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

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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.

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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 bioaccumulate in many organs, including the liver, kidney, pancreas, and testis, and adversely affect the functions of

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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 (O_2^-) 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 underline 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 100mM 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 μ L 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 μ L) was added into 75 μ L of Tris–HCl (50 mmol/L, pH 7.5) containing 10 mmol/L MnCl_2 . 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 μ L of L-arginine (0.5 mol/L, pH 9.7) at 37°C for 1 hour and was stopped by adding 400 μ L of the acid solution mixture ($\text{H}_2\text{SO}_4\text{:H}_3\text{PO}_4\text{:H}_2\text{O} = 1\text{:}3\text{:}7$).

For calorimetric determination of urea, α -is-nitrosopropiophenone (25 μ L, 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 measured with a microplate reader. The amount of urea produced, after normalization with protein, was used as an index for arginase activity.

2.5. Measurement of NO

NO content in kidney homogenates was estimated in a medium containing 400 μ L of 2% vanadium chloride (VCl_3) in 5% HCl, 200 μ L of 0.1% N-(1-naphthyl)ethylene-diaminedihydrochloride, and 200 μ L 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 VCl_3 [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 μ L of kidney homogenates, 10 μ L 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 μ L. 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, CA, USA). A standard curve was plotted for each measurement using cysteine as a standard, and the results were expressed as μ mol/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 μ L of sample, 450 μ L phosphate buffer, and 1.5 mL of 0.1mM of 5,5'-dithio-bis 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 μ mol/mg of protein.

2.8. Lipid peroxidation

Lipid peroxidation was determined as the formation of thiobarbituric acid reactive substances during an acid-heating reaction, according to Ohkawa et al [26]. Briefly, the reaction mixture consisting of 200 μ L of kidney homogenates or standard [0.03mM malondialdehyde (MDA)], 200 μ L of 8.1% sodium dodecyl sulfate, 500 μ L of 0.8% thiobarbituric acid, and 500 μ L 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 thiobarbituric acid reactive substances were expressed as μ mol 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 (RAN-DOX Laboratories Ltd, Crumlin, County Antrim, UK). The concentrations of serum cation and anion electrolytes (Na^+ , K^+ , Cl^- , and HCO_3^-) were determined by an automated selective-ion electrolyte analyzer.

2.10. Estimation of cadmium residues

For Cd^{2+} analysis, kidney tissue samples were digested according to the procedure described by Babalola et al [27]. Cd^{2+} 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. Cd^{2+} concentration in kidney tissue was expressed as μ g/g dry weight tissue.

2.11. Protein content

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

2.12. Statistical analysis

All data were expressed as mean $\pm$ 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

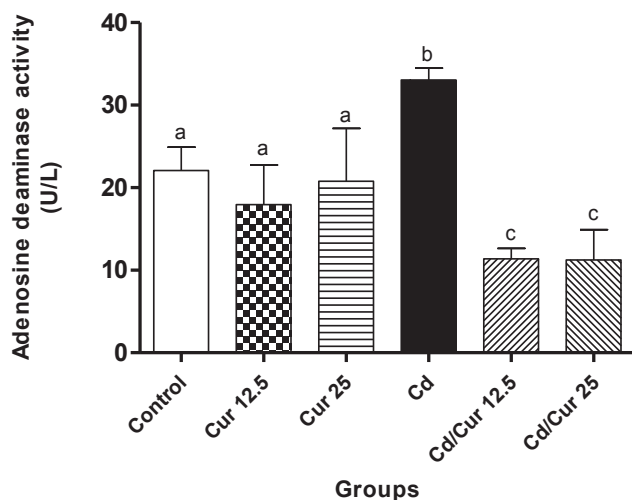


Figure 1 – Effect of curcumin on renal adenosine deaminase activity in Cd-induced renal oxidative damage. Data are presented as the mean $\pm$ SEM ($n = 5$). Bars with different letters are significantly ($p < 0.05$) different from each other. Cd = group receiving vehicle + 2.5 mg/kg cadmium; Cd/Cur 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; SEM = standard error of the mean.

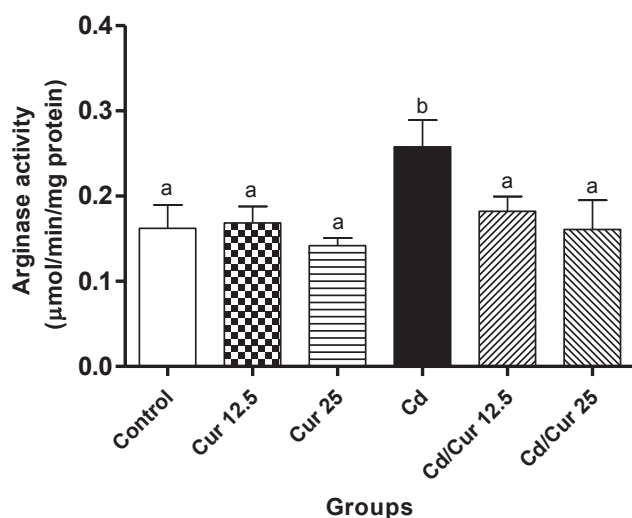


Figure 2 – Effect of curcumin on renal arginase activity in Cd-induced renal oxidative damage. Data are presented as the mean $\pm$ SEM ($n = 5$). Bars with different letters are significantly different ($p < 0.05$) from each other. Cd = group receiving vehicle + 2.5 mg/kg cadmium; Cd/Cur 12.5 = group 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; SEM = standard error of the mean.

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 (Na^+ , K^+ , Cl^- , 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 Na^+ , K^+ , Cl^- , 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

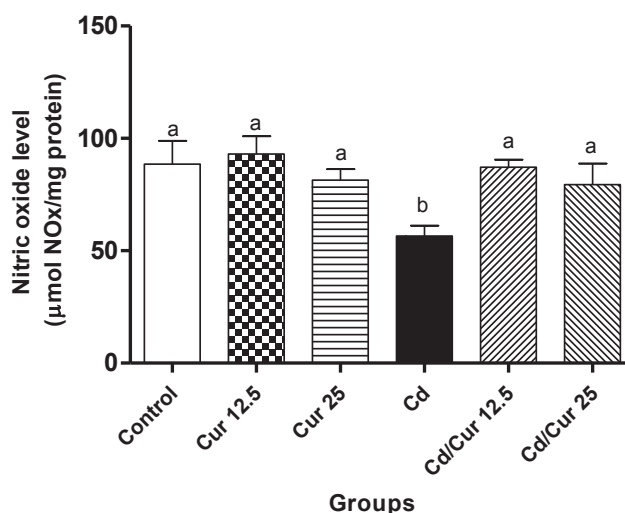


Figure 3 – Effect of curcumin on renal nitric oxide level in Cd-induced renal oxidative damage. Data are presented as the mean $\pm$ SEM ($n = 5$). Bars with different letters are significantly different ($p < 0.05$) from each other. Cd = group receiving vehicle + 2.5 mg/kg cadmium; Cd/Cur 12.5 = group 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; SEM = standard error of the mean.

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

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

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).

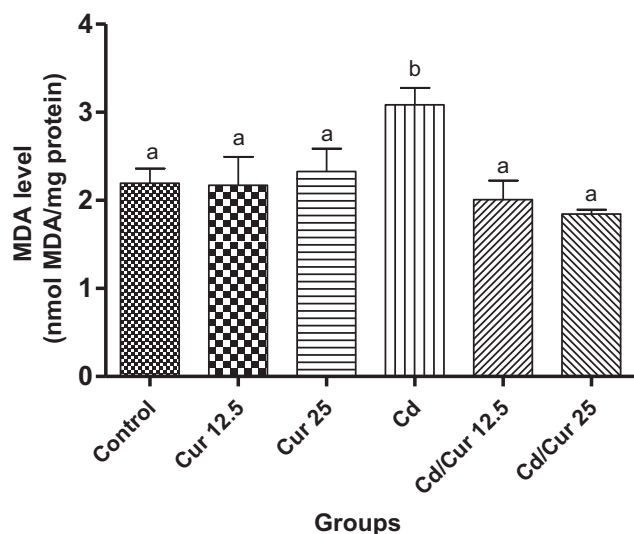


Figure 4 – Effect of curcumin on renal malondialdehyde (MDA) level in Cd-induced renal oxidative damage. Data are presented as the mean ± SEM (n = 5). Bars with different letters are significantly different ($p < 0.05$) from each other. Cd = group receiving vehicle + 2.5 mg/kg cadmium; Cd/Cur 12.5 = 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; SEM = standard error of the mean.

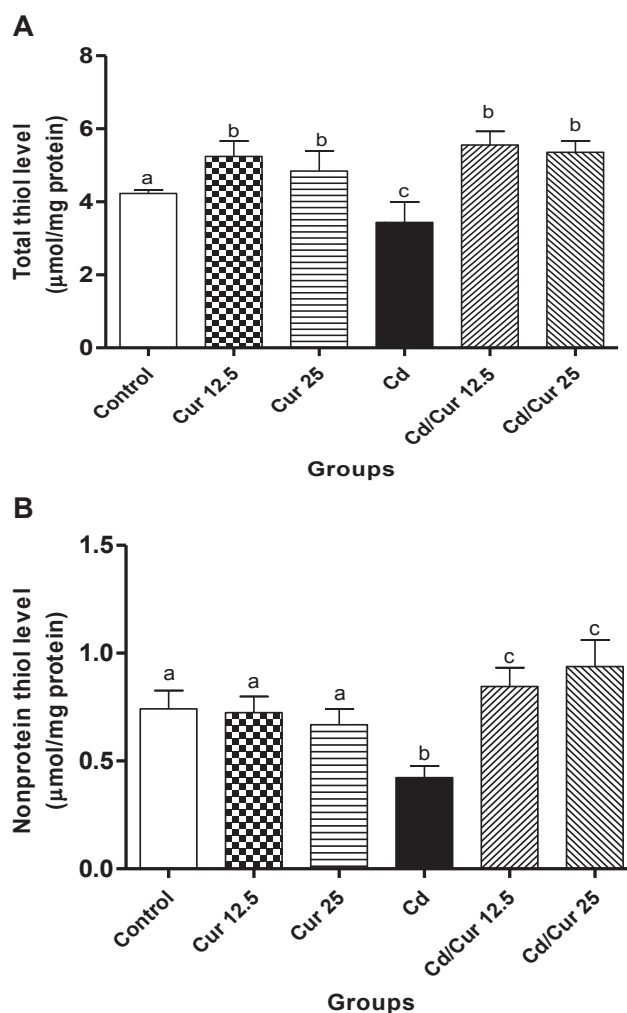


Figure 5 – Effect of curcumin on (A) renal total thiol and (B) nonprotein thiol levels in Cd-induced renal oxidative damage. Data are presented as the mean ± SEM (n = 5). Bars with different letters are significantly ($p < 0.05$) different from each other. Cd = group receiving vehicle + 2.5 mg/kg cadmium; 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; SEM = standard error of the mean.

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/metals, 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 Cd²⁺ [43]. The mechanism of Cd²⁺-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 polyunsaturated fatty acids, leading to impairment of the membrane structural and functional integrity, which is the result of an interaction

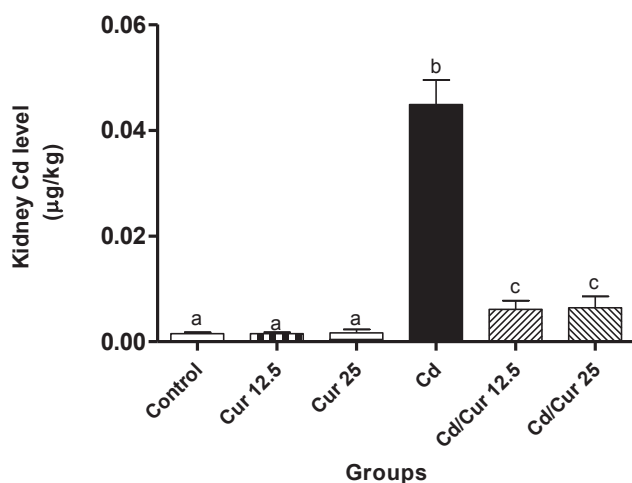


Figure 6 – Effect of curcumin on renal cadmium level in Cd-induced renal oxidative damage. Data are presented as the mean $\pm$ SEM ($n = 5$). Bars with different letters are significantly different ($p < 0.05$) from each other.

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; MDA = malondialdehyde; SEM = standard error of the mean.

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 multi-functional 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 ($O_2^{\cdot-}$) and hydroxyl radical ($\cdot OH$) through prevention of the oxidation of Fe^{2+} in Fenton's reaction [52].

The kidney is considered as one of the major target organs affected by Cd^{2+} 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.

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