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Evaluation of pretreatment with curcumin, vitamin C, or their combination on cadmium-induced toxicity on rat kidney, stomach and small intestine

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Abstract

Curcumin (Cur), a biologically active compound from turmeric, and vitamin C (VitC), act as a natural antioxidant and a potent chemopreventive agent. The present study aimed to evaluate whether the combined pretreatment with Cur and VitC offered more beneficial effects than that provided by either of them alone in reversing cadmium (Cd)-induced toxicity in rats. Male Wistar rats, equally divided into control and seven treatment groups; Cd (as $CdCl_2$ 5 mg/kg), Cur 400 mg/kg, VitC 100 mg/kg+Cd, Cur 200 mg/kg+Cd, Cur 400 mg/kg+Cd, Cur200+VitC+Cd and Cur400+VitC+Cd. Cur or VitC was given daily by oral gavage for 1 h before Cd administration. All groups were treated for 27 days. The results showed that Cd treatment significantly increased lipid peroxidation (MDA) levels and significantly decreased the reduced glutathione (GSH) levels in renal tissue. Moreover, the histological analysis revealed damages to the renal cortex, gastric mucosa and villi of the small intestine. The pretreatment with Cur alone (both 200 and 400 mg/kg) could protect from the changes induced by Cd although not statistically significant. VitC treatment alone showed no protection. However, the combined Cur and VitC pretreatment was significantly more effective in reducing such damages from Cd treatment and was able to reverse the effects to levels similar to that of control (particularly in Cur 400 mg/kg). In conclusion, our research suggests that Cur (both 200 and 400 mg/kg) significantly protects the animals to the same extent (no statistical significance) but in the presence of VitC, which showed no protective effect by itself, superiority of Cur 400 mg/kg over 200 mg/kg was demonstrated.

Keywords: *cadmium (Cd), Cd-induced toxicity, curcumin, kidney, rats, small intestine, stomach, vitamin C*

1. Introduction

Cadmium (Cd) is a toxic heavy metal with a biological half-life of more than 20 years; its level in the environment is increasing due to industrial activities, thereby increasing human exposure (El-Habit & Moneim, 2014; Johri, Jacquillet, & Unwin, 2010). It has been reported to bioaccumulate in vital organs, especially in the liver and kidney and adversely affects the functions of these organs (Ansari et al., 2017; Nazima, Manoharan, & Miltonprabu, 2015; Othman, Nada, Zaki, & Moneim, 2014). The kidney is recognized as a major target of Cd due to its preferential uptake by receptor-mediated endocytosis and metallothione in-bound Cd in the renal proximal tubule. When released freely into the cytosol, it can generate reactive oxygen species (ROS) and activate cell death pathways (Adefegha, Omojokun, & Oboh, 2015; Othman et al., 2014). Cd absorption from the gastrointestinal tract is one of the main routes of entry into humans (Klaassen, Liu, & Diwan, 2009; Ninkov et al., 2015). Therefore, oral administration of Cd can affect the structure and function of cells and tissues of the stomach and intestine through the gastrointestinal tract (Ninkov et al., 2016; Zhao, Hyun, Satsu, Kakuta, & Shimizu, 2006).

Cd-induced toxicity in living systems may be due to a rise in lipid peroxidation, which could be accredited to changes in antioxidant defense systems including the enzymes glutathione peroxidase and reduced glutathione (GSH), which generally offers protection to living systems from toxicity due to free radicals (Safhi et al., 2016). As oxidative stress is one of the important mechanisms of Cd-induced damages, it can be expected that the administration of some antioxidants should be an important therapeutic approach in Cd intoxication.

In the current study, two potent antioxidants, vitamin C and curcumin, are evaluated to assess their protective effects against Cd-induced

toxicity. Vitamin C (ascorbic acid) is a well-known antioxidant that protects the cellular compartment from the water-soluble oxygen nitrogen radicals (Cilla et al., 2012; Kalender, Uzun, Durak, Demir, & Kalender, 2010). It has anti-inflammatory effects; it prevents damages for endothelial dysfunction and also minimalizes the risk of cardiovascular diseases (El-Shafei & Saleh, 2016). Previous animal studies also suggested that vitamin C treatment may have potential protective effects on oxidative stress and environmental toxicities (Guo et al., 2016). Therefore, oral supplementation with vitamin C may protect animals from the harmful effects of Cd. Because of the health problems induced by many environmental pollutants, much effort has been expended in evaluating the relative antioxidant potency of vitamin C.

Curcumin is the principal natural polyphenol curcuminoid of turmeric (*Curcuma longa*) rhizome, a member of the ginger family (Zingiberaceae) (Anamika, 2012). It has been shown that curcumin is able to scavenge reactive oxygen species (ROS) (Trujillo et al., 2013). Furthermore, extensive animal studies have indicated that curcumin exerted a remarkable protective effect against damage induced by Cd and sodium arsenite (García-Niño & Pedraza-Chaverrí, 2014; Momeni & Eskandari, 2017; Oguzturk et al., 2012). Using the rat as a model, our groups has shown that curcumin administration alone can partially protect against Cd-induced nephrotoxicity (Tarasub, Tarasub, & Devakul Na Ayutthaya, 2011). However, studies regarding the co-effect of curcumin and vitamin C on Cd-induced toxicity are poorly documented. Therefore, it was considered of interest to investigate whether the combined pretreatment with curcumin and vitamin C offers more benefits than that provided by either of them alone in reversing Cd-induced toxicity in the kidney, stomach and small intestine of rat orally exposed to Cd.

2. Materials and methods

2.1 Chemicals

Curcumin, $CdCl_2$, Vitamin C, Bovine serum albumin and Bradford reagent were purchased from Sigma-Aldrich Chemical Company, St. Louis, USA. All other chemicals used, e.g. absolute ethanol, were purchased from Merck (Darmstadt, Germany) and were of analytical grade.

2.2 Animals

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 according to national guidelines and protocols, approved by the Institutional Animal Ethics Committee and housed in an air-conditioned animal house with constant 12 h light and 12 h dark schedule. Animals were fed standardized diet for rodents and water *ad libitum*.

2.3 Experimental design

Curcumin, vitamin C and $CdCl_2$ were dissolved in sterile distilled water. The dose of $CdCl_2$, curcumin and vitamin C used in this study was selected from the previous study (El-Demerdash, Yousef, Kedwany, & Baghdadi, 2004; Prabu, Shagirtha, & Renugadevi, 2011). The administration of vitamin C 100 mg/kg was more likely to produce effects shown in many studies (Elballat, 2016; Rana & Ahmad, 2012). It was not done in the VitC treated group. Curcumin and/or vitamin C were given daily by oral gavage for 1 h before $CdCl_2$ administration in groups 4-8. During the entire experimental period there was no mortality. Treatments were given at 08:00 each day throughout the experiment. The animals, at 180-200 g initial body weight, were randomly divided into 8 groups of 8 animals each.

Group 1 Control: control rats received only distilled water

Group 2 Cd: Normal rats orally received $CdCl_2$ 5 mg/kg BW

Group 3 Cur400: Normal rats orally received curcumin 400 mg/kg BW alone

Group 4 VitC + Cd: Normal rats orally received vitamin C 100 mg/kg BW and $CdCl_2$ 5 mg/kg BW

Group 5 Cur200+ Cd: Normal rats orally received curcumin 200 mg/kg BW and $CdCl_2$ 5 mg/kg BW

Group 6 Cur400 + Cd: Normal rats orally received curcumin 400 mg/kg BW and $CdCl_2$ 5 mg/kg BW

Group 7 Cur200+VitC+Cd: Normal rats orally received curcumin 200 mg/kg BW plus vitamin C 100 mg/kg BW and $CdCl_2$ 5 mg/kg BW

Group 8 Cur400+VitC+Cd: Normal rats orally received curcumin 400 mg/kg BW plus vitamin C 100 mg/kg BW and $CdCl_2$ 5 mg/kg BW

All groups of rats were treated by oral gavage daily for 27 days. All animals were sacrificed 24 h after the last treatment following protocols and ethical procedures. The kidney, stomach, small intestine were immediately dissected out, and washed using chilled saline solution. The kidney was homogenized and used for the determination of MDA and reduced GSH. The remaining kidney, stomach, small intestine were kept in 10% neutral phosphate buffered formalin solution for histopathological examination.

2.4 Malondialdehyde (MDA) and reduced glutathione (GSH) assays

Lipid peroxidation (LPO) level was measured by the method of Buege & Aust (1978) and evaluated by measuring the malondialdehyde (MDA) concentration which was the end product of LPO. The renal tissues were minced and homogenized (10% w/v), separately, in ice-cold 0.1 M phosphate buffer (pH 7.4) using glass homogenizer. The tissues homogenates were precipitated with trichloroacetic acid. After centrifugation (1500×g, 15 min), the supernatant was mixed with thiobarbituric acid (TBA) reagent and the mixture was kept at 100^{o}C for 15 min. The level of LPO was measured based on the formation of TBA reactive substance (TBARS) to produce a red colored complex with a peak absorbance 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 nM /mg protein.

The 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-nitrobenzonic acid) which has an intense yellow color that is measured spectrophotometrically at 412 nm (Thermo Scientific Genesis 10S spectrophotometer.) GSH levels were calculated using an extinction coefficient of 1.36×10^5 M^{-1} cm^{-1} and results were expressed as µM /mg protein.

The protein content in the supernatant was estimated by the method of Bradford (1976) using bovine serum albumin (BSA) as the standard.

2.5 Histopathological study

Immediately after sacrifice, the left kidney, stomach and small intestine were removed surgically and rinsed with ice cold physiological saline. For microscopic evaluation, these organs were fixed in 10% neutral phosphate buffered formalin solution for 48 h. Following dehydration in ascending series of ethanol steps (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 (Axioskop 40, Zeiss) and documented by digital camera (Axiocam, Zeiss).

To evaluate the degree of histological damage, 10 slides made from each treatment group were examined and assigned for severity of changes using scores on a scale of 0 to 4; 0 means no lesion, +1 means the lesions found in 1-2 rats; +2 means the lesions found in 3-4 rats; +3 means the lesions found in 5-6 rats; +4 means the lesions found in 7-8 rats. This double blinded evaluation was done by histopathologists semi-quantitatively.

2.6 Statistical analysis

All the data were expressed as means ± SEM (standard error of the means). The statistical significance was evaluated by one-way analysis of variance (ANOVA) followed by a post hoc test (Tukey's test) using SPSS version 16.0. Values were considered statistically significant when $p < 0.05$.

3. Results

3.1 Effects of curcumin, vitamin C and their combinations on MDA and reduced GSH levels in rat kidney induced by cadmium chloride

MDA levels in the renal tissue were used as a measure of lipid peroxidation. Table 1 shows the changes of MDA and reduced GSH respectively in all groups. The MDA and reduced GSH level were similar in the control and curcumin 400 mg/kg groups (p>0.05). The renal MDA level in Cd-treated rats was significantly (p<0.05) elevated when compared with the control. The MDA level in VitC+Cd group was higher than the control but there was no significant difference ($p > 0.05$) from control. This finding suggests that pretreatment with vitamin C alone was unable to prevent against cadmium induced lipid peroxidation. However, we found that the increase of MDA was diminished significantly in Cur200+Cd, Cur400+Cd, Cur200+VitC+Cd and Cur400+VitC+Cd treated groups (p<0.01) when compared with Cd-treated group and almost similar to the control. Among these four groups, the lowest MDA content was detected in Cur400+VitC+Cd treated group. In addition, significant decreases in reduced GSH level were found in Cd-treated rats (p

<0.05) when compared with the control. However, rats treated with Cur400+Cd, Cur200+VitC+Cd and Cur400+VitC+Cd (p<0.01) showed significantly elevated levels in comparison to Cd-treated group and were almost similar to the control. The highest reduced GSH was observed in Cur400+VitC+Cd treated group. Therefore, the combined pretreatment with curcumin and vitamin C was more effective in reversing Cd toxicity, particularly at the dose of curcumin 400 mg/kg.

Table 1 Effects of curcumin (Cur), vitamin C (VitC), and their combinations on lipid peroxidation, expressed as MDA and reduced GSH level in rat kidney induced by cadmium chloride (Cd)

Group	MDA (nM/mg protein)	GSH (μM/mg protein)
Control	55.19 ± 6.60	0.271 ± 0.040
Cd	70.84 ± 3.68*[a]	0.193 ± 0.020*[a]
Cur400	48.97 ± 8.88*[b]	0.266 ± 0.021*[b]
VitC+Cd	62.42 ± 6.82	0.252 ± 0.023
Cur200+Cd	53.83 ± 4.98*[b]	0.198 ± 0.021
Cur400+Cd	51.86 ± 4.49*[b]	0.265 ± 0.022*[b]
Cur200+VitC+Cd	53.90 ± 1.96*[b]	0.271 ± 0.025*[b]
Cur400+VitC+Cd	46.64 ± 3.02**[b]	0.302 ± 0.015**[b]

Results are expressed as means ± S.E.M. (n=8)
*[a] indicates significance at $p < 0.05$ compared to control group
*[b] indicates significance at $p < 0.05$ compared to cadmium group
**[b] indicates significance at $p < 0.01$ compared to cadmium group

3.2 Effects of curcumin, vitamin C and their combination on histopathological examination in rat kidney, stomach, and small intestine induced by cadmium chloride

Histopathological findings of kidney, stomach, and small intestine were evaluated by light microscopy (Figures 1-3). Incidence of lesions in the experimental groups was evaluated and summarized in Table 2. In control and curcumin-alone-treated groups, rats showed normal histological structure of kidney (Figures 1A and 1C), stomach, (Figures 2A and 2C) and small intestine (Figures 3A and 3C).

Marked histopathological changes were observed in the kidney, stomach and small intestine of Cd-treated group by light microscope examination. In the kidney of Cd-treated group, the glomeruli exhibited swelling indicated by the lack of space between the Bowman's capsule and glomerulus. The tubular epithelium increased resulting in almost no visible lumen. The dilatation of tubules, vacuolization and degeneration of tubular epithelial cells were noted. In addition, interstitial inflammation was also observed (Figure 1B). Cd treatment induced mucosal damage in the stomach, visualized as as erosions on the apical part of gastric mucosa and reduced mucus on the mucosal surface. Cytoplasmic vacuolization in the gastric gland epithelium was observed. Moreover, inflammatory infiltration with neutrophils and lymphocytes appeared in the lamina propria (Figure 2B).

In addition, Cd treatment also caused damage in the small intestine. In contrast to thin, tall villi with narrow lamina propria in control animals, shorter and thicker villi were observed following Cd consumption (Figure 3B). Fusion of some villi was visible, with necrotic areas at the top and loss of the regular brush border because of superficial desquamation of the epithelium. Massive necrosis was more often seen. Goblet-cells like vacuoles were more prominent. Mild to moderate inflammatory infiltration, which mainly involved lymphocytes, could be seen in the lamina propria (Figure 3B).

These pathological lesions induced by Cd intoxication in the kidney, stomach and small intestine remained to be observed in VitC+Cd group (Figures 1D, 2D, 3D). However, it had partial

improvement in Cur200+Cd (Figures 1E, 2E, 3E) and Cur400+Cd (Figures 1F, 2F, 3F) in comparison to the Cd-treated group. Some rats in Cur200+VitC+Cd (Figures 1G, 2G, 3G) and Cur400+VitC+Cd (Figures 1H, 2H, 3H) showed less damage in comparison to the Cd-treated group but almost similar to that of control in Cur400+VitC+Cd.

Therefore, the combined pretreatment with curcumin particularly at the dose of 400 mg/kg plus vitamin C could attenuate Cd intoxication and kept the organ almost similar to that of control. Furthermore, we also found that the observed morphological changes in all treatment groups were in correlated with the results of MDA and reduced GSH in renal tissue.

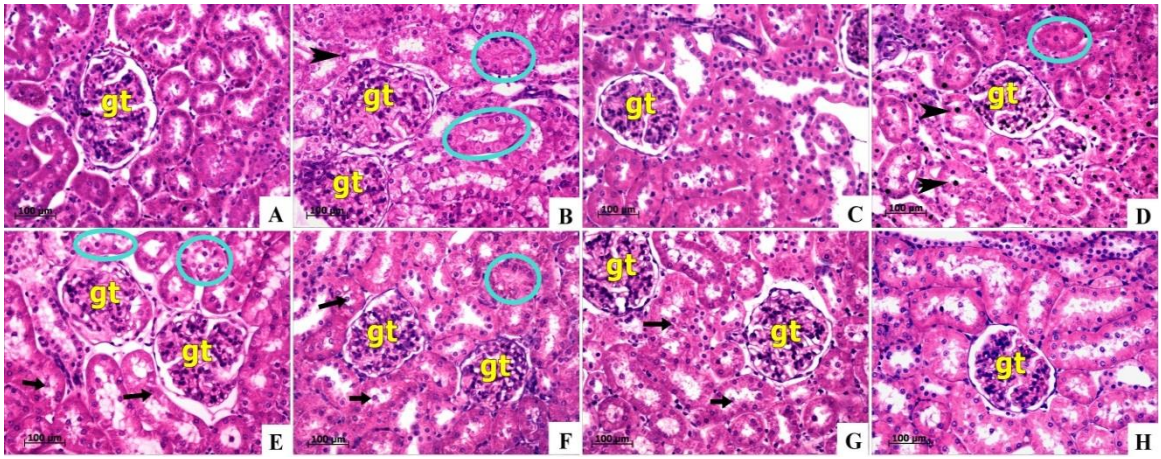

Figure 1 Representative photographs of rat renal cortex by light microscope stained with H&E at 200X magnification (A and C). Renal tissue of the control and Cur400-treated group showing the normal appearance of glomerular tuft (gt), urinary space, Bowman's capsule, proximal and distal tubules with their nuclei. (B) Cd-treated group showing the glomerular tuft swelling, tubular degeneration (circle) and pyknotic nuclei (dark arrow) (D) Vit C +Cd showing the pyknotic nuclei in renal tubules (arrow head) and tubular degeneration (circle). In three groups; (E) Cur200+Cd (F) Cur400+Cd (G) Cur200+VitC+Cd showing the lesions and the desquamation of epithelial cells (arrow) (H) Cur400+Vit C+Cd showing the improvement similar to control.

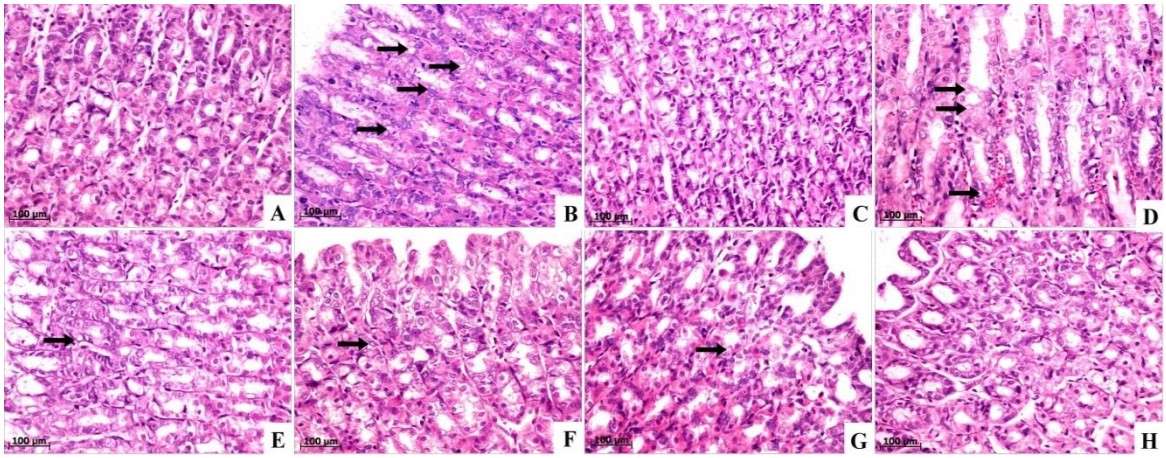

Figure 2 Representative photographs of rat stomach by light microscope stained with H&E at 200X magnification (A and C). Tissue of the control and Cur400-treated group showing the normal appearance of gastric mucosa (B) Cd-treated group showing cytoplasmic vacuolization (arrow) and gastric mucosal damage. In four groups; (D) Vit C+Cd (E) Cur200+Cd (F) Cur400+Cd (G) Cur200+Vit C+Cd showing the same lesions found in Cd-treated group but less damage, particularly in combine pretreatment with Cur200+VitC (H) Cur400+Vit C+Cd showing improvement similar to control.

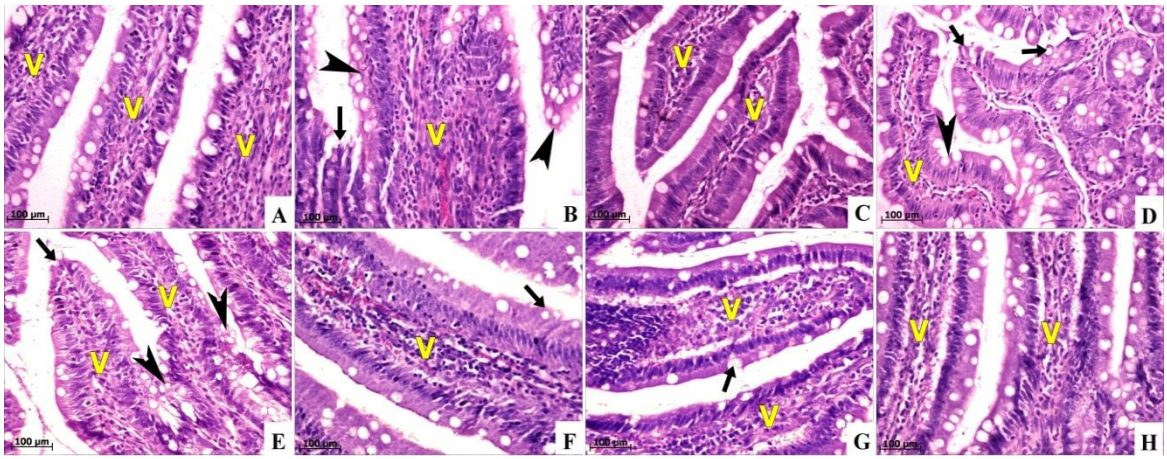

Figure 3 Representative photographs of rat small intestine by light microscope stained with H&E at 200X magnification. (A and C) Tissue of the control and Cur400-treated group showing the normal appearance of thin and tall villi (V) in the mucosa (B) Cd-treated group showing shorter and thicker villi, two-fused villi, group of goblet cell-like vacuoles (arrow head), degeneration of surface epithelium (arrow), and lymphocytic cell infiltration. The villi lost the regular brush border. In four groups; (D) Vit C+Cd (E) Cur200+Cd (F) Cur400+Cd (G) Cur200+Vit C+Cd show the same lesions found in Cd-treated group but less damage, particularly in combined pretreatment with Cur200+VitC (H) Cur400+Vit C+Cd showing improvement similar to control.

Table 2 Incidence of histopathological lesions in rat kidney, stomach and small intestine of the experimental groups

Lesions	Control	Cd	Cur400	VitC+ Cd	Cur200+ Cd	Cur400+ Cd	Cur200+VitC+ Cd	Cur400+VitC+ Cd
Kidney								
Glomerular swelling	0	+4	0	+4	+3	+2	+1	0
Proximal tubular degeneration	0	+4	0	+4	+3	+1	+1	0
Interstitial inflammation	0	+3	0	+2	+1	+1	0	0
Stomach								
Gastric pit damage	0	+4	0	+4	+3	+2	+1	0
Gastric gland damage	0	+4	0	+3	+2	+1	+1	0
Inflammation	0	+3	0	+2	+1	+1	0	0
Small intestine								
Villus damage	0	+4	0	+4	+3	+2	+1	0
Surface epithelium degeneration	0	+4	0	+3	+2	+1	+1	0
Lymphocytic infiltration	0	+3	0	+2	+1	+1	0	0

0 means no lesion; +1 means lesions found in 1-2 rats; +2 means lesions found in 3-4 rats; +3 means lesions found in 5-6 rats; +4 means lesions found in 7-8 rats

4. Discussion

In the present study, we found that subacute Cd intoxication induced renal damages, measured by increased lipid peroxidation and decreased reduced GSH. The changes of MDA and reduced GSH in renal tissue were also associated with histopathological changes in kidney, stomach and small intestine. The purpose of the present study was to evaluate the potential benefits of curcumin in combination with vitamin C on Cd-induced toxicity. The study focused on a combination treatment compared to curcumin or vitamin C treatment alone in rat kidney, stomach and small intestine. Our results indicated that pretreatment with curcumin alone (both 200 and 400 mg/kg) could protect against damage induced by Cd although not statistically significant. Protection was not seen with with vitamin C alone. However, the combined pretreatment with curcumin along with vitamin C was significantly more effective in reducing such damage from Cd treatment and reversed morphological changes to that of control, particularly in the curcumin 400 mg/kg treatment group.

Cadmium is a toxic metal which promotes oxidative stress and contributes to the development of serious degenerative changes in several tissues. Various reports have shown that Cd inducses oxidative stress by altering the antioxidative status (Erdem, Yazihan, Kocak, Sayal, & Akcil, 2015; Safhi et al., 2016). Lipid peroxidation (LPO) is one of the main manifestations of oxidative damage and has been found to play an important role in the toxicity of Cd. Also, the present results showed a significant increase in LPO in renal tissues of Cd-treated rats (Table 1), which suggests that free radicals participate in the induced oxidative cell injury, mediating the toxicity of Cd. This study was consistent with other reports in Cd- intoxicated rats (Nazima et al., 2015; Prabu, Muthumani, & Shagirtha, 2012).

Glutathione is a crucial component of the antioxidant defense mechanism and it functions as a direct reactive free-radical scavenger. The decreased level of tissue GSH in Cd-treated rats of the present study may be due to enhanced utilization during detoxification of Cd. Cd binds exclusively to sulfhydryl groups of GSH leading to its inactivation (Ninkov et al., 2015; Safhi et al., 2016). It has been suggested that the depletion of intracellular sulfhydryl groups by Cd is the prerequisite for ROS generation as well as disruption of intracellular organelles. Our findings are in consonance with the other published reports which found that GSH concentration is decreased during Cd intoxication (Renugadevi & Prabu, 2010; Safhi et al., 2016).

Our study showed that Cd induced nephrotoxicity especially glomerulusand tubular damage. The renal cortex showed clear evidence of tubulo-interstitial nephritis (Figure 1B and Table 2). This result is in accord with numerous studies (Erdem et al., 2015; Jemai, Lachkar, Messaoudi, & Kerkeni, 2010). Cd-induced nephrotoxicity is thought to be mediated through the Cd-MT complex, which is synthesized in the liver, released into circulation, and taken up by renal proximal tubule cells (Vesey, 2010). In fact, when the synthesis of MT becomes insufficient for binding all Cd ions in the liver, Cd not bound to MT produces hepatocyte injury and a Cd-MT complex is released into the bloodstream. The complex in the plasma is then filtered through the glomeruli in the kidney and taken up by the proximal tubular cells. Studies conducted on mammals have revealed kidney injury, associated with release of the metallothionein (Mt)/Cd complex in peripheral circulation, after liver saturation. On its way through the kidneys, this complex causes injury, mainly in the cortical region, reaching the proximal tubule and causing a gradual loss of the organ's function (Vesey, 2010). Our study showed that, in Cd exposed rats, this metal affected the renal cortex whereas the medulla is intact. Autoradiographical methods show that the renal cortex accumulates more Cd than the medulla and this correlates with reduced Cd damage (Smith & Thévenod, 2009).

Cd induces changes in the intestine, the tissue which is under direct influence of the metal in settings of oral exposure. It is known from animal and human studies that oral exposure to Cd causes severe irritation of gastrointestinal epithelium (ATSDR, 2012) and interrupts the integrity of intestinal epithelial barrier (Rusanov et al., 2015). Furthermore, it has been reported that a large amount of Cd taken up by enterocytes is retained in the mucosa of the small intestine after oral administration of Cd (Vesey, 2010). In the present study, we observed that oral administration of Cd resulted in significant histopathological abnormalities in the stomach and small intestine as evidenced by swelling of gastric and intestinal mucosa, including infiltration of inflammatory cells into the lamina propria (Figures 2B, 3B and Table 2). These results demonstrated that oral exposure to Cd induced an acute inflammatory response in the

gastrointestinal tract of rats. Our observations on the Cd-induced gastric mucosal and villus damage are consistent with previous reports (Ninkov et al., 2016; Tarasub, Tarasub, & Devakul Na Ayutthaya, 2010). Cd exposure induces necrosis of absorptive cells that is initiated by mitochondrial swelling. The cell debris or enzymes released from such dead cells might injure and/or kill adjacent intact cells, thus amplifying the toxicity of Cd. Such a chain reaction may be catastrophic for the intestinal mucosa, possibly leading to severe necrosis and leakage in the epithelial monolayer, which may in turn disrupt the intestinal barrier (Ninkov et al., 2015). In respect of the intestinal mucosa, we also speculate that the infiltrating neutrophils may assume the functions of phagocytosis and pinocytosis to eradicate cellular debris and enzymes released from the Cd-induced dead cells, and thus limit the dysfunction of the intestinal barrier.

Cellular damage caused by Cd exposure can be prevented by free radical scavengers or antioxidants, which further strengthen the hypothesis that free radicals play a key role in Cd toxicity. Curcumin, the most abundant curcuminoid compound in turmeric (*C. longa*), has multifunctional actions via different mechanisms. The presence of the phenolic, β-diketone, as well as the methoxy groups contribute to the free-radical-scavenging activity of curcumin (Pandey, Kulkarni, & Gaikwad, 2016). The β-diketone structure is also responsible for free radical trapping ability as a chain breaking antioxidant (Sahu, 2016). In several studies in rodents and in in vitro models, curcumin has been shown to have the potential to protect against cadmium nephrotoxicity (Deevika, Asha, Taju, & Nalini, 2012; Tarasub et al., 2011). Recent evidence has shown that curcumin can reverse the toxic effects on histopathological changes, lipid peroxidation and total antioxidant capacity of serum in sodium arsenite-treated mice (Momeni & Eskandari, 2017). The two doses of curcumin selected in this study were based on previous studies in which curcumin showed protective effect against oxidative damage in the kidney (Sankar, Telang, & Manimaran, 2012; Shailaja, Damodara Gowda, Vishakh, & Suchetha Kumari, 2017) and liver (Samarghandian et al., 2017; Tarasub et al., 2012). Numerous studies have shown that oral administration of curcumin at a dose of 200 mg/kg could protect against dimethylnitrosamine-induced hepatotoxicity (Farombi, Shrotriya, Na, Kim, & Surh, 2008) and fructose induced nephrotoxicity (Abdel-Kawi, Hassanin, & Hashem, 2016). In our study, it seems that curcumin alone (both 200 and 400 mg/kg) significantly protect the animals to the same extent (no statistical significance). It has been shown that curcumin pretreatment could decrease LPO, concomitantly restore GSH content in renal tissue and notably improve the histological lesions evoked by Cd. These results may reflect the ability of curcumin to enhance the scavenging and inactivation of H_2O_2 and hydroxyl radicals. In addition, curcumin may serve as a chelator and directly bind to Fe^{2+}, which catalyzes formation of free radicals via the Fenton reactions (Jiao et al., 2009). The ability of curcumin to chelate toxic metals was shown by previous reports (García-Niño & Pedraza-Chaverrí, 2014; Momeni & Eskandari, 2017). Curcumin may also terminate lipid peroxidation by induction of enzymatic and non-enzymatic antioxidants, such as GSH, SOD and CAT (Akinyemi et al., 2016). Accordingly, the protection afforded by curcumin against Cd-induced ROS generation is likely attributable to its antioxidant effects. Also, several studies have reported that curcumin protects kidneys through stimulation of Nrf2, a crucial part of antioxidant response, prohibition of mitochondrial dysfunction and inflammatory response, restoration of antioxidant enzymes and reduction of oxidative stress (Trujillo et al., 2013). Our findings are in agreement with those by Momeni & Eskandari, (2017) demonstrating the efficacy of curcumin in restoring GSH content in the kidney of arsenite treated mice.

The histopathological assessment was correlated with the observed results in the renal lipid peroxidation, a biochemical indicator of necrosis. In fact, this metal promotes an early oxidative stress. Afterwards it contributes to the development of various pathological aspects in renal tissues. The previous study obviously showed that histopathological changes in the kidney were associated with the increase of LPO and reduction of GSH (Abdel-Kawi et al., 2016; El-Demerdash, Yousef, & Radwan, 2009). It can be attributed to their antilipoperoxidative, antioxidant, and metal chelating properties, which significantly reduced the oxidative threat leading to reduction of pathological changes and restoration of its normal physiological function.

Vitamin C (ascorbic acid), the major non-enzymatic antioxidant has synergistic action in scavenging oxygen-derived free radicals and is likely to be most susceptible to free radical oxidation (El-

Shafei & Saleh, 2016). Several studies show that individuals with high intake of vitamin C have lower risk of a number of chronic diseases, including heart disease, cancer, eye diseases, and neurodegenerative conditions. The evidence that ascorbic acid acts as an important antioxidant in many body tissues is convincing (Du, Cullen, & Buettner, 2012). However, previous studies suggest that antioxidant effects of vitamin C depend on their concentrations and the level of free metal ions present in the system (Du et al., 2012). Our previous studies and present study show that vitamin C 100 mg/kg was unable to prevent against Cd-induced the increase of LPO and reduced GSH in renal tissue (Tarasub et al., 2012). These results may be due to the strict control of intestinal absorption of vitamin C, as oral intake is not sufficient for reaching therapeutic plasma levels. Otherwise, vitamin C may react with the other chemical substances in the stomach and are then inactivated, before absorption. Further investigation to estimate the contents of vitamin C may be needed.

Furthermore, we found that the combined pretreatment of curcumin particularly at the dose of 400 mg/kg along with vitamin C was more effective in reducing such damage from cadmium treatment and reverse the changes almost similar to that of control. This may be due to the superior antioxidant effects of curcumin, but not vitamin C which showed no protective effects by itself. Although both curcumin and vitamin C have antioxidant properties but they may not have synergistic actions on the effector organs. Recently, Elballat (2016) reported that the combination between curcumin and vitamin C have more ameliorative effects than curcumin or vitamin C alone on the sperm abnormalities induced by cisplatin treatment. In this regard, it was suggested the synergistic action between curcumin and vitamin C exist. However, our previous study has reported that curcumin (200 and 400 mg/kg) in combination with vitamin C (100 mg/kg) can prevent the cadmium-induced oxidative damage, MT expression and liver structural lesions. These findings also demonstrated that the combined treatment was more effective than with either antioxidant alone as a consequence of the antioxidant/anti-radical properties of curcumin and vitamin C (Tarasub et al., 2012). Nevertheless, in these models it was not determined whether curcumin or vitamin C gave more potent antioxidant activity.

5. Conclusion

In conclusion, it seems that curcumin (both 200 and 400 mg/kg) significantly protects animals to the same extent (no statistical significance) but in the presence of vitamin C, which showed no protective effect by itself, superiority of curcumin 400 mg/kg over 200 mg/kg was demonstrated. Therefore, the curcumin alone or the combination form of antioxidants might be very useful in protection of damage against Cd toxicity. Nevertheless, this feature needs to be further investigated.

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