Full Length Research Paper

Protective role of curcumin on cadmium-induced nephrotoxicity in rats

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Cadmium (Cd) is a well-known human carcinogen and a potent nephrotoxin. Curcumin, the yellow bioactive component of turmeric has established its antioxidant activities. The aim of this study was to investigate the protective role of curcumin against Cd induced nephrotoxicity. The rats were treated once daily by oral gavage for five days and divided into four groups of 8 rats each: control, Cd acetate 200 mg/kg BW, curcumin 250 mg/kg BW and pre-treatment with curcumin 250 mg/kg BW for one hour before administration with Cd acetate 200 mg/kg BW. After 24 h of the last treatment, we examined the level of lipid peroxidation (measured as malondialdehyde, MDA), reduced glutathione (GSH) and histological changes at the light microscopic level in renal tissues. The results showed that Cd treatment increased significantly renal lipid peroxidation ($p < 0.01$), which was associated with increased significantly reduced GSH levels ($p < 0.01$). In addition, the hydropic swelling and hypertrophy of proximal tubular cells in renal cortex was also observed by Cd treatment. The pretreatment with curcumin led to an improvement in both biochemical and histological alterations induced by Cd. A slight but not significant reduction of MDA content in renal tissue was observed in curcumin pretreated rats as compared with the Cd treated group. Interestingly, the reduced GSH levels was significantly reduced ($p < 0.01$) in curcumin pretreated rats when compared with those of Cd-treated group. In parallel, the administration of curcumin to Cd treated rats resulted in the improvement of proximal tubular cells. These results were indicated that Cd caused renal toxicity by inducing lipid peroxidation and morphological alterations. In conclusion, these results suggest that curcumin partially protect against Cd-induced nephrotoxicity. This study could be important for the further understanding of Cd toxicity in renal tissues and in the development of better treatments for people and/or animals exposed to the heavy metal.

Key words: Curcumin, cadmium, nephrotoxicity, histology of kidney, MDA, reduced glutathione.

INTRODUCTION

Cadmium (Cd) is one of the most toxic heavy metals. This metal is a serious environmental and occupational contaminant and may represent a serious health hazard to humans and other animals. Exposure to Cd can produce both acute and chronic tissue injury and can damage various organs and tissues, including liver, kidney, lung, bone, testis and blood depending on the dose, route and duration of exposure. In humans, chronic Cd exposure leads mainly to the nephrotoxicity (Trian and Trian, 1995), skeletal damage (Brzoska et al., 2008), severe damage in nervous, endocrine and immune system, linked to enhanced aging process as well as cancer (Jarup et al., 1998), whereas acute Cd exposure primarily affects the liver, inducing hepatocyte swelling and fatty change, with focal, zonal or massive necrosis (Habeebu et al., 1998).

Metals, especially transition metals, act as catalysts in the oxidative reactions of biological macromolecules; thus metal toxicities might be associated with oxidative tissue damage. Although Cd is not a redox-active metal, such

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Abbreviation: Cd, Cadmium; **ROS,** reactive oxygen species.

as iron, copper and chromium, it has been shown to stimulate the production of intracellular reactive oxygen species (ROS) due to an inhibitory effect on mitochondrial electron transport (Stohs et al., 2000). As a result of this inhibition, the electron transport chain becomes highly reduced; electrons are transferred directly to available oxygen and lead to enhanced formation of ROS. ROS may lead to cellular damage when the rate of its generation surpasses the rate of its decomposition by antioxidant defense systems, such as the enzymes superoxide dismutase (SOD), catalase (CAT), or reduced GSH. The oxidative stress induced by Cd in a biological system may be due to increased lipid peroxidation, which may be attributed to alterations in the antioxidant defense system (Jemai et al., 2007; Newairy et al., 2007). The renal impairment is the main effect observed upon chronic Cd exposure and the proximal tubules of the kidney are the primary target (Goyer and Clarkson, 2001). Several investigations report that Cd induces apoptosis in different cell types, including the renal tubular epithelial cells (Hart et al., 1999; Thevenod et al., 2000).

Trends on applying nutritional antioxidants in diseases related to oxidative stress have gained immense interest in recent years. Plant products are known to exert their protective effects by scavenging free radicals and modulating antioxidant defense system. Curcumin, an active component of turmeric (Curcuma longa Linn.) exhibits antioxidant property. It is a yellow coloured phenolic pigment yield from the rhizome of turmeric (family Zingiberaceae). The most important feature of curcumin is that it has no side effects despite being a therapeutic agent with multiple beneficial functions (Joe et al., 2004). It acts as a scavenger of free radicals. Curcumin is considered to be an effective antioxidant against oxidative tissue damage. It can significantly inhibit the generation of reactive oxygen species (ROS) both in vitro and in vivo (Biswas et al., 2005; Okada et al., 2001). Moreover, the administration of curcumin has also been reported to prevent renal lesions in streptozotocin diabetic rats (Suresh and Srinivasan,1998).

Therefore, it was considered of interest to investigate the effects of oral curcumin pretreatment in combating Cd toxicity in the rat kidney, which represent important target organs by examining the level of lipid peroxidation and reduced GSH in renal homogenate, including histopathological changes of renal tissue under light microscope.

MATERIALS AND METHODS

Chemicals

Curcumin (dissolved in glycerol) were kindly provided from Government Pharmaceutical Organization. Cd acetate (dissolved in distilled water) was purchased from Sigma-Aldrich (USA). The other chemicals used, eg. absolute ethanol was purchased from Merck (Darmstadt, Germany) and all the reagents were of analytical grade.

Animals and treatments

Adult male Wistar rats were used in the present study. The experimental animals were supplied by the National Laboratory Animal Center of Mahidol University and used for experiments after 1 week of acclimatization. The animals were maintained as national guidelines and protocols, approved by the Institutional Animal Ethics Committee and in an air-conditioned animal house with constant 12 h light and 12 h dark schedule. Animals were fed on standardized diet for rodents and water ad libitum.

The experiment was conducted over a period of 5 days. After a period of adaptation, the animals at 200 - 220 g initial body weight, were divided into four experimental groups of 8 animals each:

Group I: control rats were administered with sterile distilled water as vehicle.
Group II: rats received Cd acetate dissolved in sterile distilled water at a dose of 200 mg/kg BW.
Group III: rats received curcumin dissolved in glycerol at a dose of 250 mg/kg BW.
Group IV: rats received curcumin at a dose of 250 mg/kg BW for one hour before administration with Cd acetate 200 mg/kg BW.

All groups were treated by oral gavage once daily. The doses used in this study were selected based on preliminary experiments in our laboratory using acute treatment with Cd acetate. The selection of dose regime of Cd acetate and curcumin were based on previous published data (Athar and Iqbal, 1998; Chuang et al., 2000). All the animals were sacrificed 24 h after the last treatment following protocols and ethical procedures.

One kidney of each animal was immediately removed; weighed and washed using chilled saline solution. Tissues were minced and homogenized (10% w/v), separately, in ice-cold 0.1 M phosphate buffer (pH 7.4) in a Potter–Elvehjem type homogenizer. The homogenate was used for the determination of MDA, reduced GSH and protein content. The other kidney of each animal was fixed in 10% neutral phosphate buffered formalin solution.

Malondialdehyde (MDA) and reduced glutathione (GSH) assays

Lipid peroxidation (LPO) was measured by the method of Buege and Aust (1978). The level of LPO in the renal homogenate was measured based on the formation of thiobarbituric acid-reactive substances (TBARS). Malondialdehyde (MDA) formed adducts with thiobarbituric acid, which was measured spectrophotometrically (UV-1240 Shimadzu, Japan) at 535 nm. An extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} was applied for calculation and results were expressed as nM/mg protein.

Reduced GSH was determined according to the method by Beutler (1975) using Ellman's reagent. The procedure is based on the reduction of Ellman's reagent by SH groups to produce 5'5-dithio-bis (2-introbenzonic acid) which has an intense yellow color that is measured spectrophotometrically at 412 nm using Shimadzu Spectrophotometer. GSH levels were calculated using an extinction coefficient of 1.36×10^5 M^{-1} cm^{-1}. Results were expressed as µM/mg protein. The protein content of tissue homogenates was determined by the method of Bradford et al. (1978) using bovine serum albumin (BSA) as the standard protein.

Histopathological examination

Immediately after sacrifice, the kidney was removed surgically and rinsed with ice cold physiological saline. For microscopic evaluation kidney was fixed in 10% neutral phosphate buffered formalin
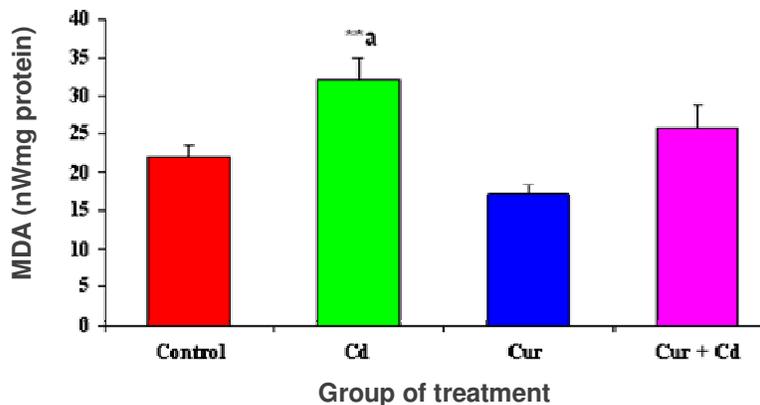
Figure 1. Lipid peroxidation, expressed as MDA level, in the kidney homogenate of control rats, curcumin treated rats at dose of 250 mg/kg BW (Cur), Cd acetate treated rats at dose of 200 mg/kg BW (Cd), curcumin 250 mg/kg BW and Cd acetate 200 mg/kg BW (Cur + Cd). Results were expressed as mean ± S.E.M from 8 animals. [**a] denote significantly different form control group at $p < 0.01$

solution for 48 h. Following dehydration in ascending series of ethanol (70, 80, 95, 100%), tissue samples were cleared in xylene and embedded in paraffin. Tissue sections of 5.0 μm were stained with hematoxylin and eosin (H and E). These sections were examined under light microscopy and documented by Ziess microphotocamera (Lillie and Fuller, 1976).

Statistical analysis

Results were expressed as mean ± standard error of means (S.E.M). One-way analysis of variance (ANOVA) followed by a post hoc test of Fisher's LSD was carried out to test for any differences between the mean values of all groups. If differences between groups were established, the values of the treated groups were compared with those of the control group. A p-value < 0.05 was considered to be significant. These statistical analyses were performed using a computer program of SPSS.

RESULTS

Effects of curcumin on renal MDA and reduced GSH level induced by Cd

MDA concentrations in the kidney tissue were used as a measure of lipid peroxidation. Figure 1 showed the results of MDA changes in all groups. The MDA concentrations were similar in the control and curcumin groups ($p > 0.05$). The administration of curcumin alone did not increase lipid peroxides compared to the control group. Cd induced a statistically significant increase in the formation of lipid peroxides as compared to the control group ($p < 0.01$). Pretreatment of rats with curcumin could reduce MDA concentrations but not significant as compared with the Cd treated group.

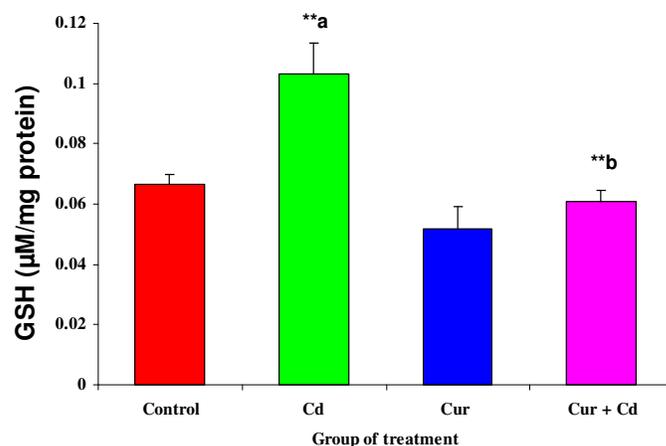Therefore, curcumin pretreatment could attenuate

Figure 2. Reduced GSH level in the kidney homogenate of control rats, curcumin treated rats at dose of 250 mg/kg BW (Cur), Cd acetate treated rats at dose of 200 mg/kg BW (Cd), curcumin 250 mg/kg BW and Cd acetate 200 mg/kg BW (Cur+Cd). Results were expressed as mean ± S.E.M from 8 animals. [**a] denote significantly different form control group at $p < 0.01$, [**b] denote significantly different form Cd acetate treated group at $p < 0.01$.

slightly Cd-induced increases in kidney MDA concentrations.

Figure 2 showed the results of reduced GSH changes in all groups. The reduced GSH were similar in the control and curcumin groups ($p > 0.05$). Cd induced a statistically significant increase in renal reduced GSH as compared to the control group ($p < 0.01$). Interestingly, the reduced GSH was depleted significantly ($p < 0.01$) in animals treated with curcumin plus Cd when compared
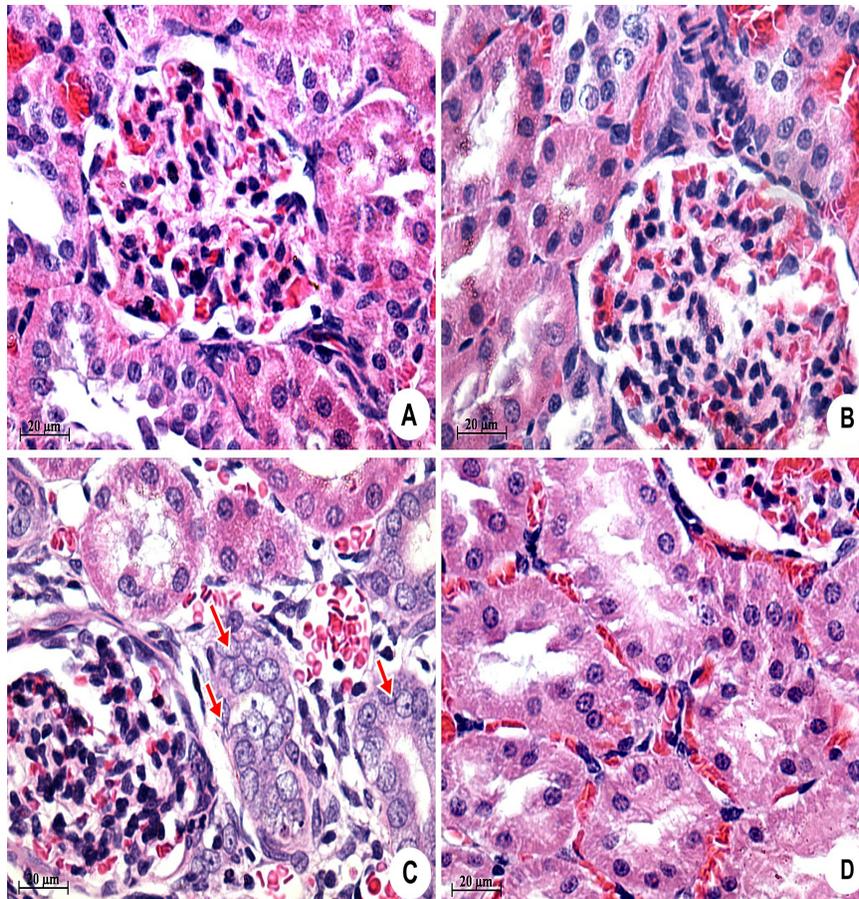
Figure 3. Representative microphotographs of rat renal cortex by light microscope with H and E staining from eight rats of each group at 400× magnification. Control (A), curcumin treated rats at dose of 250 mg/kg BW (B), Cd acetate treated rats at dose of 200 mg/kg BW (C), curcumin and Cd acetate treated rats (D). The hydropic swelling and hypertrophy of proximal tubular cells were observed in Cd-treated rats (arrow).

with the Cd group. Therefore, curcumin pretreatment could inhibit Cd-induced increase in kidney reduced GSH.

Effects of curcumin on histopathological changes of rat kidney induced by Cd

The renal cortex and medulla from all the experimental and control rats were examined with light microscope. The section of renal cortex was assessed for the appearance of glomerulus, the associated tubules and interstitial tissue.

The histological analysis of renal cortex revealed that control (Figure 3A) and curcumin-alone-treated rats (Figure 3B) showed normal morphology. In the Cd-treated rats, the hydropic swelling and hypertrophy of proximal tubular cells were observed when compared to the control group (Figure 3C). Administration of curcumin to Cd treated rats resulted in the improvement in the

structure of proximal tubular cells (Figure 3D). The histological examination supported the biochemical alterations. Therefore, the pretreatment with curcumin might reduce the Cd cytotoxicity in rat kidney.

The histological analysis of renal medulla revealed that the tubular cells and interstitial tissue in all groups of treatment had the normal appearance (Figure 4). The damages of tubular cells were not observed.

DISCUSSION

In the present study, we found that acute Cd intoxication induced renal damages, measured by increased lipid peroxidation and histopathological changes. The damage was associated with an increase significantly of reduced GSH. The purpose of the present study is to find out the ameliorating effect of curcumin on Cd-induced oxidative damage in renal tissue. The present study has shown that curcumin partially protects against lipid peroxidation
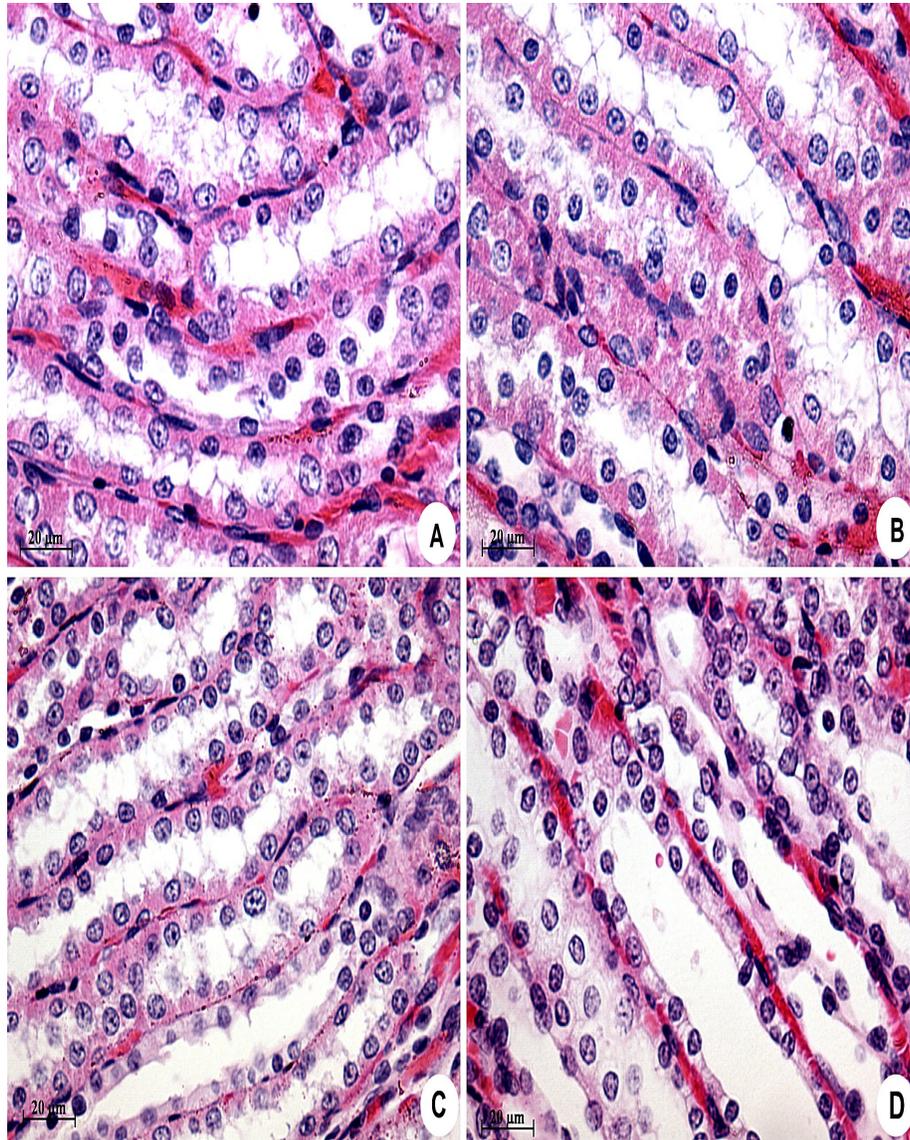
Figure 4. Representative microphotographs of rat renal medulla by light microscope with H and E staining from eight rats of each group at 400× magnification. Control (A), curcumin treated rats at dose of 250 mg/kg BW (B), Cd acetate treated rats at dose of 200 mg/kg BW (C), curcumin and Cd acetate treated rats (D). The tubular cells showed the normal appearance in all groups of treatment.

induced by Cd in the renal homogenate, as well as reduces Cd-induced structural damages in renal cortex. Accordingly, pretreatment with curcumin could reverse significantly the Cd-induced increase of reduced GSH levels ($p < 0.01$).

Cd was more highly accumulated in kidney. In animals exposed to Cd via oral routes, the kidney is by far the primary organ affected adversely by Cd. Some investigators have suggested that, under conditions of chronic exposure to Cd, complexes of Cd-metallothionein (formed in hepatocytes in response to the uptake of Cd) are released from necrotic hepatocytes and are delivered (via systemic circulation) to the kidneys, where it appears

that they are taken up and induce proximal tubular injury and death (Dorian et al., 1992).

In our experiment, the reduced GSH level increased significantly upon oral Cd administration when compared to the control group ($p < 0.01$) because kidney exhibits a high activity of γ-glutamyltranspeptidase (GGTP), the enzyme hydrolyzing GSH with the release of CysGly and cysteinylglycine dipeptidase, the enzyme hydrolyzing this dipeptide to amino acids. Kidney cells can transport GSH from the serum via the mechanism coupled with Na^+ transport. With such systems, the kidneys have practically an unlimited access to GSH and Cys in the serum. The fact that Cd causes such an increase in GSH

level in the kidney is probably related to defense against oxidative processes induced by Cd. The results are similar with the earlier observations where the increases in GSH levels were observed in kidney after injecting the rats with 0.228 mg Cd/kg for 3 days per weeks (Kamiyama et al., 1995). Moreover, Singhal et al. (1987) reported that depletion of GSH enhanced the toxicity of Cd and elevation of tissue GSH levels protected against acute Cd toxicity. Cd is an extremely toxic environmental contaminant that causes the production of ROS such as hydroxyl radicals, superoxide anions, nitric oxide and hydrogen peroxide (Stohs et al., 2000).

These ROS gives rise to lipid peroxidation. Lipid peroxidation is known to play a critical role in Cd induced renal injury and malondialdehyde (MDA) is one of its end products. Thus, measurement of MDA can be used to assess lipid peroxidation. In the present study, the MDA content in renal tissue increased significantly by acute Cd administration as compared to the control group ($p < 0.01$). This finding is in agreement with several reports demonstrating that Cd induces oxidative stress in tissues by increasing lipid peroxidation (El-Demerdash et al., 2004).

Kidney injury induced by Cd was also evaluated by a histological approach (Figures 3 and 4). Acute renal damage was shown by the hydropic swelling and hypertrophy of proximal tubular cells (panel C), as compared with control (panel A). Treatment of Cd-group with curcumin (panel D) resulted in the improvement of proximal tubular cell damage observed with Cd alone, compare panel D with panel C (Cd-group). This histopathological analysis is in agreement with the observed result in the MDA, a biochemical indicator of necrosis. This toxic metal was concentrated in the cortex, the reason of which could be explained that the metal bound to small molecule such as metallothionein was reabsorbed at proximal convoluted tubules. The toxicity of Cd may be ascribed that some of Cd metallothionein are degraded to release the Cd in toxic form (Okubo et al., 1991). This finding is in accordance with previous reports demonstrating that Cd induced nephrotoxicity (Morales et al., 2002).

Cellular damage caused by Cd exposure can be prevented by free radical scavengers or antioxidants, which further strengthens the hypothesis that free radicals play a key role in Cd toxicity. Antioxidants are the frontline of defense against free radicals (Osawa and Kato, 2005). The antioxidant mechanism of curcumin is due to its specific conjugated structure of two methoxylated phenols and an enol form of β-diketone. This structure is responsible for free radical trapping ability as a chain breaking antioxidant (Masuda et al., 2001). The ability of curcumin to chelate the toxic metals was shown by Daniel et al. (2004). They found that curcumin significantly protects against lipid peroxidation induced by heavy metals, lead and cadmium in the rat brain homogenate, as well as reduces lead-induced

structural damage in the hippocampus. Curcumin prevents free radical generation by competing with peroxidant metals for cell binding sites, which decrease the possibility of free radical formation or by maintaining the activities of antioxidant enzymes like SOD and catalase (Reddy and Lokesh, 1992). In the present study, the renal glutathione levels were statistically significantly depleted in curcumin pretreated rats when compared with Cd treated group. It is difficult to provide a complete explanation for the renal glutathione depletion by curcumin in this study. Since glutathione is involved in various biological processes that include free radical scavenging. In the present investigation, the possible curcumin may protect free radical induced damage by defending sulfhydryl groups against oxidation (Pari and Amali, 2005). Results of Tirkey et al. (2005) indicated that curcumin improved renal GSH levels in treated rats. This result is supported by other studies shown that curcumin could protect cadmium-induced oxidative damage in the liver of rats and mice (Eybl et al., 2004).

In the present investigation, a slight but not significant reduction in lipid peroxidation was observed in animals pretreated with curcumin when compared with the Cd treated group. The slight protection by curcumin in Cd nephrotoxicity could also be ascribed to the route and doses schedule used for the treatment with this antioxidant. This dose of curcumin seems to be used for the health promotion and has been used in number of previous studies. Different time of administration and solvents used for oral administration could be also considered as the reason for the absence of beneficial effects (Kalpana and Menon, 2004; Venkatesan et al., 2000). It seems reasonable to assume that curcumin is able to suppress nephrotoxicity in kidney, only in the model of moderate renal toxicity as it was demonstrated in studies with adriamycin (Venkatesan et al., 2000), gentamicin (Farombi and Ekor, 2006) and cyclosporin (Tirkey et al., 2005). In addition, the bioavailability of orally administered curcumin is very limited because in approximately 75% being excreted in the feces and only traces appeared in the urine, suggesting poor absorption of curcumin. It has been shown that curcumin is biotransformed to dihydrocurcumin, tetrahydrocurcumin and hexahydrocurcumin; subsequently, these products are converted to glucuronide conjugates (Maheswari et al., 2006), which are more polar and have better absorption than curcumin (Pan et al., 1999). Curcumin is eliminated very fast from the rat plasma. Only 5 to 31% of the maximum concentration remained in plasma 24 h after administration (Asai and Miyazawa, 2000). Therefore, it is likely that the pharmacological actions of curcumin are caused by its hydrosoluble derivatives. Hydrophilic compounds with antioxidant properties are more likely to act on GSH than on lipids, where lipid peroxidation occurs, explaining the incapacity of curcumin to prevent lipid peroxidation. Similar results on the absence of curcumin protection on Cd nephrotoxicity

were obtained by Frank et al. (2003). Since higher doses of curcumin showed protective effects against lipid peroxidation in biological assays (Venkatesan, 1998), the dose of curcumin used in this study may not have been enough to offer statistically significant protection against lipid peroxidation induced by Cd.

Conclusion

In summary, this study demonstrates that oral pre-treatment with curcumin at dose of 250 mg/kg BW might partially protect against Cd induced oxidative damages in renal tissue. The further studies are needed to investigate the nephrotoxicity by using the other biochemical markers to confirm the oxidative effects of Cd on kidney, including clarification the dose and route of curcumin treatment against Cd-induced nephrotoxicity.

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