# Effects of Curcumin on Cadmium-Induced Hepatotoxicity in Rats

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## ABSTRACT

Cadmium (Cd), an environmental contaminant, undergoes redox cycling with generation of free radicals inside the biological system. Curcumin, the yellow bioactive component of turmeric has established its antioxidant activities. The present study evaluates possible ameliorating effects of curcumin on Cd acetate induced hepatotoxicity in adult male Wistar rats. The animals were treated once daily by oral gavage for five days and divided into four groups: control, Cd acetate 200 mg/kg BW, curcumin 250 mg/kg BW and pretreatment 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, the animals were killed to determine the activities of hepatic marker enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum, the level of malondialdehyde (MDA), reduced glutathione (GSH) in liver homogenate and histological changes of liver tissues by light microscope. The results showed that Cd treatment caused a significant increase of serum AST ($p$ <0.001) and ALT ($p$ <0.05), the increased hepatic level of MDA ($p$ <0.01), the decreased hepatic level of reduced GSH ($p$ <0.05) when compared to the control group. In addition, histological examination revealed that Cd treatment also caused hydropic swelling of hepatocyte with vacuolated cytoplasm. This study could provide a possible explanation to hepatotoxicity resulting from exposure to Cd in the environment. In addition, the pretreatment with curcumin before Cd administration could not inhibit the changes against Cd toxicity. Therefore, it was concluded that curcumin at dose of 250 mg/kg BW could not prevent the toxic effects of Cd against oxidative damages in rat liver since no improvement of all parameters by curcumin treatment.

**Keywords:** Curcumin, cadmium, hepatotoxicity, lipid peroxidation malondialdehyde (MDA), glutathione (GSH), ALT, AST

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## Introduction

Cadmium (Cd) is distributed widely in the environment and is a highly toxic heavy metal. With the development of industries, the frequency of exposure of the body to Cd is gradually on the rise [1]. Studies to characterize the effect of various heavy metals including Cd on the human body and the mechanisms of toxicity have been actively performed. In acute cytotoxicity and carcinogenesis, activation of endonucleases, generation of reactive free radicals such as reactive oxygen species (ROS) and signal transduction pathways involving apoptosis play important roles[2,3]. Cd accelerates lipid peroxidation by stimulating the peroxidation chain reaction in the target organs, resulting in the generation of ROS and consequently the induction of cytotoxicity.[4]

When the body is exposed to Cd chronically, the metal accumulates in the liver and the kidney; with acute exposure, it primarily accumulates in the liver [1]. Upon acute exposure to Cd, hepatotoxicity is indicated by such changes as swelling of hepatocytes, fatty changes, and focal necrosis or necrosis in a wide area. On electron microscopy, changes such as dilatation of ribosomes, damage of membrane-bounded lysosomes, nuclear pyknosis, are seen [5].

Curcumin (diferuolymethane) from the rhizomes of turmeric (*Curcuma longa* L.), used in our study, is a well known biologically active compound. It is a natural yellow pigment that is added to curry spices. Its biological effects have been studied in recent years in many laboratories. Curcumin possesses anti-inflammatory, immunomodulatory, anti-atherogenic activities, and it is a potent inhibitor of various reactive oxygen-generating enzymes[6]. It exhibits protective effects against oxidative damage and scavenges superoxide anions, nitric oxide radicals. It is also considered to be a potent cancer chemopreventive agent [7]. The anti-cancer properties of curcumin have been proven in cultured cells, as well as in animal studies[8, 9]. As regards to the possible interaction of curcumin with toxic metals, a protective effect of curcumin has been demonstrated against iron induced oxidative tissue damage. Curcumin also modulates ferric nitrilotriacetic (Fe-NTA) induced peroxidation of microsomal membrane lipids and DNA damage[10]. The interaction of curcumin with Cd has not been extensively researched yet.

In this study, we wished to investigate the effects of oral curcumin pretreatment in combating the toxicity of Cd acetate on the activities of hepatic marker enzymes AST and ALT in serum, the level of lipid peroxidation and reduced GSH in liver homogenate, including the histopathological changes of liver tissues under light microscope in rats.

## Materials and methods

### Chemicals

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

### Animals and treatments

Adult male Wistar rats weighing 150-180 g 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 rats were separated randomly into 4 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 for five days. The doses used in this study were selected based on preliminary experiments in our laboratory using acute treatment with Cd acetate. The rats were designed to be orally administered a high dose of Cd acetate, which are close to 0.12–0.5 oral $LD_{50}$ value for Cd acetate in male rats [11]. All the animals were sacrificed 24 h after the last treatment following protocols and ethical procedures. Plasma samples were separated by centrifugation, frozen and stored at −20 °C until assayed for AST and ALT enzymes.

### Serum ALT and AST determination

The serum levels of hepatic enzymes ALT/AST from treated animals were measured and their relative activities were compared with control samples. Relative activity means activity in the serum of a rat received with Cd versus activity in the serum of a control rat during the same period. The level of AST and ALT activities was assayed using a microplate spectrophotometer (Hitachi, Japan).

### Malondialdehyde (MDA) and glutathione (GSH) assays

Lipid peroxidation (LPO) was measured by the method of Buege and Aust [12]. The level of LPO in the liver 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 X $10^5$ $M^{-1}$ $cm^{-1}$was applied for calculation and results were expressed as nmol MDA/mg protein.

GSH measurements were performed using a modification of the Ellman procedure[13]. Briefly, after centrifugation of the tissue homogenate at 3000 × *g* for 10 min, 0.5 ml of supernatant was added to 2 ml of 0.3 mol/l $Na_2HPO_4 \cdot 2H_2O$ solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/ml in 1% sodium citrate) was added and the absorbance at 412 nm was measured immediately after mixing. GSH levels were calculated using an extinction coefficient of 1.36 × $10^5$ $M^{-1}cm^{-1}$. Results were expressed as μmol GSH/mg protein.

The proteins were estimated by the method of Bradford *et al*. [14] using bovine serum albumin (BSA) as the standard protein.

### Histopathological examination

Immediately after sacrifice, the liver was removed surgically and rinsed with ice cold physiological saline. For microscopic evaluation liver was fixed in 10% neutral phosphate buffered formalin 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&E). These sections were examined under light microscopy and documented by Ziess microphotocamera[15].

### 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.

## Results

### Effects of curcumin on hepatic MDA and reduced GSH level induced by Cd acetate

The curcumin treatment alone did not exert any effects on the hepatic MDA and reduced GSH level. No significant changes on the lipid peroxidation and glutathione level were noted in rats treated with curcumin only. The MDA level was increased significantly ($p < 0.01$) and the reduced GSH level was decreased significantly ($p < 0.05$) in Cd acetate treated rats at dose of 200 mg/kg BW when compared to the control group. In addition, the increase of MDA and decrease of reduced GSH level by Cd treatment could not be prevented by the pre-treatment with curcumin 250 mg/kg BW. There was remain significantly increase of MDA ($p < 0.001$) and decrease of reduced GSH ($p < 0.05$) when compared to the control group ($p < 0.05$) (Fig. 1 and 2).

### Effects of curcumin on enzyme activities of ALT and AST induced by Cd acetate

The curcumin treatment alone did not exert any effects on the enzyme activities of ALT and AST. No significant changes on the enzyme activities of ALT and AST were observed in rats treated with curcumin only. The enzyme activities of ALT and AST were significantly increased in Cd acetate treated rats at dose of 200 mg/kg BW when compared to the control group ($p < 0.05$). The increase in enzyme activities of ALT and AST by Cd treatment could not be prevented by the pre-treatment with curcumin 250 mg/kg BW. There was remain significantly increase in the enzyme activities of ALT and AST when compared to the control group ($p < 0.05$) (Fig. 3).

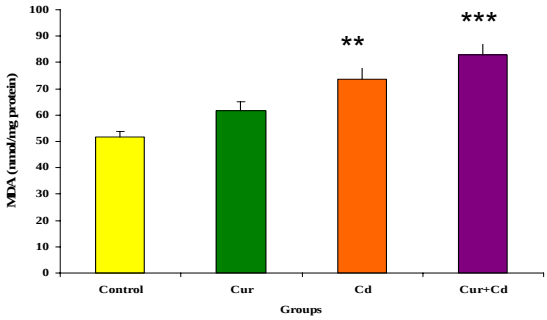


Figure 1 Lipid peroxidation, expressed as MDA level, in the liver homogenate of control rats, curcumin treated rats at dose of 250 mg/kg BW (Cur), Cd acetate treated rats at dose of 200 mg/kg BW (Cd), curcumin 250 mg/kg BW and Cd acetate 200 mg/kg BW (Cur + Cd). Results were expressed as mean ± S.E.M from 8 animals. (**) denote significantly different form control group at $p$ <0.01, (***) denote significantly different from the control group at $p$< 0.001.

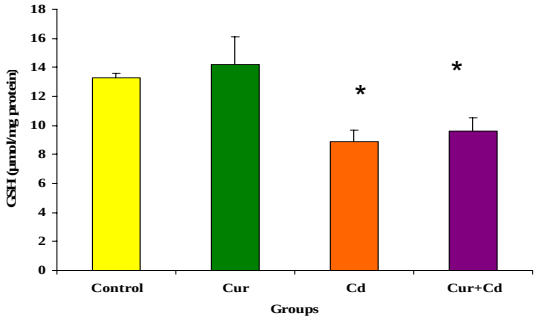


Figure 2 Reduced GSH level in the liver homogenate of control rats, curcumin treated rats at dose of 250 mg/kg BW (Cur), Cd acetate treated rats at dose of 200 mg/kg BW (Cd), curcumin 250 mg/kg BW and Cd acetate 200 mg/kg BW (Cur+Cd). Results were expressed as mean ± S.E.M from 8 animals. (*)denote significantly different form the control group at $p$ <0.05

### Effects of curcumin on histopathological changes of rat liver induced by Cd acetate

In the normal group of rats, focusing on the central vein, hepatocytes formed a cordlike arrangement (Fig. 4A). The curcumin-alone-treated rats were almost similar to control in histology, size and staining properties; no vacuolisation was seen and smooth nuclei with nucleoli were clearly visible as in the normal cells (Fig. 4B). In the Cd-treated rats, the cytoplasmic hypereosinophilia were increased, and cell damage such as nuclear hyperchromasia, pyknosis and binucleated hepatocytes were observed when compared to the control group. In addition, it had necrotic lesions and extensive vacuolisation of cytoplasm in hepatocytes

(Fig. 4C). In the pretreatment with curcumin, the cytoplasmic vacuolization was found to be significantly increased similar to the Cd-treated rats (Fig. 4D). Thus, histological examination also clearly demonstrated no protection of liver by curcumin against Cd cytotoxicity.

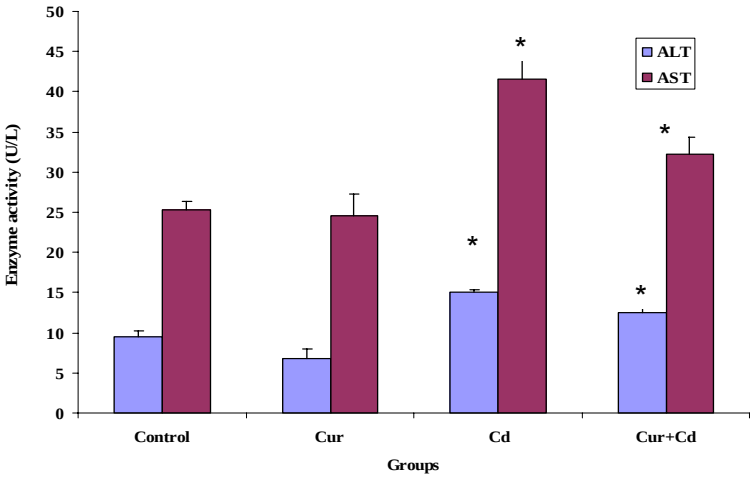


**Figure 3** Enzyme activities of serum ALT and AST in control rats, curcumin treated rats at dose of 250 mg/kg BW (Cur), Cd acetate treated rats at dose of 200 mg/kg BW (Cd), curcumin 250 mg/kg BW and Cd acetate 200 mg/kg BW (Cur+Cd). Results were expressed as mean $\pm$ S.E.M from 8 animals. (*) denote significantly different form control group at $p$ <0.05

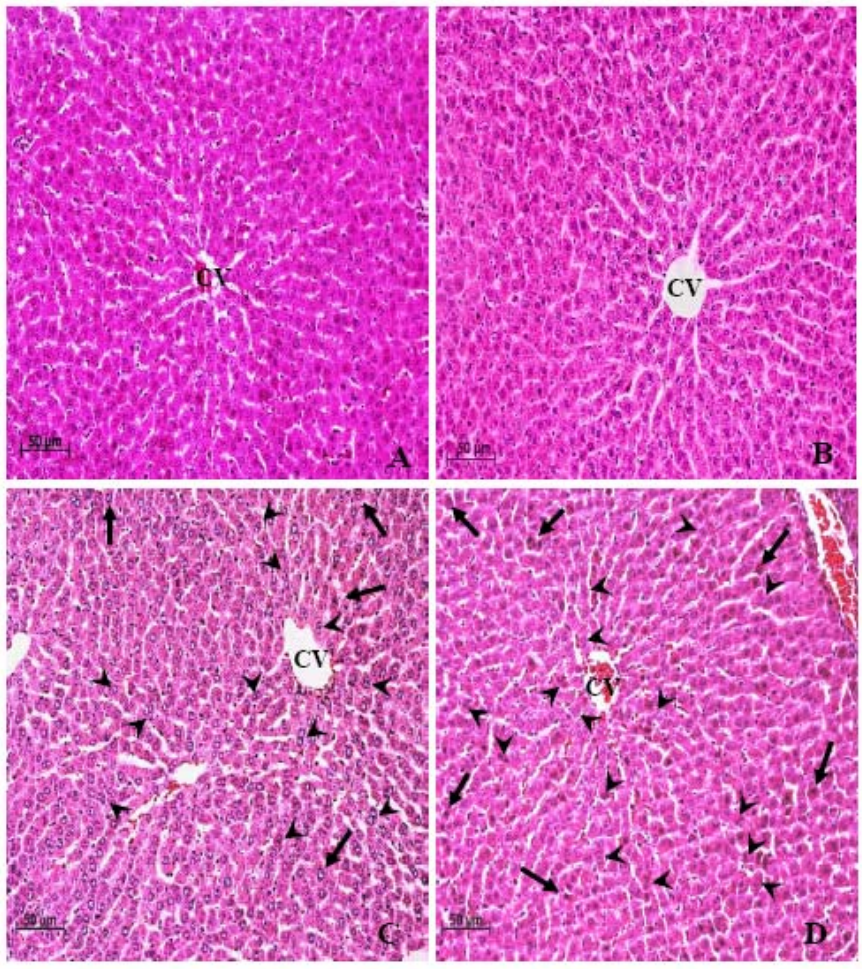


**Figure 4** Representative microphotographs of rat liver, focusing on the central vein area by light microscope with H & E staining from eight rats of each group at 200 X magnification. Control (A), curcumin treated rats at dose of 250 mg/kg BW (B), Cd acetate treated rats at dose of 200 mg/kg BW (C), curcumin and Cd acetate treated rats (D). Hepato-cytes form a cordlike arrangement in control and curcumin treated group. The nuclear hyperchromasia, pyknosis, and binucleated hepato-cytes were observed in Cd treated group and curcumin pre-treatment group. CV = Central vein; Arrowhead = Binucleated hepatocytes; Arrow = Pyknotic nucleus.

## Discussion

Cd is a toxic metal that is widely used in different industries. It promotes an early oxidative stress and afterward contributes to the development of serious pathological conditions because of its long retention in some tissues [16]. The present results have clearly demonstrated the ability of Cd to induce oxidative stress in rat liver as evidenced by increased lipid peroxidation (TBARS) after 5 days of Cd treatment (Fig1). This finding was inagreement with several reports demonstrating that Cd induces oxidative stress in tissues by increasing lipid peroxidation [17].

The liver plays a central role in Cd-toxicity [18]. Independent of the route of exposure, Cd preferentially localizes in hepatocytes after administration, and its concentration may exceed the capacity of intracellular constituents – mainly metallothionein (MT) – to bind Cd [19]. In general, AST and ALT enzymes are known to be increased by liver damaging. AST and ALT enzymes were the most specific marker of liver cell damage in mammals. Blasco and Puppo[20] reported that an increase of the activities of AST and ALT enzymes was also observed as a result of Cd cytotoxicity. Both AST and ALT are enzymes found in hepatocytes. Upon destruction of hepatocytes or due to an increase in the permeability of the hepatocyte membrane, these enzymes are released to the blood and the levels are consequently elevated. In the present study, the increased levels of AST and ALT were considered due to acute hepatocyte damage.

In the present study, the increased lipid peroxidation due to Cd was accompanied by a depletion of hepatic GSH, indicating oxidative stress at the hydrophilic level. These results are in accordance with several previous reports[21,22]. GSH, plays an important role in the maintenance of protein and lipid integrity, and provides major protection in oxidative damage. GSH is thought to be the first line of defense before induction of MT takes over. In the present study GSH level decreased significantly in the liver homogenate of Cd-treated rats when compared to the control group. This may be due to its consumption in scavenging free radicals generated by Cd. Moreover, the sulfhydryl group of cysteine moiety of GSH has a high affinity for metals, forming thermo-dynamically stable mercaptide complexes with several metals, including Cd.[23] These complexes are inert and excreted via the bile, so decreased GSH level may be due to its consumption in Cd detoxification.[24]

The microscopic observation showed that in the normal group of rats, focusing on the central vein, hepatocytes formed a cordlike arrangement. In the Cd-treated rats, the cytoplasmic hypereosinophilia were increased, and cell damage such as nuclear hyperchromasia, pyknosis, and karyorrhexis were observed. This implies that liver tissues are damaged. These histological observations supported the biochemical findings and were in accordance with other studies[25]. The mechanism of Cd-induced acute hepatotoxicity has been investigated extensively. The initial damage to the hepatocyte may be caused by a disturbance in thiol homeostasis[26] and/or the production of ROS [27]. Also since GSH is bound to Cd in an ineffective complex, the hepatocytes are more vulnerable to Cd toxicity. Cd also caused damage to the hepatocytes by disrupting their tight junction resulting in a damaged cell membrane. This causes an increase in cell permeability ensuing in hydropic swelling of the hepatocytes. Secondary liver injury occurs from inflammatory processes that are initiated by the activation of Kupffer cells.[28] Activated Kupffer cells release chemoattractants and activators of neutrophils, and the resulting neutrophil influx promotes extensive tissue damage.

It is important to point out that lipid peroxidation may produce injury by compromising the integrity of membranes and by covalent binding of reactive intermediates to important biological molecules like GSH; finally the process leads to necrosis and liver damage in general. Therefore, free radicals scavengers and antioxidants may interfere with the toxicity of Cd.

Curcumin, an antioxidant and anti-carcinogenic substance, was reported to have a protective effect against iron induced liver damage and lipid peroxidation of microsomal membrane lipids2[9]. It has been suggested that curcumin can exert antioxidative effects either directly as chemical antioxidant due to its ability to scavenge reactive oxygen and nitrogen free radicals or also by modulating cellular defenses which

themselves exert antioxidant effects[30]. We chose to investigate the possible hepatoprotective effect of curcumin because this antioxidant is a potent inhibitor of lipid peroxidation [31].

In the present investigation, the oral pre-treatment with curcumin did not inhibit liver damages induced by Cd. The absence of protection by curcumin in Cd hepatotoxicity could also be ascribed to the route and doses schedule used for the treatment with this antioxidant. The dose used in this study by gavage may not have been sufficient. Similar results on the absence of curcumin protection on Cd hepatotoxicity were obtained by Frank *et al.*[32]. Since higher doses of curcumin showed protective effects against lipid peroxidation in biological assays [33], the dose of curcumin used in this study may not have been enough to offer statistically significant protection against the biochemical and histopathological changes induced by Cd.

The present work shows that lipid peroxidation (measured as MDA) increased significantly by Cd administration. However, curcumin was not able to prevent MDA production and the decrease of hepatic reduced GSH level. The most likely explanation for the absence of protection by curcumin is also that oral consumption of curcumin in rats resulted 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, tetrahidrocurcumin, and hexahidrocurcumin; subsequently, these products are converted to glucuronide conjugates[34], which are more polar and have better absorption than curcumin[35]. Therefore, it is likely that the pharmacological actions of curcumin are caused by its hydrosoluble derivatives. Hydrophilic compounds with anti-oxidant properties are more likely to act on GSH than on lipids, where lipid peroxidation occurs, explaining the incapacity of curcumin to prevent lipid peroxidation.

In conclusion, this study demonstrates that oral pre-treatment with curcumin at dose of 250 mg/kg BW did not recover the alterations induced by Cd at a significant statistically level. This study suggests that the natural antioxidants curcumin did not offer protection against Cd-induced the toxicity of liver in rats. Further detail study will be needed to clarify the mechanism of curcumin against Cd-induced liver injury.

**Acknowledgements**

The authors are thankful to Rangsit University for supporting the equipments and funding this project. The authors would like to thanks the Government Pharmaceutical Organization for providing the curcumin in this experiment.

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