Curcumin inhibits adenosine deaminase and arginase activities in cadmium-induced renal toxicity in rat kidney

Ayodele Jacob Akinyemi a,*, Nora Onyebueke a, Opeyemi Ayodeji Faboy a, Sunday Amos Onikanni a, Adewale Fadaka a, Israel Olayide a

a Department of Chemical Science, Biochemistry Unit, Afe Babalola University, Ado-Ekiti, Nigeria
b Department of Medicine, Ekiti State University, Ado-Ekiti, Nigeria
c Department of Clinical Science, Ekiti State University Teaching Hospital, Ado-Ekiti, Nigeria

ARTICLE INFO

Article history:
Received 21 April 2016
Accepted 14 June 2016
Available online xxx

Keywords:
cadmium
curcumin
L-arginine
renal adenosine
renal biomarkers

ABSTRACT

In this study, the effect of enzymes involved in degradation of renal adenosine and L-arginine was investigated in rats exposed to cadmium (Cd) and treated with curcumin, the principal active phytochemical in turmeric rhizome. Animals were divided into six groups (n = 6): saline/vehicle, saline/curcumin 12.5 mg/kg, saline/curcumin 25 mg/kg, Cd/vehicle, Cd/curcumin 12.5 mg/kg, and Cd/curcumin 25 mg/kg. The results of this study revealed that the activities of renal adenosine deaminase and arginase were significantly increased in Cd-treated rats when compared with the control (p < 0.05). However, co-treatment with curcumin inhibits the activities of these enzymes compared with Cd-treated rats. Furthermore, Cd intoxication increased the levels of some renal biomarkers (serum urea, creatinine, and electrolytes) and malondialdehyde level with a concomitant decrease in functional sulfhydryl group and nitric oxide (NO). However, co-treatment with curcumin at 12.5 mg/kg and 25 mg/kg, respectively, increases the nonenzymatic antioxidant status and NO in the kidney, with a concomitant decrease in the levels of malondialdehyde and renal biomarkers. Therefore, our results reinforce the importance of adenosine deaminase and arginase activities in Cd poisoning conditions and suggest some possible mechanisms of action by which curcumin prevent Cd-induced renal toxicity in rats.

1. Introduction

Cadmium (Cd) is a toxic heavy metal with a biological half-life of more than 20 years; its level in the environment is increasing due to industrial activities, thereby increasing human exposure to Cd [1,2]. It has been reported to bio-accumulate in many organs, including the liver, kidney, pancreas, and testis, and adversely affect the functions of
these organs [3]. Among the various organs, the kidney is recognized as a major target of cadmium-induced renal toxicity due to its preferential uptake by receptor-mediated endocytosis and metallothionein-bound Cd in the renal proximal tubule [3]. When released freely into the cytosol, it can generate reactive oxygen species (ROS) and activate cell death pathways [3].

Epidemiological and experimental evidence suggested that acute Cd exposure induces oxidative stress through the inhibition of antioxidant enzymes, increased level of lipid peroxidation, and depletion of sulfhydryl (SH)-group-containing compounds [2,4]. However, the toxic effects of Cd are rather complex and still debated [5]. This has been the focus of much research, but there are more factors yet to be identified and explored.

Previous studies have highlighted the relationship of oxidative stress and nitric oxide (NO) production in kidney function under normal and pathological conditions [6–8]. Free radicals such as superoxide radical (O2•−) can interact with NO forming peroxynitrite (ONOO−), thereby depleting NO bioavailability.

NO is a potent, endogenous vasodilator that regulates renal function, among other functions [7]. It is produced from l-arginine by endothelial nitric oxide synthase (eNOS); however, arginase competes with this enzyme for the same substrate to l-ornithine by endothelial nitric oxide synthase (eNOS); however, function, among other functions [7]. It is produced from L-arginine metal toxicity in these organs [3]. Among the various organs, the kidney is recognized as a major target of cadmium-induced renal toxicity due to its preferential uptake by receptor-mediated endocytosis and metallothionein-bound Cd in the renal proximal tubule [3]. When released freely into the cytosol, it can generate reactive oxygen species (ROS) and activate cell death pathways [3].

Epidemiological and experimental evidence suggested that acute Cd exposure induces oxidative stress through the inhibition of antioxidant enzymes, increased level of lipid peroxidation, and depletion of sulfhydryl (SH)-group-containing compounds [2,4]. However, the toxic effects of Cd are rather complex and still debated [5]. This has been the focus of much research, but there are more factors yet to be identified and explored.

Previous studies have highlighted the relationship of oxidative stress and nitric oxide (NO) production in kidney function under normal and pathological conditions [6–8]. Free radicals such as superoxide radical (O2•−) can interact with NO forming peroxynitrite (ONOO−), thereby depleting NO bioavailability.

NO is a potent, endogenous vasodilator that regulates renal function, among other functions [7]. It is produced from l-arginine by endothelial nitric oxide synthase (eNOS); however, arginase competes with this enzyme for the same substrate to produce urea and l-ornithine [9]. In the kidney, increased arginase activity can thus reduce availability of l-arginine for eNOS, causing a decrease in NO production and a rise in superoxide generation due to uncoupling of eNOS [9].

Studies have also implicated the endogenous signaling molecule adenosine in kidney function. Adenosine is produced by enzymatic phosphohydrolysis of its precursor molecules, particularly Adenosine triphosphate (ATP) and Adenosine monophosphate (AMP) [10–12]. However, adenosine deaminase (ADA), an enzyme, that is present in the kidney, catalyzes the irreversible hydrolytic deamination of adenosine to inosine and 2-deoxyadenosine to 2-deoxyinosine, thereby depleting the level of adenosine production.

Therefore, inhibition of ADA activity has been suggested to be a good therapeutic approach for the management/prevention of kidney dysfunction.

Curcumin is the principal natural polyphenol curcuminoid of turmeric (Curcuma longa) rhizome, a member of the ginger family (Zingiberaceae) [13]. Curcumin has a wide spectrum of therapeutic properties, and it has been shown to possess antioxidant, anti-inflammatory, anticancer, antiangiogenesis, chemopreventive, and chemotherapeutic properties [14–18]. Studies on the effect of curcumin on enzymes involved in degradation of renal adenosine and l-arginine metal toxicity in animal models are scarce. Therefore, the present study highlighted other significant aspects that underlie Cd-curcumin exposure. Hence, we investigated the effect of curcumin on arginase and ADA activities in Cd-induced renal toxicity in rats.

2. Materials and methods

2.1. Chemicals

Cadmium sulfate was obtained from Oxford Laboratory, Mumbai, India, and curcumin was purchased from Sigma-Aldrich, St Louis, MO, USA. All other reagents used in this study were of analytical grade, and water was glass distilled.

2.2. Animals and experimental design

Adult male albino rats (weighing 150–180 g) were obtained from the animal breeding unit at Afe Babalola University, Ado-Ekiti, Nigeria, and were housed in cages, at room temperature (25–28°C), relative humidity 60–70%, and 12-hour light/dark cycle. Food (pellet rat chow) and water were available ad libitum. Animals were cared according to US National Institute of Health ethical guidelines.

After 2 weeks of acclimatization, animals were randomly divided into six groups of six animals each: saline/vehicle, saline/curcumin 12.5 mg/kg, saline/curcumin 25 mg/kg, Cd/vehicle, Cd/curcumin 12.5 mg/kg, and Cd/curcumin 25 mg/kg. In the present study, Cd sulfate was administered orally to rats as described by Zalups and Ahmad [19], and the choice of Cd dosage was according to the study of Goncalves et al [20], where it induced renal damage, while the choice of the curcumin doses (12.5 mg/kg and 25 mg/kg) was made based on previous works that reported beneficial results of this compound in rats [21]. Both solutions were administered for a period of 7 days. Curcumin was administered 30 minutes after Cd, and the solutions were freshly prepared. Cd was diluted in saline and the curcumin in 0.1% ethanol, and both were administered (1 mL/kg).

It is important to note that controls for all ex vivo tests were performed to correct vehicle (0.1% ethanol) interference. However, no significant differences were observed between the results obtained for the vehicle (0.1% ethanol) and the control (saline) regarding the parameters analyzed in this study (data not shown).

After the treatment period, animals were fasted overnight and sacrificed 24 hours after the last dose under light ether anesthesia. Blood samples were obtained by heart puncture and centrifuged at 3000g for 10 minutes. The clear non-hemolyzed sera were stored at −20°C till subsequent measurements. The kidneys were quickly excised and washed in cold saline solution, blotted on filter papers to remove adhering blood, and homogenized in 100 mM potassium phosphate, pH 7.5. The homogenates were centrifuged at 10,000g for 20 minutes at 4°C, and the supernatant was used for subsequent enzymatic assays.

2.3. Determination of ADA activity

ADA activity determination was performed as described by Guisti and Galanti [22], which is based on the direct measurement of the formation of ammonia, produced when ADA acts in an excess of adenosine. In brief, 50 μL of kidney homogenates reacted with 21 mmol/L of adenosine, pH 6.5, and was incubated at 37°C for 60 minutes. The protein content used for the experiment was adjusted to between 0.7 mg/mL and 0.9 mg/mL. The results obtained were expressed in units per liter (U/L). One unit (1 U) of ADA is defined as the amount of enzyme required to release 1 mmol of ammonia per minute from adenosine at standard assay conditions.
2.4. **Determination of arginase activity assay**

The arginase activity of kidney homogenates was assayed as described by Romero et al [23]. Briefly, tissue lysate (50 μL) was added into 75 μL of Tris–HCl (50 mmol/L, pH 7.5) containing 10 mmol/L MnCl₂. Heating the lysate at 55–60°C for 10 minutes activated arginase. The hydrolysis reaction of L-arginine by arginase was performed by incubating the mixture containing activated arginase with 50 μL of L-arginine (0.5 mol/L, pH 9.7) at 37°C for 1 hour and was stopped by adding 400 μL of the acid solution mixture (H₂SO₄, H₃PO₄, H₂O = 1:3:7).

For calorimetric determination of urea, N-iso-nitrosopropiophenone (25 μL, 9% in absolute ethanol) was then added and the mixture was heated at 100°C for 45 minutes. After placing the sample in the dark for 10 minutes at room temperature, the urea concentration was determined spectrophotometrically, with the absorbance at 550 nm being used with a microplate reader. The amount of urea was determined by the method of Ellman[25]. Briefly, the reaction mixture consisted of 40 μL of 2% vanadium chloride (VCl₃) in 5% HCl, 200 μL of 0.1% N-(1-naphthyl)ethylene-diaminedi hydrochloride, and 200 μL of 2% sulfanilamide (in 5% HCl). After incubating at 37°C for 60 minutes, nitrite levels, which correspond to an estimative of levels of NO, were determined spectrophotometrically at 540 nm, based on the reduction of nitrate to nitrite by VCl₃ [24]. Kidney nitrite and nitrate levels were expressed as nanomoles of NO per milligram of protein.

2.5. **Measurement of NO**

NO content in kidney homogenates was estimated in a medium containing 400 μL of 2% vanadium chloride (VCl₃) in 5% HCl, 200 μL of 0.1% N-(1-naphthyl)ethylene-diaminedihydrochloride, and 200 μL of 2% sulfanilamide (in 5% HCl). After incubating at 37°C for 60 minutes, nitrite levels, which correspond to an estimative of levels of NO, were determined spectrophotometrically at 540 nm, based on the reduction of nitrate to nitrite by VCl₃ [24]. Kidney nitrite and nitrate levels were expressed as nanomoles of NO per milligram of protein.

2.6. **Determination of total thiol content**

Total thiol content was determined according to the method previously described by Ellman [25]. Briefly, the reaction mixture consisted of 40 μL of kidney homogenates, 10 μL of 10mM 5,5’-dithio-bis-2-nitrobenzoic acid (DTNB), and 0.1M potassium phosphate buffer (pH 7.4) in a final volume of 200 μL. The mixture was incubated for 30 minutes at an ambient temperature and then the absorbance was read at 412 nm using a SpectraMax plate reader (Molecular Devices). A standard curve was plotted for each measurement using cysteine as a standard, and the results were expressed as μmol/mg protein.

2.7. **Determination of nonprotein thiol content**

Nonprotein thiol levels were determined by the method of Ellman [25]. Briefly, an aliquot of kidney homogenates was mixed (1:1) with 10% trichloroacetic acid. Subsequent to precipitation of protein, the resulting solution was centrifuged at 10,000g for 5 minutes at 4°C, and the free SH groups were determined in the supernatant. The reaction mixture consisting of 50 μL of sample, 450 μL phosphate buffer, and 1.5 mL of 0.1mM of 5,5’-dithiobis 2-nitrobenzoic acid was incubated for 10 minutes at 37°C. The absorbance was measured at 412 nm using a SpectraMax plate reader (Molecular Devices). Nonprotein thiol levels were expressed as μmol/mg of protein.

2.8. **Lipid peroxidation**

Lipid peroxidation was determined as the formation of thio barbituric acid reactive substances during an acid-heating reaction, according to Okahawa et al [26]. Briefly, the reaction mixture consisting of 200 μL of kidney homogenates or standard [0.03mM malondialdehyde (MDA)], 200 μL of 8.1% sodium dodecyl sulfate, 500 μL of 0.8% thiobarbituric acid, and 500 μL of acetic acid solution (2.5M HCl, pH 3.4) was heated at 95°C for 1 hour. The absorbance was measured at 532 nm using a SpectraMax plate reader (Molecular Devices). Tissue levels of thio barbituric acid reactive substances were expressed as μmol MDA/mg of protein.

2.9. **Determination of renal function markers**

The levels of serum urea and creatinine were measured according to supplier's directions from commercial kits (Randox Laboratories Ltd, Crumlin, County Antrim, UK). The concentrations of serum cation and anion electrolytes (Na⁺, K⁺, Cl⁻, and HCO₃⁻) were determined by an automated selective-ion electrolyte analyzer.

2.10. **Estimation of cadmium residues**

For Cd²⁺ analysis, kidney tissue samples were digested according to the procedure described by Babalola et al [27]. Cd²⁺ concentration in the digested tissues was measured using an atomic absorption spectrophotometer (PerkinElmer 2380). Analytical blanks were run in the same way as the samples, and standard solutions for the calibration curve were prepared in the same acid matrix. Cd²⁺ concentration in kidney tissue was expressed as μg/g dry weight tissue.

2.11. **Protein content**

Protein was measured using the Coomassie blue method according to Bradford [28] using serum albumin as the standard.

2.12. **Statistical analysis**

All data were expressed as mean ± the standard error of the mean. The statistical analyses used were one- and two-way ANOVA, followed by Duncan’s multiple range tests; p < 0.05 was considered to represent a significant difference in both analyses used.

3. **Results**

3.1. **Effect of curcumin on kidney ADA and arginase activities, and NO level in Cd-induced renal oxidative damage**

ADA and arginase activities in kidney tissues of the experimental animals are shown in Figures 1 and 2. The results revealed that oral Cd administration significantly altered the activities of ADA and arginase enzymes compared with the control rats (p < 0.05). However, co-treatment with curcumin (12.5 mg/kg and 25 mg/kg) prevented these alterations by...
inhibiting the activities of ADA and arginase enzymes, compared with the Cd-treated rats. In addition, our results demonstrated that the NO level was significantly decreased in Cd-treated rats when compared with the control \((p < 0.05)\) (Figure 3). Co-treatment with curcumin at 12.5 mg/kg and 25 mg/kg resulted in an increase of renal NO level when compared with Cd-treated rats.

### 3.2. Effect of curcumin on Cd-induced changes in renal function indices

Table 1 depicts the effect of Cd intoxication on serum urea and creatinine levels as well as electrolyte concentration. Exposure to Cd caused a significant increase \((p < 0.05)\) in serum urea and creatinine levels when compared with the control (Table 1). In addition, the concentrations of serum cation and anion electrolytes \((\text{Na}^+, \text{K}^+, \text{Cl}^-, \text{and HCO}_3^-)\) were altered in Cd-treated rats when compared with the control. However, co-treatment with curcumin (12.5 mg/kg and 25 mg/kg) significantly \((p < 0.05)\) reduced the elevated levels of serum urea and creatinine, as well as prevented alterations in serum electrolyte concentration by reducing \(\text{Na}^+, \text{K}^+, \text{Cl}^-, \text{and HCO}_3^-\) concentrations (Table 1).

### 3.3. Effect of curcumin on Cd-induced changes in renal oxidative stress biomarkers

As presented in Figure 4, the renal MDA levels in Cd-treated rats was significantly \((p < 0.05)\) elevated when compared with the control. Cd = group receiving vehicle + 2.5 mg/kg cadmium; Cd/Cur 12.5 = group receiving 12.5 mg/kg curcumin + 2.5 mg/kg cadmium; Cd/Cur 25 = group receiving 25 mg/kg curcumin + 2.5 mg/kg cadmium; Control = group receiving saline/vehicle; Cur 12.5 = group receiving 12.5 mg/kg curcumin only; Cur 25 = group receiving 25 mg/kg curcumin only; SEM = standard error of the mean.

---

**Please cite this article in press as:** Akinyemi AJ, et al., *Curcumin inhibits adenosine deaminase and arginase activities in cadmium-induced renal toxicity in rat kidney*, *Journal of Food and Drug Analysis* (2016), http://dx.doi.org/10.1016/j.jfda.2016.06.004
with the control, indicating that the treatment of Cd caused obvious oxidative damage to rats. However, we found that the increase was diminished by co-treatment with curcumin (12.5 mg/kg and 25 mg/kg). In addition, oral administration of Cd caused a significant decrease in total and nonprotein thiols (-SH) when compared with the control (p < 0.05), an indicator of depletion of nonenzymatic antioxidant status (Figure 5A and B). However, co-treatment with curcumin at 12.5 mg/kg and 25 mg/kg body weight, respectively, was able to cause a significant increase in total and nonprotein thiols (-SH) levels when compared with the control (p < 0.05) (Figure 5A and B).

Table 1 – Effect of curcumin on renal function biomarkers and serum electrolytes in Cd-induced renal oxidative damage.

| Group                  | Urea (mg/dL) | Creatinine (mg/dL) | Na⁺ (mmol/L) | K⁺ (mmol/L) | Cl⁻ (mmol/L) |
|------------------------|--------------|--------------------|--------------|-------------|--------------|
| Control                | 63.1 ± 6.1   | 2.5 ± 0.59         | 126.4 ± 4.1  | 3.9 ± 0.1   | 78.5 ± 1.2   |
| Cur 12.5               | 66.1 ± 9.1   | 2.2 ± 0.41         | 122.5 ± 3.9  | 3.7 ± 0.1   | 79.7 ± 1.3   |
| Cur 25                 | 68.6 ± 7.1   | 2.5 ± 0.51         | 120.9 ± 2.9  | 3.9 ± 0.3   | 78.7 ± 2.1   |
| Cd                     | 91.2 ± 4.1   | 4.5 ± 0.11         | 140.5 ± 1.5  | 5.4 ± 0.2   | 90.6 ± 1.3   |
| Cd/Cur 12.5            | 70.1 ± 8.1   | 2.5 ± 0.40         | 124.1 ± 3.6  | 4.1 ± 0.4   | 80.5 ± 2.1   |
| Cd/Cur 25              | 69.1 ± 6.9   | 1.9 ± 0.51         | 121.5 ± 3.2  | 3.6 ± 0.1   | 79.2 ± 2.4   |

Data are presented as the mean ± SEM (n = 5). Values with different letters along the same column are significantly different (p < 0.05) from each other.

Cd = group receiving vehicle + 2.5 mg/kg cadmium; Cd/Cur 12.5 = group receiving 12.5 mg/kg curcumin + 2.5 mg/kg cadmium; Cd/Cur 25 = group receiving 25 mg/kg curcumin + 2.5 mg/kg cadmium; Control = group receiving saline/vehicle; Cur 12.5 = group receiving 12.5 mg/kg curcumin only; Cur 25 = group receiving 25 mg/kg curcumin only.
3.4. Effect of curcumin on concentration of Cd in the kidney of Cd-treated rats

Figure 6 demonstrates accumulation of cadmium in the kidney of Cd-treated animals. Oral administration of cadmium (2.5 mg/kg) for 7 days caused a significant increase in the accumulation of Cd in the kidney of Cd-treated rats when compared with the control (p < 0.05). This accumulation was influenced by curcumin treatment resulting in a decrease Cd level in the kidney when compared with Cd-treated rats (Figure 6).

4. Discussion

At present, therapeutic modalities to prevent or treat acute renal injury related to metal poisoning are extremely limited, and the search for novel therapeutic interventions is an area of intense investigation. Recent studies implicate the endogenous signaling molecule adenosine in kidney function/protection. As such, enzymatic production of adenosine from its precursor molecules ATP and AMP, and regulation of its level by ADA play a critical role in attenuating renal damage and preserving kidney function during episodes of renal metal poisoning [11,12].

The result of the present study demonstrated that ADA activity was significantly increased in the kidney of Cd-treated rats when compared with the control (p < 0.05) (Figure 1). Previous studies have demonstrated upregulation in the ADA activity in renal injury [29–32]. The increase in ADA activity found in this study could result in a decrease in the level of adenosine, a renoprotector molecule [11,12]. Extracellular adenosine primarily functions as a signaling molecule and can signal through four adenosine receptors (ARs): A1AR, A2AAR, A2BAR, and A3AR [33]. Several studies have implicated the levels of adenosine in hypoxia, inflammation, or acute renal injury [11,34,35].

It is interesting to note that co-treatment with curcumin was able to prevent an increase in ADA activity in Cd-intoxicated rats (Figure 1). This suggests that curcumin has a protective role against Cd poisoning, and the probable mechanism could be due to their inhibitory effect on renal ADA activity, thereby resulting in an increase in the level of adenosine. Previous studies have implicated curcumin and adenosine levels via modulation of the purinergic system in various pathological conditions such as Alzheimer's disease, diabetes, stroke, hypertension, and inflammation [21,36].

Arginase enzyme has been implicated to play a significant role in kidney function. This study shows that pharmacological inhibition of renal arginase activity (Figure 2) in curcumin-treated rats mediates renal tissue protection, as proved by a reduction in serum urea, creatinine, and electrolyte levels (Table 1) during Cd poisoning. These findings further reveal an important role of arginase activity in the pathogenesis of renal injury and provide evidence for arginase inhibition as a potential therapeutic modality for treating metal poisoning.

An elevated arginase activity has been linked to several vascular problems including hypertension, atherosclerosis, and end-stage kidney damage [37–39]. Previously, we showed activation of arginase as a key mediator of kidney injury (Figure 2). Enhanced arginase activity can impair endothelium-dependent vasorelaxation by decreasing L-arginine availability to eNOS, thereby reducing NO production and uncoupling eNOS function [9]. We observed a significant decrease in the level of NO in Cd-induced renal damage (Figure 3). The result is in agreement with Figure 2, where we observed an increase in arginase activity that can deplete NO production. However, treatment with curcumin, the principal active ingredient in turmeric, restores the level of NO in Cd-intoxicated rats. This increase in NO could be a result of the fact that curcumin exhibited an inhibitory effect on arginase activity [40]. There is increasing evidence that NO, a potent vasodilator, is one of the most important paracrine modulators and mediators in the control of renal functions, such as overall and regional renal blood flow, renal autoregulation, glomerular filtration, renin secretion, and salt excretion [41,42]. NO also plays an important role in the pathogenesis of several renal disease states, such as diabetic nephropathy, inflammatory glomerular disease, acute renal failure, and nephrotoxicity of drugs/metal, conveying both beneficial effects via its hemodynamic functions [41,42].

Oxidative stress has recently been reported as one of the important mechanisms of toxic effect of Cd [43]. The mechanism of Cd–induced oxidative stress involves an imbalance between generation and removal of ROS in tissues and cellular components, causing damage to membranes, DNA, and proteins [43]. ROS generated by Cd initiate lipid peroxidation of the membrane-bound polysaturated fatty acids, leading to impairment of the membrane structural and functional integrity, which is the result of an interaction...
between free radicals of diverse origins and unsaturated fatty acids that are typical in membrane lipids. Degradation of polyunsaturated fatty acids in cell membranes by ROS, induced by Cd, results in the destruction of membranes and the formation of thiobarbituric acid reactive species, MDA, or conjugated dienes as indicators of lipid peroxidation [44]. In the present study, the level of MDA was measured as an indicator of lipid peroxidation (Figure 4). The level of MDA was significantly increased in the kidney tissue of Cd-intoxicated rats. Our experimental findings suggest that oxidative stress plays an important role in cadmium-induced renal injuries. Our result is in accordance with other findings reporting Cd-induced oxidative damage [43,45,46]. The increased MDA levels in renal tissue is an indication of overaccumulation of lipid peroxides in tissue, causing overconsumption and depletion of functional thiol (-SH) groups in several antioxidant enzymes, as observed in Figure 5.

Reduced glutathione (GSH) or non-protein thiol is a multifunctional intracellular nonenzymatic antioxidant, which is considered to be the major thiol—disulfide redox buffer of the cell. It serves many vital physiological functions, including protection of cells from ROS, detoxification of exogenous compounds, and amino acid transport with the help of the SH group present in them, which is essential for its antioxidant activity against some forms of ROS in the cells [47]. Decreased GSH and total thiol levels, as observed in the Cd-treated rats (Figure 5), are due to enhanced utilization of these antioxidants for scavenging free radicals. GSH forms complexes with Cd through the free SH group, while ROS generated by Cd deplete the intracellular SH groups as well as disrupt intracellular organelles, thereby altering Cd distribution and excretion [48]. However, treatment with curcumin enhances the level of GSH and effectively provides thiol groups for the possible GSH-mediated detoxification reactions. The antioxidant mechanism of curcumin has been attributed to its conjugated structure, which includes two methoxylated phenols and an enol form of β-diketone [49]. The structure showed a typical radical-trapping ability as a chain-breaking antioxidant [49]. Furthermore, it has been reported that curcumin is a bifunctional antioxidant, because of its ability to react directly with reactive species and to induce upregulation of various cytoprotective and antioxidant proteins via activation of the Keap1/Nrf2/ARE pathway [50,51]. Curcumin is able to inhibit generations of superoxide anion (O2−) and hydroxyl radical (·OH) through prevention of the oxidation of Fe2+ in Fenton’s reaction [52].

The kidney is considered as one of the major target organs affected by Cd2+ toxicity. Cd can accumulate in the kidneys and cause severe tissue damage in these organs, as observed from the results reported in this study. It is noted that the Cd content in the whole kidney of the group that received Cd was significantly increased (p < 0.05) when compared with the control. Numerous studies have unraveled the exact pathways by which cadmium enters the renal epithelial cells and the possible mechanism by which it causes toxicity in the kidney [3,45,46]. Interestingly, this study has found that the Cd contents in the kidney of Cd-intoxicated groups were decreased in rats receiving curcumin (Figure 6). Based on the electrochemical studies, it has been suggested that there might be a metal–ligand interaction between Cd and curcumin, thereby reducing the heavy metal load in the body and the toxic effects of Cd [53]. Moreover, it is possible that curcumin might interfere with the gastrointestinal absorption of Cd, thereby causing a reduction in Cd concentration in the tissues. This suggests the potential chelating effect of curcumin, as reported in some recent studies [51,54,55].

5. Conclusion

In conclusion, curcumin attenuated Cd-induced renal oxidative damage by inhibiting ADA and arginase activities, as well as increasing endothelial NO and functional SH groups in the kidney. In addition, curcumin inhibits the accumulation of Cd in the kidney probably due to its chelating ability. Therefore, these activities further suggest some possible mechanisms of action for their renoprotective potential.

Conflicts of interest

The authors report no conflicts of interest related to this study.

Compliance with ethical standards

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

REFERENCES

[1] Johri N, Jacquillet G, Unwin R. Heavy metal poisoning: the effects of cadmium on the kidney. BioMetals 2010;23:783–92.
[2] El-Habit O, Abdel Moneim AE. Testing the genotoxicity, cytotoxicity, and oxidative stress of cadmium and nickel and their additive effect in male mice. Biol Trace Elem Res 2014;159:364–72.
[3] Othman MS, Nada A, Zaki HS, Abdel Moneim AE. Effect of Physalis peruviana L. on cadmium-induced testicular toxicity in rats. Biol Trace Elem Res 2014;159:278–87.
[4] Rikans LE, Yamano T. Mechanisms of cadmium mediated acute hepatotoxicity. J Biochem Mol Toxicol 2000;14:110–7.
[5] Lopez E, Figueroa S, Oset-Gasque MJ, Gonzalez MP. Apoptosis and necrosis: two distinct events induced by cadmium in cortical neurons in culture. Br J Pharmacol 2003;138:901–11.
[6] Modlinger PS, Wilcox CS, Aslam S. Nitric oxide, oxidative stress, and progression of chronic renal failure. Semin Nephrol 2004;24:354–65.
[7] Araujo M, Welch WJ. Oxidative stress and nitric oxide in kidney function. Curr Opin Nephrol Hypertens 2006;15:72–7.
[8] Tsuchiya K, Tomita S, Ishizawa K, Abe S, Ikeda Y, Kihiara Y, Tamaki T. Dietary nitrite ameliorates renal injury in L-NNAME-induced hypertensive rats. Nitric Oxide 2010;22:98–103.
[9] Kim JH, Bugui LJ, Oh YJ, Bivalacqua TJ, Ryoo S, Soucy KG, Santhanam L, Webb A, Camara A, Sikka G, Nyhan D, Shoukas AA, Ilies M, Christianson DW, Champion HC, Berkowitz DE. Arginase inhibition restores NOS coupling and reverses endothelial dysfunction and vascular stiffness in old rats. J Appl Physiol 2009;107:1249–57.
Babalola OO, Okonji RE, Atoyebi JO, Sennuga TF, Raimi MM, Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in hepatic fibrogenesis. J Biol Chem 2012;287:36341–55.

Anamika B. Extraction of curcumin. J Environ Sci Toxicol 2012;1:1–16.

Strimpakos AS, Sharma RA. Curcumin: preventive and therapeutic properties in laboratory studies and clinical trials. Antioxid Redox Signal 2008;10:511–45.

Venkatesan N, Punithavathi D, Arumugam V. Curcumin prevents adriamycin nephrotoxicity in rats. Br J Pharmacol 2000;129:231–4.

Kuhad A, Pilkhwals S, Sharma S, Turkey N, Chopra K. Effect of curcumin on inflammation and oxidative stress in cisplatin induced experimental nephropathy. J Agric Food Chem 2007;55:10150–5.

Nabavi SF, Moghadam AH, Esami S, Nabavi SM. Protective effects of curcumin against sodium fluoride-induced toxicity in rat kidneys. Biol Trace Elem Res 2012;145:369–74.

Trujillo J, Chirino YI, Molina-Jijón E, Andélica-Romero AC, Tapia E, Pedraza-Chaverri J. Renoprotective effect of the antioxidant curcumin: recent findings. Redox Biol 2013;1:448–56.

Zalups RK, Ahmad S. Molecular handling of cadmium in transporting epithelia. Toxicol Appl Pharmacol 2003;186:163–88.

Goncalves JF, Duarte MMMF, Fiorenza AM, Spanevello RM, Mazzanti CM, Schetinger MRC, Morsch VM. Apyrase and 5′-nucleotidase activities in rat synaptosomes and platelets. Int J Dev Neurosci 2007;25:1581–90.

Akinyemi AJ, Thome GR, Morsch VM, Stefanello N, da Costa P, Cardoso A, Goulart JF, Bello-Klein A, Akindahunsi AA, Oboh G, Schetinger MR. Effect of dietary supplementation of ginger and turmeric rhizomes on ectonucleotidases, adenosine deaminase and acetylcholinesterase activities in synaptosomes from the cerebral cortex of hypertensive rats. J Med Food 2015;18:792–803.

Zhang C, Hein TW, Wang W, Miller MW, Fossum TW, McDonald MM, Humphrey JD, Kuo L. Upregulation of vascular arginine in hypertension decreases nitric oxide-mediated dilation of coronary arterioles. Hypertension 2004;44:935–43.

Ming XF, Barandier C, Viswambharan H, Kwak BR, Mach F, Mazzoli L, Hayoz D, Ruffieux J, Rusconi S, Montani JP, Yang Z. Thrombin stimulates human endothelial arginine enzymatic activity via Rhoph/KoC pathway: implications for atherosclerotic endothelial dysfunction. Circulation 2004;110:3708–14.

Cowan AW, Mori T, Mattson D, Zou AP. Role of renal NO production in the regulation of medullary blood flow. Am J Physiol Regul Integr Comp Physiol 2003;284:R370–8.
[42] Ghasemi M, Nematbakhsh M, Daneshmand F, Moeini M, Talebi A. Role of nitric oxide in kidney and liver (as distance organ) function in bilateral renal ischemia-reperfusion: effect of L-arginine and NG-nitro-L-arginine methyl ester. Adv Biomed Res 2015;4:233.

[43] Yang H, Shu Y. Cadmium transporters in the kidney and cadmium-induced nephrotoxicity. Int J Mol Sci 2015;16:1484–94.

[44] Esrefoglu M, Gul M, Ates B, Yilmaz I. Ultrastructural clues for the protective effect of ascorbic acid and N acetylcysteine against oxidative damage on caerulein induced pancreatitis. Pancreatology 2006;6:477–85.

[45] Sompamit K, Kukongviriyapan U, Donpunha W, Nakmareong S, Kukongviriyapan V. Reversal of cadmium-induced vascular dysfunction and oxidative stress by meso-2,3-dimercaptosuccinic acid in mice. Toxicol Lett 2010;198:77–82.

[46] El-Boshy ME, Risha EF, Abdelhamid FM, Mubarak MS, Hadda TB. Protective effects of selenium against cadmium induced haematological disturbances, immunosuppressive, oxidative stress and hepatorenal damage in rats. J Trace Elem Med Biol 2015;29:304–10.

[47] Cnubben NH, Rietjens IM, Wortelboer H, van Zanden J, van Bladeren PJ. The interplay of glutathione-related processes in antioxidant defense. Environ Toxicol Pharmacol 2001;10:141–52.

[48] Ranas V, Verma S. Protective effects of GSH, vitamin E and selenium on lipid peroxidation in cadmium fed rats. Biol Trace Elem Res 1996;51:161–8.

[49] Masuda T, Maekawa T, Hidaka K, Bando H, Takeda Y, Yamaguchi H. Chemical studies on antioxidant mechanism of curcumin: analysis of oxidative coupling products from curcumin and linooleate. J Agric Food Chem 2001;49:2539–47.

[50] Gonzalez-Reyes S, Guzmán-Beltrán S, Medina-Campos ON, Pedraza-Chaverri J. Curcumin pretreatment induces Nrf2 and an antioxidant response and prevents hemin-induced toxicity in primary cultures of cerebellar granule neurons of rats. Oxid Med Cell Longev 2013;2013:801418.

[51] García-Niño WR, Pedraza-Chaverri J. Protective effect of curcumin against heavy metals-induced liver damage. Food Chem Toxicol 2014;69:182–201.

[52] Reddy AC, Lokesh BR. Effect of dietary turmeric (Curcuma longa) on iron-induced lipid peroxidation in the rat liver. Food Chem Toxicol 1994;32:279–83.

[53] Eybl V, Kotyzova D, Koutensky J. Comparative study of natural antioxidants—curcumin, resveratrol and melatonin—in cadmium-induced oxidative damage in mice. Toxicology 2006;225:150–6.

[54] Jiao Y, Wilkinson J, Di X, Wang W, Hatcher H, Kock ND, D’Agostino D, Kovch MA, Torti FM, Torti SV. Curcumin, a cancer chemopreventive and chemotherapeutic agent, is a biologically active iron chelator. Blood 2009;113:462–9.

[55] Du XX, Xu HM, Jiang H, Song N, Wang J, Xie JX. Curcumin protects nigral dopaminergic neurons by iron-chelation in the 6-hydroxydopamine rat model of Parkinson’s disease. Neurosci Bull 2012;28:253–8.