



AMELIORATIVE EFFECT OF CURCUMIN AGAINST CADMIUM-INDUCED HEPATOTOXICITY IN RATS

Attia M. Gabr¹, Tarek A. Salem^{2*}, Hesham S. Ata³ and Abdel Raheim M. Meki⁴

¹Department of Pharmacology, Faculty of Medicine, Suez Canal University, Egypt.

²Departments of Biochemistry, College of Medicine, Qassim University, KSA.

³Departments of Pathology, College of Medicine, Qassim University, KSA.

⁴Department of Biochemistry, Faculty of Medicine, Assiut University, Egypt.

ABSTRACT

Health hazards from increased cadmium (Cd) exposure as a result of industrial and environmental pollution are recognized. For thousands of years, curcumin (Cur) has been used in the Orient as a healing agent for a wide range of inflammatory, neoplastic and other conditions. In recent years, extensive *in vitro* and *in vivo* studies suggested that curcumin has anticancer, antiviral, antiarthritic, anti-amyloid, antioxidant, and anti-inflammatory properties. The present study investigated the effect of Cd on the oxidative status biomarkers in tissue of liver of rats. Meanwhile, the potential protective effect of Cur against Cd hepatotoxicity was investigated. Cadmium group exhibited a marked decline in liver tissue of superoxide dismutase (SOD) and glutathione (GSH). However, the treatment with Cur ameliorated Cd-induced malondialdehyde (MDA) and oxidative stress in liver tissue as they provoked the antioxidant defense system more significantly. The histological examination of liver proved the liver injury induced by Cd where it showed focal inflammatory cell infiltration, congestion, ballooning degeneration and nuclear fragmentations. While, co-treatment of Cd with Cur ameliorated the cellular texture of the liver. These findings show that Cd induces hepatic oxidative damage. Also, results suggest the curative action of Cur since it exhibited the ability to resist the harmful action of Cd and to protect the liver from oxidative damage.

Key words: Cadmium, Curcumin, Hepatotoxicity, Histopathology, Oxidative status.

INTRODUCTION

Cadmium (Cd) is an important heavy metal and widely known as an environmental contaminant. Generally, exposure of the Cd is being via consumptions of contaminated food and drinking water. In part, Cd exposure in humans seems to be related to their occupation in battery and paint manufacture utilizing Cd and also due to consumption of cigarette and alcoholic beverages (El-Demerdash *et al.*, 2004; de Souza Predes *et al.*, 2010). It was reported that exposure to Cd can produce wide range of biochemical and physiological dysfunctions, can cause both acute and chronic tissue injury and can damage various organs, including liver in

human beings and experimental animals (Sarkar *et al.*, 1995). Liver, responsible for maintaining the body's metabolic homeostasis, has been considered as the target organ for the toxic effects of Cd (Eybl *et al.*, 2006). The adverse effects of this metal include oxidative damage within the tissues and inflammation. These effects are considered an early sign of its toxicity and have been linked with carcinogenesis (Waalkes, 2000).

The generation of reactive oxygen species (ROS) followed by development of oxidative stress in the target organs is one of several mechanisms through which Cd exerts its toxicity (Casalino *et al.*, 2002). The scavenging potential of liver is normally maintained by adequate levels of antioxidants (Shaikh *et al.*, 1999). Long term exposure to Cd leads to an increase in lipid peroxidation and causes inhibition of superoxide dismutase (SOD)

*Corresponding Author

Prof. Tarek Abdulraoof Salem

Email: tareksalem@qumed.edu.sa

activity indicating oxidative damage in liver (Casalino *et al.*, 1997 and Elmissiry and Shalaby, 2000). The increase in lipoperoxidation may be attributed to alterations in the antioxidant defense system. This defense system includes the glutathione (GSH) and the enzymes, catalase (CAT) and superoxide dismutase (SOD), which normally protect against radical toxicity (Rana and Verma, 1996). Recently, our study showed that Cd toxicity increased significantly the plasma levels of tumor necrosis factor alpha and interleukin-6 as well as systemic oxidative stress (Alghasham *et al.*, 2013).

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical disease. More attention has been paid to the protective effects of natural antioxidants against chemically induced toxicities (Jurenka, 2009). Nowadays, curcumin is considered as a source of dietary constituents endowed with biological and pharmacological activities with potential benefits to human health. Curcumin is an important natural phytochemical compound and is found in turmeric, a yellow curry spice with a long history of use in traditional Indian diets and herbal medicine. It has several pharmacological and biological properties including anticancer, anti-inflammatory, antimicrobial, antiviral, antifungal and antioxidant (Aggarwal *et al.*, 2007; Ciftci *et al.*, 2010). Recent studies showed that curcumin exerts powerful oxygen free radical scavenger effects and increases intracellular glutathione concentration, thereby protects against lipid peroxidation (Ciftci *et al.*, 2011a, b; Ciftci *et al.*, 2012a, b). Accordingly, the present study was designed to investigate the toxicity of Cd in the liver of rats and to clarify the curative action of curcumin against the hepatotoxic action of cadmium.

MATERIALS AND METHODS

Chemicals

Cadmium chloride (CdCl₂, 98% purity) was purchased from Sigma-Aldrich Company (St. Louis, MO, USA). Other chemicals used in the present study were of analytical grade. Fine crude ground curcumin was purchased from the local market of Buraidah City, Qassim, KSA.

Animal treatment

Sixty healthy male Sprague–Dawley rats (about 170–200 g body weight) were purchased from Animal House, College of Pharmacy, King Saud University. All animals were conditioned at room temperature at a natural photoperiod for 2 weeks before experiment execution. A commercial balanced diet and tap water ad libitum were provided. The duration of experiment was 6 weeks. The rats were randomly divided into 4 groups (15 rats each) as the following: Group I (control group), received distilled water as sole drinking source (negative control). Group II: The rats were orally administrated 50 mg/kg of curcumin

daily (Cur-group) (Eybl *et al.*, 2004). Group III: Animals were received 40 mg CdCl₂/Lin drinking water, daily (Cd-group) (Eybl *et al.*, 2004). Group IV: Animals were received 40 mg CdCl₂/Lin drinking water and 50 mg/kg of curcumin daily (Cd+Cur-group).

After 6 weeks of treatments, the animals of all groups were weighted and then anaesthetized using diethyl ether and sacrificed. Blood specimens were collected on heparin-containing tubes. After centrifugation at 2500 rpm for 10 min., plasma were separated and kept in small aliquots for further laboratory assays. Livers were excised immediately, washed in physiological saline and weighted. The liver organ was divided into two parts. One part was prepared for histology. Livers were stored in 10% neutral buffered formalin and processed for histopathological examination. The second part was homogenized in ice-cold 100mM phosphate buffer (pH 7.4) using a Potter-Elvehjem homogenizer fitted with a Teflon Plunger. Homogenates were centrifuged at 11,000 g for 20 min and resulting supernatants were divided into aliquots and stored at –80°C.

Biochemical analysis of liver function

Plasma alanine transaminase (ALT) and aspartate transaminase (AST) activities were estimated according to the method of Reitman and Frankel (1957). Alkaline phosphatase (ALP) activities were measured in plasma according to the method of kind and king (1954). Total proteins levels in plasma and in tissue homogenates of liver were determined by the method of Lowry *et al.* (1951).

Determination of hepatic oxidative status markers

Superoxide dismutase (SOD) activity in tissue homogenates of liver was determined according to the procedure of Nishikimi *et al.* (1972). Levels of reduced glutathione (GSH) in tissue homogenates were estimated by using the method mentioned by Dutta *et al.* (1995). The levels of the end product of lipid peroxidation, malondialdehyde (MDA) were measured in liver homogenate according to the method of Ohkawa *et al.* (1979).

Histological Examination

After autopsy of all animals, livers were dissected out and fixed in 10% neutral buffered formalin and rinsed with 70% ethanol, dehydrated in serial dilutions of ethanol before embedding in paraffin wax. Paraffin blocks of the tissues were sectioned at 5 μm thickness in a rotary microtome. Sections of each block were processed stained with haematoxylin and eosin for histopathological evaluation by method described by (Bogdanovic *et al.*, 2008).

Statistical Analysis

The results were expressed as mean ± standard error (SE). Differences between groups were assessed by

one-way analysis of variance (Bonferroni test) using the Prism version 5 software package for Windows. Spearman rank correlation analysis was performed to investigate the relationship among the parameters studied. The level of significance was accepted with $P < 0.05$.

RESULTS

Liver and body weight

The Cd-treated rats were found to be significantly higher in liver weight and significantly lower in body weights in comparison with the control rats. Treatment of rats with combination of Cd and curcumin reversed the effects caused by Cd alone (Table 1).

Effect of cadmium on liver enzymes

Liver enzymes, ALT, AST and ALP activities were significantly elevated in Cd group in comparison with controls. These enzymes were significantly reduced in Cur+Cd group comparing with Cd group (Table 2).

Levels of malondialdehyde, glutathione and superoxide dismutase in liver homogenates

The levels of MDA in the tissues homogenates of liver were significantly higher in Cd-group than control group. In the Cur+Cd group, the levels of MDA in the studied tissues were significantly reduced comparing to Cd group. The levels of GSH in tissues of liver were significantly declined in Cd-group comparing with controls. In the Cur+Cd group, the levels of GSH were

significantly elevated in comparing with Cd-group in the studied tissues. The SOD activities in tissue homogenates of liver were significantly decreased in Cd group comparing with controls. Moreover, in the Cur+Cd group, the SOD activities in liver tissues were significantly higher than their activities in Cd group (Table 3).

Histopathological examination of rat liver

Livers of Cd-treated rats for 6 weeks showed many pathological alterations in the form of focal inflammatory reactions proved by mononuclear cell infiltration, cellular degeneration including hydropic (ballooning), hyaline degeneration and fatty change, disorganization by blurring of the normal lobular architecture, congestion, focal necrosis and apoptosis proved by cellular swelling, deep cytoplasmic acidophilia, nuclear pyknosis, caryorrhexis and nuclear fragmentation (Figures 1A, B, C, and D). These changes were found throughout the sections without any specific zonal localization. Livers of rats that treated with combination of cadmium and curcumin for 6 weeks showed some pathological changes in the form of focal inflammatory cell infiltration, cellular degeneration including hydropic (ballooning) degeneration and fatty change. Normal lobular architecture was preserved but still there was venous congestion and focal necrosis (Figures 2A, B). Control rats and curcumin- treated ones showed no abnormal findings (Figure 3).

Table 1. Effect of curcumin and Cd on the weights of the body and liver of different groups of rats

Groups	Body Weight (g)		Liver Weight (g)
	Start	End	
Controls	152 ± 1.78	164 ± 1.78	3.2 ± 0.09
Cur group	150 ± 1.45	167 ± 2.17	2.8 ± 0.08
Cd group	151 ± 1.58	136 ± 2.02 ^{**}	4.3 ± 0.07 [*]
Cur + Cd group	152 ± 1.47	161 ± 2.43	3.1 ± 0.03 [§]

Values are shown as mean ± SE for 15 rats. ^{*}; $P < 0.05$ and ^{**}; $P < 0.01$, respectively, as compared to control group. [§] $P < 0.05$ as compared to Cd-treated group.

Table 2. Plasma levels of bio-indices of liver function in different groups of male rats

Plasma level (Bio-indices)	Controls	Cur group	Cd group	Cur + Cd group
Total protein (g/dL)	7.58 ± 0.49	7.92 ± 0.54	4.50 ± 0.26 ^{***}	6.88 ± 0.37 ^{§§}
AST (U/L)	9.5 ± 0.41	8.7 ± 0.23	31.7 ± 1.65 ^{***}	13.4 ± 1.09 ^{§§}
ALT (U/L)	13.6 ± 0.54	12.1 ± 0.49	45.3 ± 2.46 ^{***}	15.8 ± 0.90 [§]
Alk phosphate (U/L)	97.6 ± 2.30	94.3 ± 2.95	198.5 ± 3.39 ^{***}	121.4 ± 3.75 ^{§§}

Values are means ± SE for 15 rats. P values are shown as ^{***}, $P < 0.001$ for comparison Cd-treated group versus controls. [§] $P < 0.05$ and ^{§§}, $P < 0.01$ for comparison Cur+Cd group versus Cd-treated group.

Table 3. Hepatic oxidative status markers in different groups of the rats

Markers	Controls	Cur-group	Cd group	Cur + Cd group
MDA (nmol / mg protein)	0.99 ± 0.09	0.88 ± 0.04	2.59 ± 0.19 ^{***}	1.66 ± 0.15 ^{§§§}
GSH (nmol / mg protein)	31.57 ± 3.08	35.60 ± 1.65	19.90 ± 1.88 ^{**}	24.84 ± 1.48 [§]
SOD (mU / mg protein)	2.58 ± 0.19	2.09 ± 0.16	1.36 ± 0.21 ^{***}	1.61 ± 0.17

Values are means ± SE for 15 rats. P values are shown as ^{***}, $P < 0.001$ for comparison Cd-treated group and Cur-treated group versus controls. [§], $P < 0.05$ and ^{§§§}, $P < 0.001$ for comparison Cd+Cur group versus Cd-treated group.

Figure 1. Hematoxylin and Eosin staining of rats' liver after treatment with cadmium (0.4%) for 6 weeks. (A) Shows marked fatty change and focal necrosis; (B) shows fatty change, pyknotic nuclei and loss of lobular pattern; (C) shows marked necrosis, apoptosis, fatty change, loss of lobular pattern and venous congestion and (D) shows apoptotic bodies

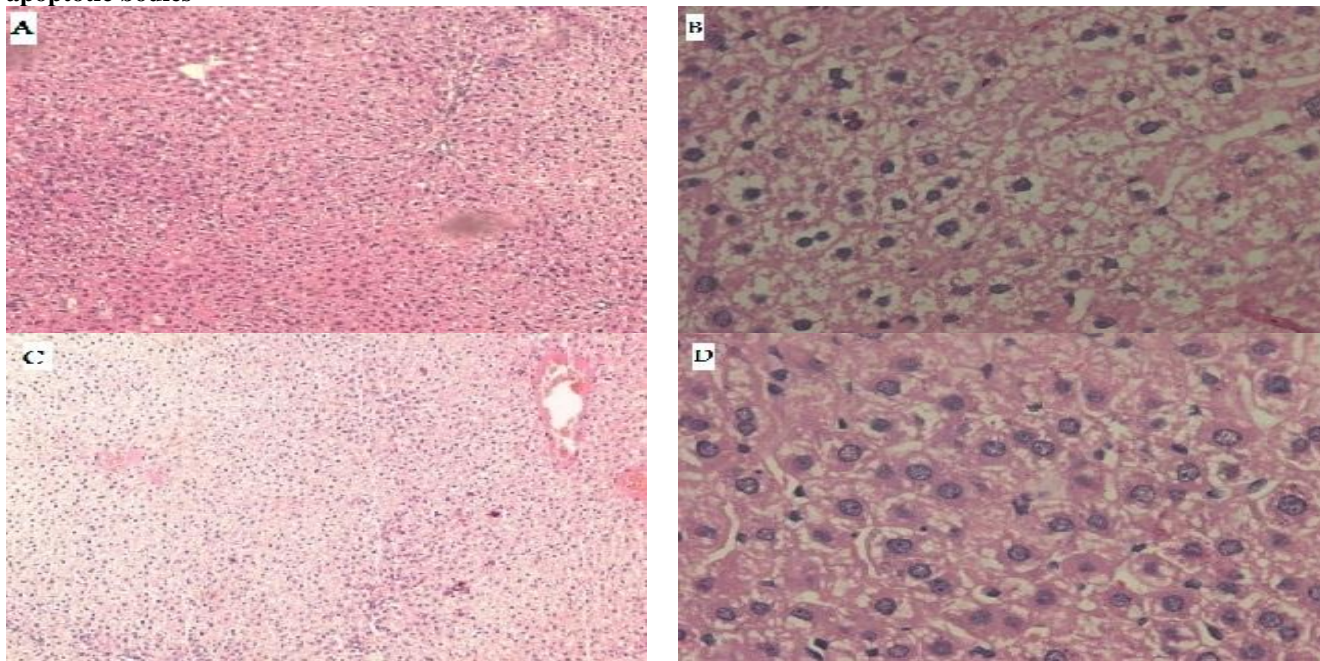


Figure 2. Hematoxylin and Eosin staining of rats' liver after cotreatment with cadmium (0.4%) and curcumin (50 mg/kg body weight) for 6 weeks. (A) and (B) Show foci of hepatocellular necrosis and fatty change with preservation of the lobular pattern

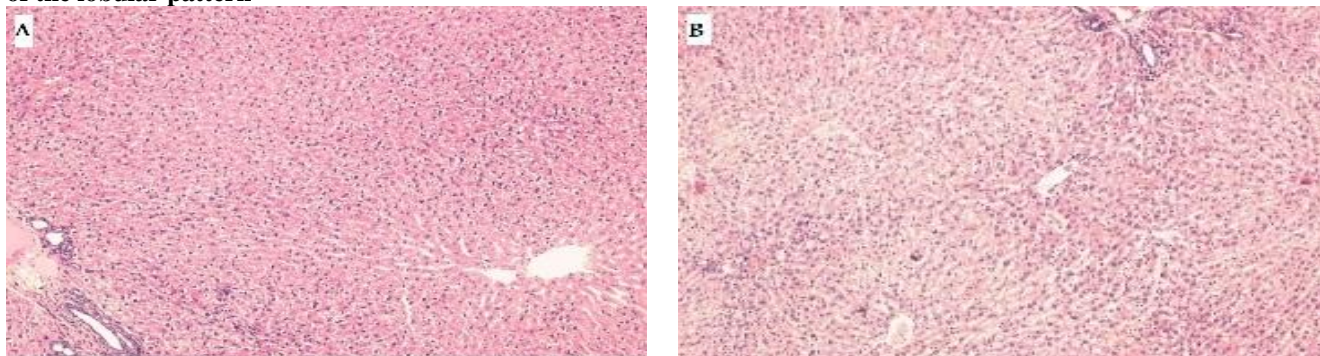
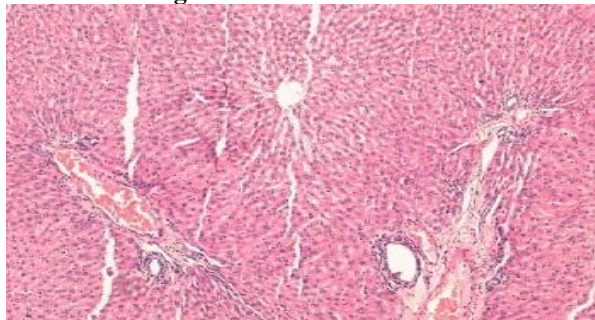


Figure 3. Hematoxylin and Eosin staining of liver tissues of normal rats shows normal liver architecture



DISCUSSION

Cadmium (Cd) represents a dangerous environmental and industrial pollutant. Cd is found in foods (vegetables, grains, and cereals), water, and tobacco leaves. It accumulates unevenly in human tissues, and is concentrated primarily in lungs, liver, kidneys, brain, heart, and testes (Gerhardsson *et al.*, 2002). Cadmium is a very toxic heavy metal since it cannot undergo biotransformation along with low excretion rate. Therefore, it has a longer biological half-life ranged from 15 to 30 years and hence accumulates primarily in the liver and kidneys with concentrations higher than those present in erythrocytes, lungs, pancreas, thyroid, testis, salivary glands and placenta (Bhattacharyya *et al.*, 2000; Henson and Anderson, 2000). The level of Cd used in this study corresponds to human occupational exposure or human environmental exposure in heavily contaminated areas (Brzoska *et al.*, 2004; Brzoska *et al.*, 2005). Liver is one of the most critical organs for the toxicity of Cd and is a target organ for cadmium toxicity (Nordberg and Nordberg, 1975; Suzuki, 1980). It can be subject to distinct pathological (Muller, 1986; Theocharis *et al.*, 1991; Koyu *et al.*, 2006) and morphological (Mitsumori *et al.*, 1998) changes under Cd effect.

The present study revealed clear signs of Cd toxicity with reference to decreased body weight and increased liver weight (Table 1). These findings are in agreement with previous reports demonstrating that Cd toxicity leads to abnormal body and organ weight (Deepti *et al.*, 2010; Zhang *et al.*, 2013). Moreover, the present study has also clearly demonstrated the hepatotoxic effect of Cd as it elevated the activities of ALT, AST and ALP in plasma of rats after oral administration of Cd (Table 2). Our results are in agreement with others who found similar increase in AST and ALT in the blood due to cadmium administration (Asagba and Eriyamremu (2007); Guilhermino *et al.*, (1998); Kowalczyk *et al.*, (2003). Other investigators, also found oxidative stress, liver dysfunction and liver damage in animals administered oral cadmium. (Zhang *et al.*, 2013; Arafa *et al.*, 2014). Khaled *et al.*, (2008) reported that the free radicals attack hepatic cells, leading to hepatic toxicity and dysfunction that are supported by the increase in ALT, AST and ALP activities. Previous results suggested that oxidative stress may be involved in the mechanism leading to changes in the plasma concentration of cytokines after exposure to Cd for 6 weeks (Alghasham *et al.*, 2013).

The hepatotoxicity of Cd may be associated with the production of reactive oxygen species (ROS) (Szuster-Ciesielska *et al.*, 2000). Lipid peroxidation is a manifestation of oxidative damage and has been found to play an important role in the toxicity of many xenobiotics (Flora *et al.*, 2009). Lipid peroxidation is also considered as the primary mechanism for Cd toxicity despite its

inability to directly generate free radicals (Eneman *et al.*, 2000). The displacement of iron and copper from various intracellular sites by Cd increases the concentrations of ionic iron and copper which in turn causes oxidative stress through Fenton reaction producing hydroxyl radical species which are believed to initiate lipid peroxidation (Yinn *et al.*, 1999). Decomposition products of lipid hydroperoxides such as malondialdehyde (MDA) and 4-hydroxynonenal can cause chaotic cross linkage with proteins and nucleic acids which plays an important role in Cd-induced hepatotoxicity.

Our results revealed that the levels of MDA were significantly higher while GSH and SOD were significantly lower in the tissues homogenates of liver in Cd-group than control group (Table 3). Zhang *et al.* (2013) observed similar effects of Cd on SOD activity and MDA levels in liver tissues. The imbalance between antioxidant/oxidant induced by Cd was also observed by other authors (Eriyamremu *et al.*, 2008), where, it caused an increase in hydroxyl radicals (Liu *et al.*, 2008), superoxide anions, nitric oxide and hydrogen peroxide production (Hsu *et al.*, 2007; Chen *et al.*, 2008; Liu *et al.*, 2008).

Cadmium is known to produce oxidative damage in the liver by enhancing peroxidation of membrane lipids, a deleterious process solely carried out by free radicals (Hassoun and Stohs, 1996). Our results revealed diminished total GSH with Cd treatment. GSH is known to play a major role in the regulation of intracellular levels of reactive oxygen species by direct reaction, scavenging, or via the GSH peroxidase/GSH system. Thus, GSH is a major cellular antioxidant and protects cells against oxidative damage. Direct binding of Cd by GSH may also be important in reducing toxicity.

The results of our previous study (Alghasham *et al.*, 2013) confirmed that drinking (40 mg CdCl₂/L) Cd-containing water for 6 weeks resulted in significant elevations in the plasma levels of TNF- α and IL-6 as compared to those of control group. These findings may be due to the immune-modulatory action of Cd on the immune cells. In this respect, involvement of TNF- α in the development of an acute inflammatory response characterized by a rise of acute phase reactants following sub-chronic intravenous Cd administration in mice was implicated and its role in Cd hepatotoxicity has been proposed (Kayama *et al.*, 1995). Chen *et al.* (2003) suggested that the massive release of TNF- α induced by Cd loading may play an important role in the induction of malfunction of multiple systems or organs in rats.

Curcumin, an antioxidant and anti-carcinogenic substance, was reported to have a protective effect against iron-induced liver damage and Fe-NTA-induced peroxidation of microsomal membrane lipids as well as DNA damage in kidneys (Pulla Reddy and Lokesh, 1996;

Iqbal *et al.*, 2003a,b). A protective action of curcumin was referred also against adverse effects of cisplatin (Antunes *et al.*, 2000). In the current study, the significant elevation of MDA levels and the significant reduction of the activity of SOD and GSH levels in the liver clearly indicate that Cd is able to induce oxidative stress during repeated administration. In contrast, curcumin administration with Cd decreased significantly the levels of MDA and increased significantly the antioxidant indices (SOD activity and GSH levels) in the liver tissue homogenates. 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 (Priyadarsini *et al.*, 2003).

The hepatotoxic effect of Cd was confirmed by histopathological examination of liver section where it is revealed that orally administration of Cd resulted in liver injury that includes foci of inflammation, congestion and various degrees of focal necrosis. On a cellular level, toxicity included hepatocyte swelling, pyknosis, and karyorrhexis (Figure 1C). These findings may interpret the elevation of liver enzymes in plasma. The previous studies of several investigators supported our results where they proved the liver injury after exposure to Cd (Dudley *et al.*, 1982; Eric and Curtis, 2002). Other researchers observed that long-term Cd exposure was related to hepatic lesions, such as small focal necrosis, fatty degeneration, and fibrosis (Kaufmann *et al.*, 1984). In our study, we administered Cd for a relatively short period which induced inflammation, congestion, and necrosis.

The present study indicated that the harmful effects of Cd on histopathological of liver were prevented variably by co-treatment with curcumin. As we explained before, the co-treatment with curcumin ameliorated the toxic effects of Cd; the levels of MDA were significantly reduced while the levels of GSH and SOD were significantly elevated in tissues of liver in comparison with Cd-group (Table 3).

Several studies have demonstrated that curcumin was able to modulate the production of various inflammatory mediators, thereby exhibiting potent anti-inflammatory activity (Alghasham *et al.*, 2013). In diabetic rats, chronic treatment with curcumin significantly reduced serum TNF- α level, attenuating cognitive deficit, oxidative stress and inflammation (Kuhad and Chopra, 2007). In the carbon tetrachloride rat

model, curcumin protected the rat liver from CCl₄-induced injury and fibrogenesis by reducing inflammatory cytokines levels in the liver (Fu *et al.*, 2008). Previous studies of several investigators showed that curcumin is a potent antioxidative compound, and because of this property, it strongly protects tissues against the oxidative stress (Ilbey *et al.*, 2009; Sharma and Singh, 2010; Ciftci *et al.*, 2011a).

Our findings revealed that curcumin treatment alleviated the toxic effects of Cd on liver histology, when given together with Cd. Similarly, Aktas *et al.* (2012) investigated the histopathologic effects of curcumin against Cd toxicity, and they reported that Cd leads to severe histological damage, including apoptotic cell death, and curcumin treatment partially reversed these effects of Cd. It was assumed that there is a correlation between the histological findings and oxidative status, and the histopathological effects may reflect the oxidative stress in liver tissue induced by Cd. For this reason, we claim that curcumin may exert a protecting effect against Cd-induced liver injury due to its ameliorating effects of elevated oxidative stress and preventive effects of histological damage in liver tissue when Cd toxicity occurs.

CONCLUSION

The present findings revealed that cadmium causes an oxidative stress in the tissue of liver. The oxidative stress may be involved in the mechanism of the Cd toxicity. Such events may be, at least in part, responsible for the morphological changes in liver tissues associated with Cd exposure. The findings of the current study also illustrate that exogenously administered curcumin is capable of reversing the oxidative toxic effects of cadmium. These data suggest that curcumin, by preventing hepatotoxicity, may enhance the selectivity of this herb in the patients who occupationally exposed to cadmium.

Conflict of interest

The authors declare that there are no conflicts of interest.

ACKNOWLEDGMENT

This study was supported by grant (number SR-S-011-20) from Deanship of scientific research, SABIC, Qassim University, Saudi Arabia. We are grateful to our lab specialists for helping in experiment done in the lab.

REFERENCES

- Aggarwal BB, Sundaram C, Malani N, Ichikawa H. Curcumin: The Indian solid gold. *Adv Exp Med Biol*, 595, 2007, 1–75.
- Aktas C, Kanter M, Erbog M, Ozturk S. Anti-apoptotic effects of curcumin on Cd-induced apoptosis in rat testes. *Toxicol Ind Health*, 28(2), 2012, 122-30.

- Alghasham A, Salem TA, Meki AR, Effect of cadmium-polluted water on plasma levels of tumor necrosis factor- α , interleukin-6 and oxidative status biomarkers in rats: protective effect of curcumin. *Food Chem Toxicol*, 59, 2013, 160-4.
- Antunes LM, Araujo MC, Darin JD, Bianchi ML. Effects of the antioxidants curcumin and vitamin C on cisplatin-induced clastogenesis in Wistar rat bone marrow cells. *Mutat Res*, 465, 2000, 131-137.
- Arafa MH, Mohammad NS, Atteia HH. Fenugreek seed powder mitigates cadmium-induced testicular damage and hepatotoxicity in male rats. *Exp Toxicol Pathol*, 66(7), 2014, 293-300.
- Asagba SO, Eriyamremu GE. Oral cadmium exposure alters haematological and liver function parameters of rats fed a Nigerian-like diet. *J Nutr Environ Med*, 16, 2007, 267-274.
- Bhattacharyya MH, Wilson AK, Rajan SS, Jonah M. Biochemical pathways in cadmium toxicity, in: Zalup, R.K, Koropatnick, J, (Eds.), *Molecular biology and toxicology of metals*. London: Taylor and Francis, 2000, 34-74.
- Bogdanovic M, Janeva AB, Bulat B. Histopathological changes in rat liver after a single high dose of Aluminum. *Arch Indust Hyg Toxicol*, 59, 2008, 97-101.
- Casalino E, Calzaretti G, Sblano C, Landriscina C. Molecular inhibitory mechanisms of antioxidant enzymes in rat liver and kidney by cadmium. *Toxicol*, 30, 2002, 37-50.
- Casalino E, Sblano C, Landriscina C. Enzyme activity alteration by cadmium administration to rats: the possibility of iron involvement in lipid peroxidation. *Arch Biochem Biophys*, 346, 1997, 171-179.
- Chen L, Liu L, Huang S, Cadmium activates the mitogen-activated protein kinase (MAPK) pathway via three inductions of reactive oxygen species and inhibition of protein phosphatases 2A and 5. *Free Rad Biol Med*, 45(7), 2008, 1035-44.
- Chen L, Zhou J, Gao W, Jiang YZ, Action of NO and TNF- α release of rats with cadmium loading in malfunction of multiple system organ. *Sheng Li Xue Bao*, 55(5), 2003, 535-40.
- Ciftci O, Tanyildizi S, Godekmerdan A. Protective effect of curcumin on immune system and body weight gain on rats intoxicated with 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). *Immunopharmacol Immunotoxicol*, 32, 2010, 99-104.
- Ciftci O, Aydin M, Ozdemir I, Vardi N. Quercetin prevents 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin-induced testicular damage in rats. *Andrologia*, 44(3), 2012a, 164-73.
- Ciftci O, Beytur A, Cakir O, Gurbuz N, Vardi N. Comparison of reproductive toxicity caused by Cisplatin and novel platinum-N-heterocyclic carbene complex in male rats. *Basic Clin Pharmacol Toxicol*, 109, 2011a, 328-333.
- Ciftci O, Ozdemir I, Aydin M, Beytur A. Beneficial effects of chrysin on the reproductive system of adult male rats. *Andrologia*, 44(3), 2012b, 181-6.
- Ciftci O, Ozdemir I, Tanyildizi S, Yildiz S, Oguzturk H. Antioxidative effects of curcumin, b-myrcene and 1,8-cineole against 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced oxidative stress in rats liver. *Toxicol Ind Health*, 27, 2011b, 447-453.
- Deepti G, Shabad P, Dua K. Chronic cadmium toxicity in rats: Treatment with combined administration of vitamins, amino acids, antioxidants and essential metals. *J Food Drug Analysis*, 18(6), 2010, 464-470.
- De-Souza PF, Diamante MA, Dolder H. Testis response to low doses of cadmium in Wistar rats. *Int J Exp Pathol*, 91, 2010, 125-131.
- Dudley RE, Svoboda DJ, Klaassen CD. Acute exposure to Cd causes severe liver injury in rats. *Toxicol Appl Pharmacol*, 65, 1982, 302-313.
- Dutta P, Seirafi J, Halpin D, Pinto J, Rivlin R. Acute ethanol exposure alters hepatic glutathione metabolism in riboflavin deficiency. *Alcohol*, 12, 1995, 43 - 47.
- El-Demerdash FM, Yousef MI, Kedwany FS, Baghdadi HH. Cadmium induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats: protective role of vitamin E and β -carotene. *Food Chem Toxicol*, 42, 2004, 1563-1571.
- El-Missiry MA, Shalaby F. Role of β -carotene in ameliorating the cadmium induced oxidative stress in rat brain and testis. *J Biochem Mol Toxicol*, 14, 2000, 238-243.
- Eneman JD, Potts RJ, Osier M, Shukla GS, Lee CH, Chin JF. Suppressed oxidant-induced apoptosis in cadmium adapted alveolar epithelial cells and its potential involvement in cadmium carcinogenesis. *Toxicol*, 147, 2000, 215-228.
- Eric BH, Curtis DK. Analysis of strain difference in sensitivity to Cd-induced hepatotoxicity in Fischer 344 and Spargue-Dawley rats. *Toxicol Sci*, 67, 2002, 329-340.
- Eriyamremu GE, Ojिमogho SE, Asagba SO, Osagie VE. Palm oil induced changes in ocular tissue lipid peroxidation, antioxidant enzymes and ATPases of rabbits in cadmium toxicity. *Food Chem Toxicol*, 46(9), 2008, 3155-8.
- Eybl V, Kotyzová D, Bludovská M. The effect of curcumin on cadmium induced oxidative damage and trace elements level in the liver of rats and mice. *Toxicol Lett*, 151(1), 2004, 79-85.

- Eybl V, Kotyzová D, Lesetický L, Bludovská M, Koutenský J. The influence of curcumin and manganese complex of curcumin on Cd-induced oxidative damage and trace elements status in tissues of mice. *J Appl Toxicol*, 26, 2004, 207-212.
- Flora SJS, Mehta A, Gupta R. Prevention of arsenic induced hepatic apoptosis by concomitant administration of garlic extraction in mice. *Chem Biol Interact*, 177, 2009, 227-233.
- Fu Y, Zheng S, Lin J, Ryerse J, Chen A. Curcumin protects the rat liver from CCl₄-caused injury and fibrogenesis by attenuating oxidative stress and suppressing inflammation. *Mol Pharmacol*, 73, 2008, 399-409.
- Gerhardsson L, Englyst V, Lundstrom NG, Sandberg S, Nordberg G. Cadmium, copper and zinc in tissues of deceased copper smelter workers. *J Trace Elem Med Biol*, 16, 2002, 261-266.
- Guilhermino L, Soares AMVM, Carvalho AP, Lopes MC. Effects of cadmium and parathion exposure on hematology and blood biochemistry of adult male rats. *Bull Environ Contam Toxicol*, 60, 1998, 52-59.
- Hassoun EA, Stohs SJ. Cadmium induced production of superoxide anion and nitric oxide, DNA single strand breaks and lactate dehydrogenase leakage in J774A1 cell cultures. *Toxicol*, 112, 1996, 219-226.
- Henson MC, Anderson MB. The effects of cadmium on placental endocrine function. *Recent Res Dev Endocrinol*, 1, 2000, 37-47.
- Hsu CY, Chan YP, Chang J. Antioxidant activity of extract from *Polygonum cuspidatum*. *Biol Res*, 40, 2007, 13-21.
- Ilbey YO, Ozbek E, Cekmen M, Simsek A, Otunctemur A, Somay A. Protective effect of curcumin in cisplatin induced oxidative injury in rat testis: mitogen-activated protein kinase and nuclear factor-kappa B signaling pathways. *Human Reprod*, 24, 2009, 1717-1725.
- Iqbal M, Okazaki Y, Okada S. In vitro curcumin modulates ferric nitrilotriacetate (Fe-NTA) and hydrogen peroxide-induced peroxidation of microsomal membrane lipids and DNA damage. *Teratog Carcinog Mutagen*, 23(1), 2003b, 151-160.
- Iqbal M, Sharma SD, Okazaki Y, Fujisawa M, Okada S. Dietary supplementation of curcumin enhances antioxidant and phase II metabolizing enzymes in ddY male mice: possible role in protection against chemical carcinogenesis and toxicity. *Pharmacol Toxicol*, 92, 2003a, 33-38.
- Jurenka JS. Anti-inflammatory properties of curcumin, a major constituent of *Curcuma longa*: a review of preclinical and clinical research. *Altern Med Rev*, 14(2), 2009, 141-53.
- Kaufmann I, Höpker WW, Deutsch-Diescher OG, Seitz HK, Götz R, Kommerell B. Liver damage caused by chronic cadmium poisoning. *Leber Magen Darm*, 14, 1984, 103-106.
- Kayama F, Yoshida T, Elwell MR, Luster MI. Role of tumor necrosis factor-alpha in cadmium-induced hepatotoxicity. *Toxicol. Appl. Pharmacol*, 131(2), 1995, 224-34.
- Khaled H, Serge C, Fatma, A, Hatem M, Abdelfattah F. Positive effects of green tea on hepatic dysfunction, lipid peroxidation and antioxidant defense depletion induced by Cd. *Biol Res*, 41, 2008, 331-339.
- Kind PR, King EJ. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. *J Clin Pathol*, 7(4), 1954, 322-326.
- Kowalczyk E, Kopff A, Fijalkowski P, Kopff M, Niewiarok J, Blaszczyk J, Kedziora J, Tyslerowicz P. Effect of anthocyanins on selected biochemical parameters in rats exposed to cadmium. *Acta Biochim Pol*, 50, 2003, 543-548.
- Koyu A, Gokcimen A, Ozguner F, Bayram DS, Kocak A. Evaluation of the effects of cadmium on rat liver. *Mol Cell Biochem*, 284(1-2), 2006, 81-85.
- Kuhad A, Chopra K. Curcumin attenuates diabetic encephalopathy in rats: behavioral and biochemical evidences. *Eur J Pharmacol*, 576, 2007, 34-42.
- Liu J, Qian SY, Guo Q, Jiang J, Waalkes MP, Mason RP, Kadiiska MB. Cadmium generates reactive oxygen- and carbon-centered radical species in rats: Insights from in vivo spin-trapping studies. *Free Rad. Biol. Med*, 45, 2008, 475-81.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem*, 193, 1951, 265-275.
- Mitsumori K, Shibusaki M, Sato S, Onodera H, Nakagawa J, Hayashi Y, Ando M. Relationship between the development of hepato-renal toxicity and cadmium accumulation in rats given minimum to large amounts of cadmium chloride in the long term: preliminary study. *Arch Toxicol*, 72(9), 1998, 545-552.
- Muller L. Consequences of cadmium toxicity in rathepatocytes: mitochondrial dysfunction and lipid peroxidation. *Toxicol*, 40, 1986, 285-295.
- Nishikimi M, Rao NA, Yog K. Colorimetric determination of superoxide dismutase activity. *Biochem Biophys Res Commun*, 46 (1), 1972, 849-851.
- Nordberg M, Nordberg GF. Distribution of metallothionein-bound cadmium and cadmium chloride in mice: preliminary studies. *Environ Health Perspect*, 12, 1975, 103-108.

- Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Anal Biochem*, 95(2), 1979, 351-358.
- Priyadarsini KI, Maity DK, Naik GH, Kumar MS, Unnikrishnan MK, Satav JG, Mohan H. Role of phenolic O-H and methylene hydrogen on the free radical reactions and antioxidant activity of curcumin. *Free Rad Biol Med*, 35, 2003, 475-484.
- Pulla-Reddy AC, Lokesh BR. Effect of curcumin and eugenol on iron-induced hepatic toxicity in rats. *Toxicol*, 107, 1996, 39-45.
- Rana SVS, Verma S. Protective effects of GSH, vitamin E and selenium on lipid peroxidation in cadmium-fed rats. *Biol Trace Elem Res*, 51, 1996, 161-168.
- Reitman S, Frankel SA. Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am J Clin Path*, 28(1), 1957, 56-63.
- Sarkar S, Yadav P, Trivedi R, Bansal AK, Bhatnagar D. Cadmium induced lipid peroxidation and status of antioxidant system in rats' tissues. *J Trace Elem Med Biol*, 9, 1995, 144-149.
- Shaikh ZA, Vu TT, Zaman K. Oxidative stress as a mechanism of chronic cadmium- induced hepatotoxicity and renal toxicity and protection by antioxidants. *Toxicol Appl Pharmacol*, 154, 1999, 256-263.
- Sharma P, Singh R. Protective role of curcumin on lindane induced reproductive toxicity in male Wistar rats. *Bull Environ Contam Toxicol*, 2010, 84, 378-84.
- Suzuki Y. Cadmium metabolism and toxicity in rats after long-term subcutaneous administration. *J Toxicol Environ Health*, 6(3), 1980, 469-482.
- Szuster-Ciesielska A, Lokaj I, Kandeferszerszeń M. The influence of cadmium and zinc ions on the interferon and tumor necrosis factor production in bovine aorta endothelial cells. *Toxicol*, 145, 2000, 135.
- Theocharis S, Margeli A, Fasitsas C, Loizidou M, Deliconstantinos G. Acute exposure to cadmium causes time-dependent liver injury in rats. *Comp Biochem Physiol*, 99 (1-2), 1991, 127-130.
- Waalkes MP. Cd carcinogenesis in review. *J Inorg Biochem*, 79, 2000, 241-244.
- Yinn SJ, Chem CL, Sheu JY, Tseng WC, Lin TH. Cadmium induced renal lipid peroxidation in rats and protection by selenium. *J Toxicol Environ Health*, 57, 1999, 403-13.
- Zhang J, Song J, Zhang J, Chen X, Zhou M, Cheng G, Xie X. Combined Effects of Fluoride and Cadmium on Liver and Kidney Function in Male Rats. *Biol Trace Elem Res*, 155, 2013, 396-402.