

Protective Effect of Curcumin on Cadmium-Induced Liver Apoptosis in Rats

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ABSTRACT

Objective: Cadmium (Cd), which is used for many industrial purposes, is a toxic agent. Cd accumulates in the liver; therefore, exposure to toxic doses of Cd results in hepatic damage. Studies in rats have shown that Cd induces apoptosis in hepatocytes. Curcumin is a natural compound isolated from *Curcuma longa*. It has a powerful anti-inflammatory effect and scavenges reactive oxygen radicals. Additionally, it has been shown to have an anti-apoptotic effect in a dose-dependent manner. The aim of the present study was to evaluate the effects of therapeutic doses of curcumin on Cd-induced hepatic apoptosis as well as hepatic biochemical and inflammatory changes in Sprague-Dawley rats.

Methods: In this study, 24 female Sprague-Dawley rats (6 months old) were randomly assigned to four main groups (n=6): control, Cd, Cd +curcumin, and curcumin. At the end of the experiment, after collecting blood samples from the heart, the rats were sacrificed and their livers were removed for histopathological and biochemical examinations. The number of apoptotic cells, total anti-oxidant status, total oxidant status, and thiol and MPO levels were measured in liver tissue; interleukin-6 and procalcitonin levels were measured in sera.

Results: Chronic Cd administration induced apoptosis. The number of apoptotic cells was significantly increased in the Cd group (almost 2 fold) compared to that in the control group. However, in the Cd+curcumin group, the number of apoptotic cells was significantly decreased (almost 2 fold) compared to that in the Cd group. However, there were no statistically significant differences.

Conclusion: We suggest that curcumin protects the liver against toxin-induced apoptosis.

Keywords: Apoptosis, cadmium, curcumin, liver

Introduction

Cadmium (Cd), a heavy metal, is a highly toxic environmental agent with carcinogenic effects. The main sources of Cd that are often used in industries are fossil fuels, wastewater, mineral deposits, and tobacco products (1). Cd enters the human body through air, water, and nutrients and accumulates in various organs (2, 3). Because its half-life is very long and it cannot be reduced in the body, it accumulates in various organs such as the liver, kidney, lung, heart, and bone, leading to the damage of DNA, activation of proto oncogenes, and inhibition of DNA repair mechanisms by inducing oxidative stress formation in organs at the cellular level (4-6). Studies have shown that Cd induces apoptosis in many organs such as the liver and kidney (7, 8). However, the signaling pathway through which Cd acts is not yet completely clear. Cd toxicity has been reported to lead to various diseases such as renal and hepatic failure, osteoporosis, arteriosclerosis, anemia, and cancer (9-12).

Curcumin is obtained from yellow turmeric (*Curcuma longa* L.), which is used as a spice in foods. It is known that curcumin has anti-inflammatory, anti-microbial, and antioxidant properties (13-18). The anti-carcinogenic effect of curcumin has been demonstrated in cell cultures and experimental animal models (19-21).

In this study, the effect of chronic Cd application on the rate of apoptosis in the liver of adult rats was investigated. In addition, the total oxidant status (TOS), total antioxidant capacity (TAC), thiol, and myeloperoxidase (MPO) levels were measured in liver tissues. Serum interleukin-6 (IL-6) and procalcitonin (PCT) levels

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were also determined. This study aimed to investigate the effect of curcumin, which was applied at therapeutic doses, on apoptotic, biochemical, and inflammatory changes following Cd application.

Methods

Method of Experiment

Twenty-four 6-month-old female Sprague Dawley rats were used. The rats were randomly divided into the following four groups: Group I (n=6), control group; Group II (n=6), Cd group; Group III (n=6), Cd+curcumin group (Cd+Cur); and Group IV (n=6), curcumin group (Cur). The rats received Cd intraperitoneally (ip.) at a dose of 0.5 mg/kg/day, and received Cr ip. at a dose of 50 mg/kg/day for 10 days. Cr was applied for 10 days after application of cadmium at the co-treated group. Curcumin dissolved in olive oil. Curcumin, prepared in the same way, was intraperitoneally administered at a dose of 50 mg/kg to the rats in the Cur group for 10 days. Olive oil was intraperitoneally administered to rats in the control group at a dose of 0.5 mL for 10 days. At the end of the experiment period, general anesthesia was administered to all the rats using 80 mg/kg ketamine (Ketalar, Pfizer, Turkey) and 5 mg/kg xylazine HCl (Rompun, Bayer, Turkey), and blood samples were obtained from the heart. In addition, liver tissue samples were also obtained. Half of the tissues were temporarily preserved at -80°C in a freezer until biochemical tests were performed. The other half was placed in 10% formaldehyde-buffered solution for use in the immunofluorescence methods.

This study was conducted at the same center following the approval of the Bezmi Alem Vakif University Research Center, Experimental Animals Ethics Committee.

TUNEL Method

Liver specimens fixed with 10% neutral buffered formalin for microscopic examination were embedded in paraffin with a routine histological follow-up. The TUNEL method (terminal deoxynucleotidyl transferase-mediated dUTP-X nick end labeling assay, Roche-11 684 795 910-kit) was applied to the sections taken from paraffin blocks with a thickness of 4 μm , according to the recommended procedure in the kit. The number of apoptotic cells was obtained by counting TUNEL positive cells in the immunofluorescence microscope (Nikon Eclipse 80i) in randomly selected 20 fields at $\times 20$ magnification on each section.

Biochemical Analysis

Liver samples were weighed and homogenized for 3 min at 16000 rpm in Tris-HCl buffer (pH 7.4). Homogenization was achieved in an ice bucket. The homogenate was centrifuged at 5000 $\times g$ for 1 h (at $+4^{\circ}\text{C}$) to obtain the supernatant. TAC, TOS, thiol, and MPO

levels of the supernatant samples were determined using the Erel method (Rel Assay Diagnostics, Gaziantep, Turkey).

Determination of Serum Inflammatory Factor Levels

Blood samples obtained from the heart were centrifuged at 3000 rpm for 5 min to obtain the serum. Serum samples were stored at -80°C until further analysis. IL-6 and PCT levels were measured using a commercial kit (Rat IL-6 Platinum ELISA, eBioscience, Bender Med-Systems GmbH, Vienna, Austria), (Rat PCT Elisa Kit, Eastbio Pharm, Hangzhou East Biopharm Co., Hangzhou, China). The method was performed according to the procedures in the company kit.

Statistical analysis

All statistical analysis was performed using the Graph-Pad Prism 6 (USA) program. The Dunnett's multiple comparison test was used as post-hoc test. The results were evaluated at the significance level of $p < 0.05$.

Results

Apoptosis Findings

The mean numbers of apoptotic cells were 132.50 ± 58.52 in the control group, 242.66 ± 170.02 in the Cd group, 112.33 ± 15.38 in the Cd+Cur group, and 75.16 ± 26.55 in the Cur group. Although the numbers of apoptotic cells in all the groups were quite different, there was no statistically significant difference. It was remarkable that the values of the control and Cd+Cur group were very close. The number of apoptotic cells was lower in the Cur group than in the control group (Table 1, Figures 1, 2).

Oxidative Stress Parameters

The mean TOS levels were 8.26 ± 2.55 in the control group, 11.31 ± 3.56 in the Cd group, 9.70 ± 2.42 in the Cd+Cur group, and 12.80 ± 1.99 in the Cur group. The mean TAC levels were 1.13 ± 1.55 in the control group, 1.08 ± 0.87 in the Cd group, 1.00 ± 1.20 in the Cd+Cur group, and 0.59 ± 0.60 in the Cur group. Thus, the TOS level increased in the Cd group compared with the control group, while the TAC level decreased. TOS and TAC levels decreased in the Cd+Cur group compared with the Cd group. The highest TOS and lowest TAC levels were in the Cur group. These results were not statistically significant (Figure 3, 4).

The mean thiol levels were 0.60 ± 0.46 in the control group, 0.36 ± 0.24 in the Cd group, 0.45 ± 0.22 in the Cd+Cur group, and 0.33 ± 0.12 in the Cur group. Thus, the thiol level decreased in the Cd group compared with the control group, whereas it increased in the Cd+Cur group compared with the Cd group. The thiol level was lowest in the Cur group. These results were not statistically significant (Figure 5).

Table 1. Mean apoptotic cell numbers of all groups

Groups	Apoptosis (mean±SD)
Control	132.50±58.52
Cd	242.66±170.02
Cd+Cur	112.33±15.38
Cur	75.16±26.55

Cd: cadmium; Cur: curcumin; SD: standard deviation

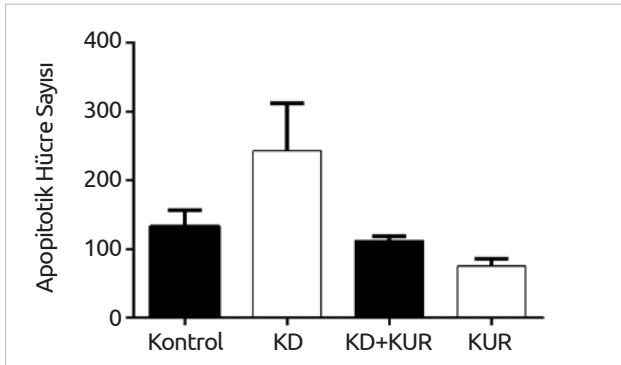


Figure 1. The average number of apoptotic cells in all groups

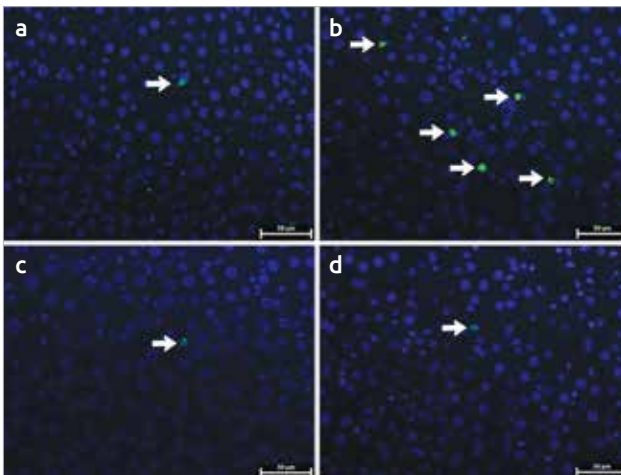


Figure 2. (a-d). Apoptotic cells are shown in hepatic liver sections (arrow) (a) control group; (b) Cd group; (c), Cd+Cur group; (d), Cur group (Tunel Method at 40× magnification; Bar, 50 µm)

The mean MPO levels were 1449.89±868.12 in the control group, 1739.92±901.75 in the Cd group, 1269.58±690.12 in the Cd+Cur group, and 1719.35±529.82 in the Cur group. The MPO level was found to be the highest in the Cd group and the lowest in the Cd+Cur group. These results were not statistically significant (Figure 6).

The mean values of the biochemical data are given in Table 2.

Inflammatory Marker Parameters

The mean IL-6 levels were 55.39±39.23 in the control group, 66.96±20.56 in the Cd group, 53.96±29.58

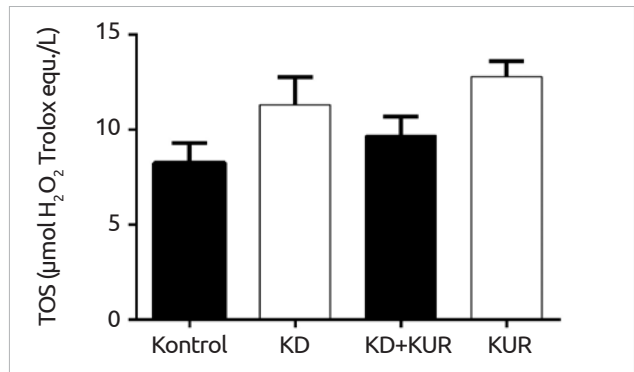


Figure 3. TOS averages of all groups
TOS: total oxidant status

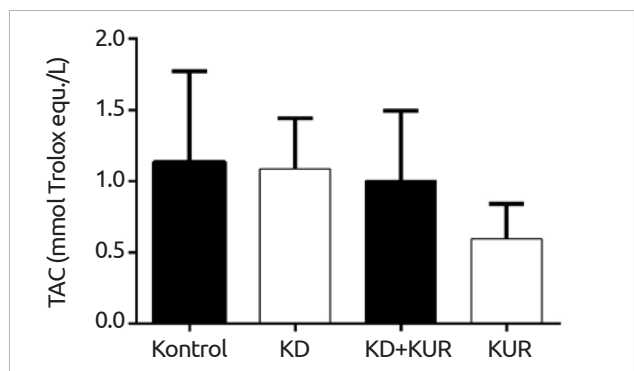


Figure 4. TAC averages of all groups
TAC: total antioxidant capacity

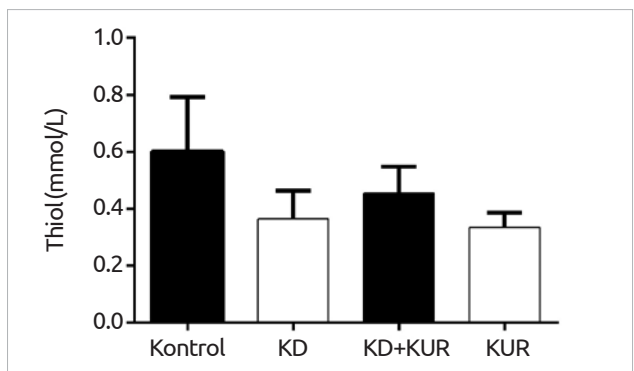


Figure 5. Thiol averages of all groups

in the Cd+Cur group, and 87.50±59.71 in the Cur group. The highest IL-6 level was observed in the Cur group. Compared with the control group, the IL-6 level increased in the Cd group and was the lowest in the Cd+Cur group. These results were not statistically significant (Figure 7).

The mean PCT levels were 480.90±321.48 in the control group, 1750.19±250.38 in the Cd group, 694.13±434.54 in the Cd+Cur group, and 681.10±342.87 in the Cur group. The highest PCT level was observed in the Cd group. The PCT level decreased in the Cd+Cur group.

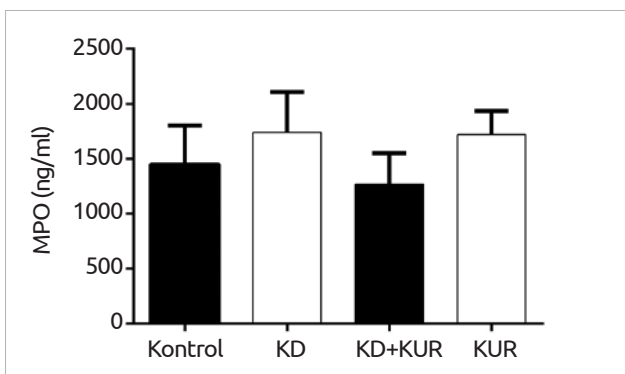


Figure 6. MPO averages of all groups
MPO: myeloperoxidase

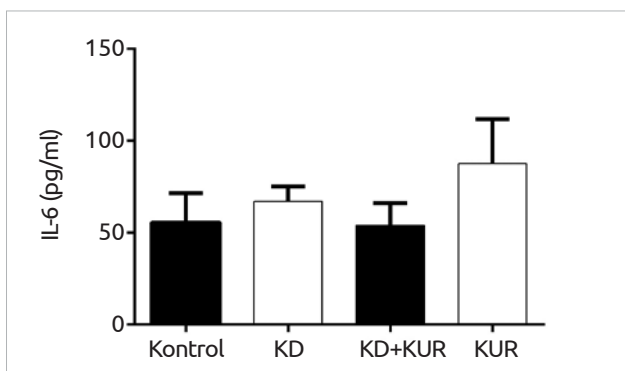


Figure 7. IL-6 averages of all groups

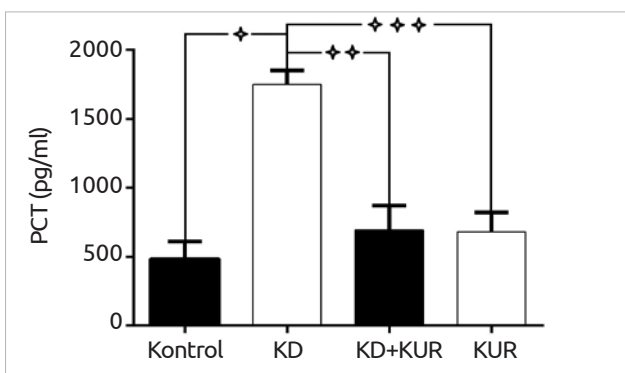


Figure 8. PCT averages of all groups
PCT: procalcitonin
*, **, ***p<0.000

These results were statistically significant ($p<0.000$; Figure 8).

The mean values of the parameters of the inflammatory markers are given in Table 3.

Discussion

A large amount of Cd entering the body is absorbed through the intestines and transported to the liver by portal circulation (22). Cd accumulating in hepatocytes produces a toxic effect through two different pathways.

Table 2. Mean biochemical values of all groups

Groups	Mean Biochemical Values (mean±SD)			
	TAC (mmol Trolox equ./L)	TOS (µmol H ₂ O ₂ equ./L)	Thiol (mmol/L)	MPO (ng/mL)
Control	1.13±1.55	8.26±2.55	0.60±0.46	1449.89±868.12
Cd	1.08±0.87	11.31±3.56	0.36±0.24	1739.92±901.75
Cd+Cur	1.00±1.20	9.70±2.42	0.45±0.22	1269.58±690.12
Cur	0.59±0.60	12.80±1.99	0.33±0.12	1719.35±529.82

Cd: cadmium; Cur: curcumin; SD: standard deviation; TAC: total antioxidant capacity; TOS: total oxidant status; MPO: myeloperoxidase

Table 3. Results of the mean inflammatory markers of all groups

Groups	Inflammatory Markers (mean±SD)	
	IL-6 (pg/mL)	PCT (pg/mL)
Control	55.39±39.23	480.90±321.48*
Cd	66.96±20.56	1750.19±250.38*, **, ***
Cd+Cur	53.96±29.58	694.13±434.54**
Cur	87.50±59.71	681.10±342.87***

Cd: cadmium; Cur: curcumin; SD: standard deviation; PCT: procalcitonin
*p<0.000; Control and Cd groups
**p<0.000; Cd and Cd+Cur groups
***p<0.000; Cd and Cur groups

In the primary pathway, Cd causes direct damage. Cd binds to the sulfhydryl groups of some of the important molecules in the mitochondria and induces oxidative stress by inactivating the thiol groups. In the secondary pathway, it is the inflammatory pathway that is achieved by inducing the activation of Kupffer cells with the secretion of cytotoxic and inflammatory mediators (23). Studies have demonstrated that Cd induces apoptosis in many organs such as the liver and kidney (7, 8). However, the signaling pathway through which Cd acts is not completely clear.

Acute exposure to Cd causes severe liver damage (23). In addition, it causes cellular damage in many organs such as kidneys, testes, pancreas, and bone. Thus, cells die because of necrosis or apoptosis (24-27). Apoptosis is a highly complex cell death mechanism, which is induced by many intracellular signal pathways. Previous studies have shown that Cd increases oxidative stress and induces apoptosis (28-31). In our study, it was observed that the number of apoptotic cells in the liver of rats of the Cd group was two times higher than that

in liver of rats of the control group, and the TOS level increased, whereas the TAC level decreased. It is known that curcumin has anti-proliferative, apoptosis-inducing, anti-tumorigenic effects (32-34). However, studies conducted in the field of cell culture have shown that curcumin inhibits chemotherapy-induced apoptosis by inhibiting the formation of reactive oxygen species in human breast cancer cells, and it inhibits apoptotic biochemical changes in human epidermoid carcinoma cells (35, 36). Similarly, in our study, the increased number of apoptotic cells in the Cd group was observed to decrease by half in the Cd+Cur group.

Under normal conditions, free radicals that are degradation products of oxygen used during cell metabolism are destroyed by the intracellular antioxidant system and are prevented from damaging the cell. However, the fact that the increasing amount of free radicals in various pathological conditions cannot be neutralized by the suppressed antioxidant system can lead to cell death. In our study, the Cd group had a higher TOS level and lower TAC level than the control group. These data suggest that Cd leads to oxidative stress. The TOS level decreased in the Cd+Cur group. It can be said that curcumin is successful in reducing the TOS level in hepatocytes, but it does not have much effect in increasing the antioxidant capacity because the administration of curcumin does not lead to a significant increase in the mean TAC levels.

Neutrophils that migrate to the inflammatory region significantly contribute to oxidative damage by generating free radicals. Increased MPO levels in the tissue indicate inflammation and thus, oxidative stress. Similarly, thiol levels vary depending on the oxidative stress. Free radicals cause the oxidation of thiol groups in proteins, resulting in oxidative protein damage. The thiol and oxidative damage levels are inversely proportional. In our study, the mean MPO level of the Cd group was higher than that of the control group. This level was lower in the Cd+Cur group than in the Cd group. It was observed that the thiol level in the Cd group decreased compared with that in the control group and increased in the Cd+Cur group compared with that the Cd group. In the light of these data, it can be said that inflammation and oxidative stress increased in the Cd group and that curcumin decreased the cell damage by suppressing the inflammation.

In addition, the high average TOS level and low TAC level in the Cur group suggest that curcumin administration as an antioxidant in healthy people may induce oxidative stress by acting as a prooxidant, which is true for many other antioxidants. Any antioxidant that acts as an antioxidant and free radical scavenger in tissues where oxidative stress is intense may cause oxidative damage as a reverse prooxidant in healthy tissues. Our

results are supported by the average MPO and thiol levels observed in the Cur group. MPO and thiol levels of the Cur group were also very close to the Cd group.

Curcumin is a compound that has antioxidant properties. However, studies have shown that curcumin has both cytoprotective and cytotoxic effects. Studies conducted in the field of cell culture have shown that when H_2O_2 and curcumin were simultaneously added to the culture medium of NG108-15 nerve cells, curcumin protected the cells from H_2O_2 -induced oxidative damage. However, it could not tolerate the cytotoxic effect of H_2O_2 in the cells that were first treated with curcumin and in which damage was produced with H_2O_2 later (37). In different studies, it has been reported that curcumin is a dose-dependent prooxidant (38, 39).

IL-6 is a multifunctional cytokine that is involved in the regulation of the immune response, hematopoiesis, and inflammation. Various toxins, bacterial and viral infections, and cancers lead to an increase in IL-6 levels and in many other cytokines (40). Souza et al. (41) have shown that $CdCl_2$ in cell cultures increases the IL-6 expression in HepG2 cells. In different studies, it has been shown that Cd increases and curcumin decreases IL-6 levels (42, 43). Our study demonstrated that the serum IL-6 level increased in the Cd group (254.09 ± 40.27) and decreased in the Cd+Cur group (209.77 ± 39.92).

PCT is a calcitonin hormone precursor that is produced by neuroendocrine cells in the intestines, pancreas, lung, and in the C cells of the thyroid and is an early marker of inflammation (44). While the PCT level in the Cd group increased, it decreased in the Cd+Cur group ($p < 0.000$). The similar course of PCT and IL-6 levels may be interpreted as Cd damage increases inflammation and curcumin decreases it.

Conclusion

Thus, curcumin reduced Cd-induced apoptotic cell death and suppressed inflammation and oxidative stress. We also concluded that curcumin, whose prooxidant characteristic was identified and which is planned to be used in healthy people as a prophylactic agent, should be considered, and further studies should be conducted in this regard.

Ethics Committee Approval: Ethics committee approval was received for this study from the central ethics committee for laboratory animals of Bezmialem University.

Informed Consent: Informed consent is not required in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - N.B., M.E., M.K., M.I., S.K., E.K.; Design - N.B., M.E., M.K., M.I., S.K., E.K.; Supervision -

N.B., M.E., M.K., M.I., S.K., E.K.; Funding - N.B., M.E., M.K., M.I., S.K., E.K.; Materials - N.B., S.K.; Data Collection and/or Processing - N.B., M.K., S.K.; Analysis and/or Interpretation - N.B., M.E., M.I., E.K.; Literature Review - N.B., M.K.; Writing - N.B., M.E.; Critical Review - N.B., M.E., M.K., M.I., S.K., E.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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