O str at 1600 cm^{-1}, aromatic C-O str at 1250 cm^{-1}, aliphatic C-O str at 1180 cm^{-1} and =C-H bending at 980 and 900 cm^{-1}. C=O str appeared at low frequency due to enolization and intermolecular H-bonding. In case of CURNPs, the spectra showed that C=O str of ester appeared at 1740cm^{-1}. Also, O-H stretch of CUR was completely disappeared. This could be attributed to the formation of intermolecular hydrogen bonding between O-H of CUR and C=O of PLGA. This results agree with Gosh *et al*. [60] which found that FTIR analysis revealed no drug polymer interactions in the nanoparticles formulation, and this indicates that CUR can be incorporated in the nanoparticles without altering its individual structural identity. Also, Chereddy *et al*. [61]observed that the characteristic peaks of FTIR spectra of CUR was observed at 3524 cm^{-1} (O-H stretch). The spectrum of PLGA showed the C=O absorption band at 1760 cm^{-1}which was also seen in PLGA-CURNPs. The O-H absorption band of CUR was not observed whereas the aromatic signature of CUR was found in PLGA-CURNPs spectrum [62].The spectrum of CUR for similar study displayed the characteristic intensities of the O-H stretch at 3508 cm^{-1}[59]. The spectrum of PVP (Polyvinylpyrrolidone) displayed the C=O absorption band at 1662 cm^{-1}. In the case of CURNPs, the C=O absorption band was shifted to a lower wave number and the O-H absorption band of CUR was completely absent. This could be due to the formation of intermolecular hydrogen bonds between the O-H band of CUR and the C=O band of PVP. Tantishaiyakul *et al*. [63] have previously pointed out that hydrogen bonding can influence the conversion of drug crystals.

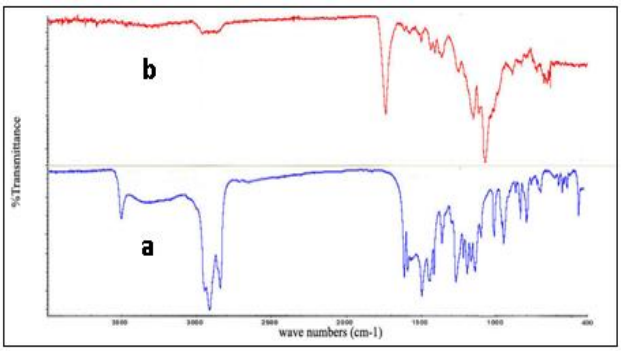

Fig. 4: FTIR spectra of (a) original Curcumin and (b) Curcumin nanoparticles

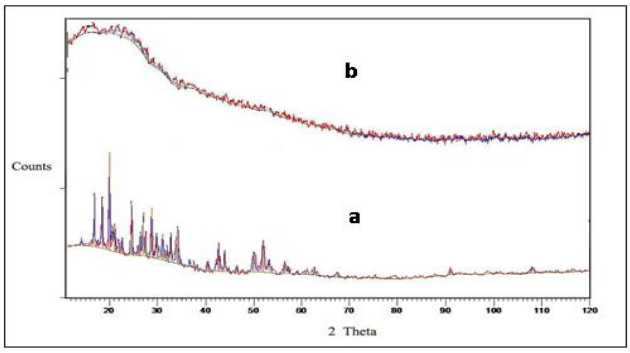

Fig. 5: X-ray diffraction pattern of (a) original Curcumin and (b) Curcumi nanoparticles

X-ray diffraction analysis

Powder XRD analysis was applied to investigate the crystal transformation of nanoparticle system. Fig. 5 indicates the XRD patterns of CUR and CURNPs. The characteristic peaks of CUR appeared at a diffraction angle of 2θ (14.20°, 17.53°, 18.44°, 22.55°, 24.54°, 25.86° and 27.01°), may be that the drug possessed a highly crystalline structure. On the other hand, no characteristic peaks appeared in the patterns of lyophilized CURNPs, suggesting a conversion of CUR from a highly crystalline condition to an amorphous state. This results agree with other study which observed that the characteristic peaks in spectrum of CUR are originated from its crystalline structure [61]. In the case of PLGA-CUR NP, there were no characteristic peaks of CUR in the spectrum. In other previous reports, the XRD pattern peaks of CUR are presented at (21.26°, 23.35° and 24.68°). This patterns represent characteristics of the crystalline structure of CUR while this characteristics was not observed in the nano-CUR formulation [58]. This data suggest that the intermolecular interactions between PLGA/PVA/PLL polymer chains offer an amorphous nature to CUR after encapsulation. Curcumin encapsulated PLGA nanoparticles were indicated in amorphous stage. Detailed compatibility studies also suggest a better compatibility of CUR with PLGA polymer. Similar phenomenon was observed in most of the crystalline drugsposes amorphous nature when encapsulating into PLGA NPs [64][65] PVP probably suppressed the crystal aggregates of CUR during the preparation of its nanoparticle system, thus promoting the formation of an amorphous complex [59].The characteristic peaks of CUR appeared at a diffraction angle of 2θ (8.89°, 14.48°, 17.22°, 18.18°, 23.33°, 24.60°, and 25.52°). In contrast, no characteristic peaks appeared in the patterns of the lyophilized CURN, suggesting a conversion of CUR from a highly crystalline condition to an amorphous state. A similar finding has been demonstrated for piroxicam dispersed into PVP, which also displayed an amorphous state [66].

Biological study

Effect of CUR and CURNPs on Urea and Creatinine

Table 1 shows the effect of CUR and CURNPs on serum urea and creatinine. The concentrations of urea and creatinine were significantly increased ($p<0.05$) in the cisplatin alone treated group (PC) (114.61mg/dl) and (1.46 mg/dl) respectively, compared to normal control (NC) (24.44 mg/dl) and (0.74 mg/dl) respectively. Serum urea and creatinine in groups treated with CUR (60, 30mg/kg b.w) + CDDP and CURNPs(60, 30mg/kg b.w) + CDDP were reduced to 55.88%, 12.49%, 66.63% and 28.81% in urea and 43.15%, 49.32%, 21.23% and 35.62% in creatinine concentrations, respectively, with respect to the PC.

Our results are corroborated by previous studies reported by other investigators on CDDP induced nephrotoxicity in normal rats [67-71].Safirstein *et al.* [72] has been suggested that binding of CDDP to the renal base transport system and the following peroxidation of membrane lipids may account for its nephrotoxicity. There is evidence suggesting that CDDP exerts its nephrotoxic effects by the generation of free radicals [73, 74]. However, although these studies suggest lipid peroxidation as a pathway in the onset of CDDP induced renal damage. Its causal role in CDDP induced renal damage has been queried. It has been suggested that oxidative and nitrosative stresses are the two main cascades involved in CDDP induced nephrotoxicity [7, 8]. CDDP is known to generate ROS such as superoxide anion and hydroxyl radicals, and stimulates renal lipid peroxidation [75, 76]. Intravenous CDDP administration caused abnormal renal functions in all rats. In the present study, a single dose of CDDP induced nephrotoxicity which was observed by biochemical parameters as a significant increase in serum creatinine. Earlier studies have also shown that a 5 mg/kg (i.p) dose of CDDP was sufficient to induce nephrotoxicity in rats [77]. Kersten *et al.* [67] and Husain *et al.* [70] also observed that a single dose of CDDP resulted in renal failure 3 days following CDDP injection. Several investigations have shown that CDDP nephrotoxicity is associated with lipid peroxidation in

radical-mediated chain reaction that damages cell membranes, and the inhibition of this process by CUR is mainly attributed to the ability of scavenger free radicals [78]. In the present investigation, significant reduction in lipid peroxidation was observed in animals treated with CUR plus CDDP when compared with the CDDP group.CUR significantly and dose-dependently improved urea and creatinine, and decreasing the elevated levels of serum urea and creatinine provides convincing evidence for participation of reactive oxygen species (ROS) in CDDP induced renal dysfunction. It may also be possible that CUR, due to its potential antioxidant properties, improves renal function via attenuating the oxidative stress [79, 80].

Table 1: Effect of CUR (60, 30mg/kg b.w) and CURNPs (60, 30mg/kg b.w) on serum Urea and Creatinine concentrations in male rats treated with Cisplatin (6mg/kg b.w)

Treatments	Urea (mg/dl)	Creatinine(mg/dl)
Negative Control (NC)	24.44±0.50^{f}	0.74±0.01^{f}
DMSO	22.64±0.66^{h}	0.83±0.01^{d}
CUR 60mg	24.07±0.56^{fg}	0.73±0.01^{g}
CUR 60mg + CDDP	51.71±0.84^{d}	0.83±0.01^{d}
CURNPs 60mg	22.72±0.65^{h}	0.71±0.01^{h}
CURNPs 60mg + CDDP	38.24±0.84^{e}	0.74±0.01^{f}
CUR30mg	24.76±0.31^{f}	0.76±0.00^{e}
CUR30mg + CDDP	100.29±0.62^{b}	1.15±0.01^{b}
CURNPs 30mg	23.25±0.37^{gh}	0.75±0.00^{f}
CURNPs 30mg + CDDP	81.59±0.75^{c}	0.94±0.01 ^{c}
CDDP (PC)	114.61±1.75^{a}	1.46±0.02^{a}
LSD	0.928	0.009

Each value represents mean ±SD of six animals, CUR= original curcumin, CURNPs= curcumin nanoparticles, CDDP (PC)= cisplatin (positive control). Statistically significant at P < 0.05 as compared to negative Control (NC) (One-way ANOVA followed by Fischer's LSD test).

Effect of CUR and CURNPs on TBARS

The activity of TBARS was given in Table 2. The activity of TBARS was increased significantly ($p<0.05$) in PC compared to NC. Administration of CUR (60, 30mg/kg b.w) + CDDP and CURNPs (60, 30mg/kg b.w) + CDDP were decreased significantly ($p<0.05$) compared to PC. The treatments of CUR (60, 30mg/kg b.w) and CURNPs (60, 30mg/kg b.w) gave results near to the NC.

Table 2: Effect of CUR (60, 30mg/kg b.w) and CURNPs (60, 30mg/kg b.w) on serum TBARS activity in male rats treated with Cisplatin (6mg/kg b.w)

Treatments	TBARS (µM)
Negative Control (NC)	8.38±0.01^{f}
DMSO	8.38±0.01^{f}
CUR 60mg	8.36±0.01^{f}
CUR 60mg + CDDP	10.80±0.07^{d}
CURNPs 60mg	8.36±0.01^{f}
CURNPs 60mg +CDDP	8.90±0.09^{e}
CUR30mg	8.39±0.01^{f}
CUR30mg +CDDP	12.59±0.17^{b}
CURNPs 30mg	8.37±0.01^{f}
CURNPs 30mg +CDDP	11.75±0.22^{c}
CDDP (PC)	24.79±0.39^{a}
LSD	0.172

Each value represents mean ±SD of six animals, CUR= original curcumin, CURNPs= curcumin nano particles, CDDP (PC)= cisplatin (positive control). Statistically significant at P < 0.05 as compared to negative Control (NC)(One-way ANOVA followed by Fischer's LSD test).

Malondialdehyde (MDA), a degradation product from lipid hydroperoxide, provides an index of the peroxidation of lipids in biological tissue. It is well documented that CDDP causes lipid peroxidation in the kidneys via ROS generation [76]. Earlier we observed an increased production of MDA measured as TBARS in the kidneys of CDDP-treated rats [10]. In this study, CUR significantly and dose-dependently attenuated

lipid peroxidation in CDDP treated rats, providing convincing evidence for the involvement of ROS in CDDP-induced lipid peroxidation. Rukkumani *et al*. [81] reported a protective effect of CURon circulating lipids and lipid peroxidation. CUR attenuates oxidative DNA damage in the mouse epidermis [82], and in cultured mouse fibroblast cells [5].

CONCLUSIONS

CDDP-induced nephrotoxicity was confirmed by our study. CUR and CURNPs (60, 30mg/kg b.w) had effect on the biochemical factors (urea and creatinine) and TBARS. We found that CURNPs (60, 30mg/kg b.w)are more effective on CDDP-induced nephrotoxicity than CUR(60, 30mg/kg b.w). Therefore, CURNPs has proved to be an effective free radical quencher. In addition, CURNPs formulation approach resulted in improved oral bioavailability, enhanced efficacy and protect the rat kidney from CDDP-induced nephrotoxicity. Though further studies on quantitating bioavailability remains to be done CURNPs in oral route might be a promising antioxidant alternative to prevent kidney.

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