International Journal of Occupational Medicine and Environmental Health 2010;23(1):55–62
DOI 10.2478/v10001-010-0002-y

ALTERATION IN THE ACTIVITY OF OXIDATIVE ENZYMES IN THE TISSUES OF MALE WISTAR ALBINO RATS EXPOSED TO CADMIUM

SAMUEL O. ASAGBA

Delta State University, Abraka, Nigeria
Department of Biochemistry, Faculty of Science

Abstract
Objective: The objective of the present study was to investigate the effect of cadmium (Cd) on the activities of some oxidative enzymes [viz Aldehyde oxidase, AO (E.C. 1.2.3.1); Xanthine oxidase, XO (E.C. 1.2.3.2); Sulphite oxidase, SO (E.C.1.8.3.1.); and Monoamine oxidase, MO (E.C. 1.4.3.4)] in the liver and kidney. **Materials and methods:** Male Wistar albino rats were administered 1, 2 and 4 mg Cd^{2+}/kg body weight for one and three months. The activities of the oxidative enzymes were subsequently analyzed in the liver and kidney after both periods of exposure. **Results:** There was a dose dependent increase in liver and kidney Cd concentration in the test rats as compared to control after both periods of treatment with the liver retaining higher concentration of Cd than the kidney for each of the exposure dose. The oxidative enzymes were decreased in a dose dependent manner in the liver and kidney after both periods of treatment. The percentage inhibition of these enzymes was less in the liver of rats treated with Cd for three months relative to the one month treated rats for each of the exposure dose. Conversely, the inhibition of the activities of these enzymes in the kidney of rats in all the treatment groups was more pronounced after three months relative to the trend in the one month treated rats. However, the activities of the oxidative enzymes were higher in the liver as compared to the kidney in all the treatment groups after both durations of Cd treatment. **Conclusion:** Based on the results obtained, it can be concluded that the inhibition of the oxidative enzymes by Cd may disturb metabolism of bioactive endogenous substances, exogenous components of food and some xenobiotics.

Key words:
Cadmium, Oxidative enzymes, Xenobiotics, Metabolism, Rat

INTRODUCTION

Cadmium is a nonessential trace element which is present as a contaminant in the general environment. Because of its widespread nature, cadmium can either be ingested via contaminated foods or inhaled. Cadmium accumulates mostly in the liver and kidney and therefore there is a high potential for toxicity of the metal in these organs [1]. One of the basis of cadmium toxicity is its negative influence on enzymatic systems of cells, owing to substitution of other metal ions (mainly Zn^{2+} and Cu^{2+}) in metalloenzymes and its very strong affinity to biological structures containing SH groups [2,3].

Some of the important enzymes whose activities have been negatively influenced by cadmium are the xenobiotics metabolizing enzymes [4]. Foreign compounds are enzymatically transformed by these enzymes to less harmful excretable compounds. This biotransformation process occurs mostly in the hepatic tissues and to a lesser extent, some extra hepatic tissues [3]. Oxidation reactions are probably the most common phase I reactions in xenobiotic biotransformation and for these processes, a group of non-specific, cytochrome P-450 dependent mixed function oxidases (MFO) are required. Studies have shown that exposure to cadmium decreased cytochrome P-450 activity in rodents [5–7]. Other enzymes involved in the oxidation of xenobiotics are aldehyde, xanthine, and sulphite oxidases, all of which are molybdenum and haem containing soluble

Received: September 22, 2009. Accepted: January 27, 2010.
Address reprint request to S.O. Asagba, Department of Biochemistry, Faculty of Science, Delta State University, Abraka PMB 1, Abraka, Nigeria (e-mail: asagbabch@yahoo.com).

enzymes that are present in the liver and other tissues [8–10]. Monoamine oxidase is also important as it is involved in the biotransformation of aromatic monoamines, including classical neurotransmitters such as serotonin, adrenalin, histamine and dopamine.

Despite the role played by these important oxidative enzymes in the biotransformation of xenobiotics, animal studies on the effect of cadmium on these enzymes are missing. Thus, the present study examines the effect of the exposure to cadmium on the activities of these oxidative enzymes (viz aldehyde, xanthine, sulphite and monoamine oxidases) in the liver and kidney using the rat as animal model.

MATERIALS AND METHODS

Experimental design

Eighty male albino rats (Wistar strain) with a mean weight 104±3 g were used for this study. The rats were distributed into four groups with twenty rats per group such that the average weight difference between the groups was less than 0.5 g. Rats in three of these groups were injected subcutaneously once a week with 1, 2 or 4 mg Cd/kg body weight, respectively, using $CdCl_2$ (May & Baker, Dangeham, England) in same volume of saline. Rats in the last group served as the control and were treated with same volume of Cd-free saline. Half of the animals in each group were given this treatment for one month, while the remaining half was similarly treated for three months and the animals were allowed free access to food and water. All the treatment procedures were approved by the Ethics Committee for Animal Experiments of the University of Benin Teaching Hospital, Benin-City, Nigeria.

During the treatment period, water intake, food consumption and dry fecal output were measured daily, while weight gain was recorded weekly. All these animal treatments were carried out in accordance with the principles of laboratory animal care of the NIN guide for laboratory animal welfare as contained in the NIN guide for grants and contracts. At the end of the specified treatment period, the animals were fasted for three hours before sacrifice under chloroform anesthesia. While under anesthesia, the liver and kidney were quickly excised, placed on ice and subsequently weighed. Portions of the liver and kidney were homogenized to give 20% homogenates and centrifuged at 10 000 g for 15 min to obtain clear supernatants for biochemical analysis.

Digestion of samples

Weighed samples of the liver and kidney of each rat were digested separately in beakers with 20 ml of acid mixture ($HNO_3/HClO_4$; 4:1 v/v). The digestion was facilitated by heating at 100°C after which the samples were allowed to cool and then diluted with deionised water to give a final volume of 100 ml. Before use, all glass and plastic utensils were washed in dilute nitric acid and rinsed with deionised water.

Cadmium analysis

The cadmium concentrations in the digests were measured by atomic absorption spectrophotometry (Varian AA 1475 Spectrophotometer). The test metal was dissolved in deionised water and used as standard. The analytical method was checked by using reference material V-10 (Hays) obtained from the International Atomic Energy Agency (IAEA).

The cadmium concentration obtained for the reference sample (0.03±0.002 mg Cd/kg) was in agreement with the certified value (0.03±0.001 mg Cd/kg). In all the determinations, blanks were prepared to determine the effect of reagent purity on the metal levels.

Biochemical analysis

The supernatants obtained from the kidney and liver were used for the determination of the activities of Monoamine oxidase, MO (E.C. 1.4.3.4), Aldehyde oxidase, AO (E.C. 1.2.3.1.), Sulphite oxidase, SO (E.C. 1.8.3.1) and Xanthine oxidase, XO (E.C. 1.2.3.2). MO activity was assayed by the method of Tabor et al. [11] based on the oxidative deamination of benzylamine to benzaldehyde. The activity of the enzyme is expressed in units per gramme tissue weight and one unit of the enzyme is defined as the amount of enzyme that is required for the production of one micromole of benzaldehyde per minute. The activity

of AO was monitored by the method of Johns [12] which is based on the oxidation of benzaldehyde to benzoate using 2,6-dichloroindolephenol (DCIP) as the electron acceptor. The activity of the enzyme is given in units per gramme tissue weight and one unit is the amount of enzyme that produces one micromole of benzoate per minute. The activity of XO was determined by the method of Stirpe and Della Corte [13] using xanthine as the substrate and oxygen as electron acceptor. The enzyme activity is expressed in XO units per gramme tissue and each XO unit is the amount of the enzyme that produces one micromole uric acid. SO activity was determined by the method of Macleod et al. [14]. The principle is based on the oxidation of sulphite to sulphate by the enzyme using ferricyanide as electron acceptor. The activity of the enzyme is also expressed in units per gramme tissue and one unit represents the amount of the enzyme that reduces one micromole of ferricyanide in one minute.

Statistical analysis

The values are reported as means ±SD. Statistical differences for the biochemical values were determined using analysis of variance (ANOVA) and differences in the means were tested by Duncan's multiple range test [15].

RESULTS

Table 1 shows the feed consumption, weight gain, feed efficiency and dry fecal output of rats exposed for three months to varying doses of Cd. There was a dose dependent decrease in weight gain of cadmium treated rats relative to the control, although the feed consumption was not significantly different in all experimental groups. Conversely, there was a dose dependent increase in dry fecal output of cadmium treated rats relative to the control. This is a reflection of the inferior feed efficiency of the Cd treated rats relative to the control.

The status of rat tissue Cd-load, organ/bodyweight ratio, AO, SO, MO and SO activities at the end of one month Cd treatment is presented in Table 2. There was a dose dependent increase in liver and kidney Cd concentration in the test rats as compared to control. However, the liver retained higher concentration of Cd than the kidney for each exposure dose. The liver/body weight ratio of the test rats was significantly increased in a dose dependent manner relative to the control. Similarly the kidney/body weight ratio was significantly increased only in rats treated with the highest dose (0.4 mg Cd^{2+}/kg body weight) of the metal. The oxidative enzymes in the liver were decreased in a dose dependent manner. These enzymes were also decreased in a dose-dependent manner in the kidney of Cd treated rats as compared to control. The level of these enzymes was higher in the livers of rats in each treatment group when compared to the kidney.

The changes in the oxidative enzymes after three months of Cd treatment are shown in Table 3. Like in rats treated with Cd for one month, there was a dose dependent decrease in the activities of the oxidative enzymes in the liver of the test rats relative to the control. However, the percentage inhibition was less in rats treated with Cd for three months relative to the one month treated rats for each of

Table 1. Values of food consumption, weight gains, feed efficiency and dry fecal output of rats treated with cadmium for three months

Parameter	Dose (mg Cd/kg b.w.)			
	Control	+Cd (1.0)	+Cd (2.0)	+Cd (4.0)
Food intake (g/rat/day)	26.8±4.5[a]	25.7±5.4[a]	26.5±5.7[a]	25.2±4.8[a]
Weight gain (g/rat/day)	1.8±0.2[a]	1.6±0.3[a]	1.2±0.3[b]	0.7±0.04[c]
Feed efficiency (g b.w. / g feed)	0.07	0.06	0.05	0.03
Dry fecal output (g/day/rat)	1.3±0.04[a]	1.6±0.04[a]	2.4 ±0.05[b]	2.7±0.05[c]

b.w. — body weight.
Values are given as mean ±SD.
[a-c] Means with matching superscripts in each row are not significantly different at $p < 0.05$.

Table 2. Status of rat tissue Cd-load, organ/body weight ratio, AO, SO, MO and XO activities at the end of one month cadmium treatment

Organ/parameter	Dose (mg Cd/kg b.w.)			
	Control	+Cd (1.0)	+Cd (2.0)	+Cd (4.0)
Liver				
Cadmium load (μg/g tissue)	0.005±0.0007[a]	25.7±2.5[b]	55.4±5.2[c]	115.8±12.6[d]
Organ/b.w. ratio ($\times 10^{-2}$)	2.9±0.3[a]	3.6±0.4[b]	4.8±0.4[c]	5.8±0.8[d]
Aldehyde oxidase (AO)	85.7±8.5[a]	70.2±5.2[b] (18.1)	56.8±6.5[c] (33.7)	40.6±5.5[d] (52.7)
Xanthine oxidase (XO)	75.50±4.5[a]	60.4±5.4[b] (20)	49.3±4.7[c] (34.7)	28.6±5.0[d] (62.1)
Monoamine oxidase (MO)	157.0±10.5[a]	138.2±8.5[b] (12)	87.0±8.2[c] (44.9)	63.0±5.5[d] (60)
Sulphite oxidase (SO)	685.4±45.8[a]	583.9±40.7[b] (14.8)	424.9±34.6[c] (38)	322.0±36.5[d] (53)
Kidney				
Cadmium load (μg/g tissue)	0.007±0.0004[a]	13.6±2.3[b]	28.3±4.6[c]	50.4±9.2[d]
Organ/b.w. ratio ($\times 10^{-2}$)	0.42±0.04[a]	0.47±0.04[a]	0.45±0.05[a]	0.56±0.05[b]
Aldehyde Oxidase (AO)	18.4±2.1[a]	16.7±2.6[a]	15.6±2.2[b] (15.2)	14.3±2.2[b] (22.3)
Xanthine Oxidase (AO)	50.4±7.5[a]	38.6±10.4[b] (23.4)	37.5±7.6[b] (25.6)	22.6±5.8[c] (55.2)
Monoamine oxidase (MO)	52.0±5.4[a]	44.4±5.5[b] (14.6)	37.5±4.4[c] (27.9)	30.6±5.2[d] (41.2)
Sulphite Oxidase (SO)	254.6±25.4[a]	239.5±30.5[a]	215.8±32.5[b] (15.2)	205±30.5[b] (16.4)

Abbreviations as in Table 1.
The activities of the oxidative enzymes are in units/g tissue.
Figures in parenthesis represent percentage inhibition relative to control.

the exposure doses. Again, the activity of these enzymes in the kidney was also similarly decreased in a dose dependent manner. Unlike in the liver, the inhibition of the activities of these enzymes in the kidney of rats in all treatment groups was more pronounced after three months relative to the trend in the one month treated rats. However, as was observed in the one month exposed rats, the activities of the oxidative enzymes were higher in the liver as compared to the kidney in all the treatment groups.

The changes in other parameters under study and the Cd-load in the organs after three months Cd treatment are also presented in Table 3. The organ/body weight ratio for the liver and kidney of the test rats were not significantly different, but were increased significantly ($p < 0.05$) relative to the control. There was a dose dependent increase in tissue Cd-load of test rats relative to control. As observed in rats treated with Cd for one month, the liver Cd concentration for each exposure dose remained higher than that of the kidney. The Cd concentration in the tissues of the three months treated rats was higher as compared to that of the one month exposed rats for each exposure concentration.

DISCUSSION

This study provides evidence for the pattern of accumulation of cadmium over time and its effect on some oxidative enzymes in the tissues of the rat.

Table 3. Status of rat tissue Cd-load, organ/body weight ratio, AO, SO, MO and XO activities at the end of three month cadmium treatment.

Organ/parameter	Cadmium dose (mg/kg b.w.)			
	Control	+Cd (1.0)	+Cd (2.0)	+Cd (4.0)
Liver				
Cadmium load (μg/g tissue)	0.02±0.003[a]	78.5±10.2[b]	156.6±12.5[c]	308±15.4[d]
Organ/b.w. ratio (× 10^{-2})	3.4±0.3[a]	4.9±0.5[b]	4.6±0.5[b]	4.8±7.6[b]
Aldehyde oxidase (AO)	95.4±10.5[a]	80.5±8.2[b] (15.6)	68.5±7.0[c] (28.2)	53.7±8.5[d] (43.7)
Xanthine oxidase (XO)	79.7±5.6[a]	68.5±5.2[b] (18.8)	60.6±4.7[c] (31.9)	48.4±4.8[d] (52.4)
Monoamine oxidase (MO)	120.6±12.5[a] (10.4)	108.0±10.6[b] (29.5)	85.0±7.5[c] (49.6)	60.8±7.8[d]
Sulphite oxidase	858.8±54.6[a]	794.5±40.5[b] (7.5)	625.4±30.5[c] (27.2)	586.5±36.7[d] (31.7)
Kidney				
Cadmium load (μg/g tissue)	0.03±0.002[a]	30.0±5.4[b]	56.4±6.5[c]	76.8±8.0[d]
Organ/b.w. ratio (× 10^{-2})	0.45±0.03[a]	0.52±0.04[b]	0.58±0.06[b]	0.56±0.05[b]
Aldehyde oxidase (AO)	40.8±5.5[a]	30.5±4.8[b] (25.2)	26.4±4.2[b] (35.3)	18.0±3.4[c] (55.9)
Xanthine oxidase (XO)	60.5±7.4[a]	42.4±5.6[b] (29.9)	32.6±5.5[c] (46.1)	24.4±4.2[d] (59.7)
Monoamine oxidase (MO)	70.6±8.6[a]	58.0±7.5[b] (17.8)	40.6±8.5[c] (42.5)	27.8±3.6[d] (60.6)
Sulphite oxidase (SO)	176.4±12.0[a]	150.5±15.6[b] (14.7)	130.4±10.4[c] (26.1)	112.0±14.5[d] (36.5)

Abbreviations as in Table 1 and 2.

The decrease in weight gain of the rats is in consonance with previous reports on the effect of cadmium on weight gain of rats [16,17]. The decrease in weight gain and the corresponding increase in dry fecal output of the Cd treated rats is also consistent with previous reports on the inhibitory effect of Cd on digestive and absorption enzymes in rats [18–20]. The inhibitory effect of Cd on the digestive and absorption enzymes may also account for the decreased feed efficiency observed in the Cd treated rats. The increased accumulation of Cd observed in the liver and kidney of Cd exposed rats is in consonance with earlier reports [16,21,22]. Examination of the cadmium levels in the liver of rats after both periods of exposure to cadmium shows that it was consistently higher than the level in kidney. Available reports indicate that when Cd was administered orally and subcutaneously, more was deposited in the liver than in the kidneys [16,23,24]. Experimental evidence also indicates that the transport and distribution of Cd to tissues is aided by metallothionein (MT) [25,26]. MTs are a family of low molecular weight heavy metal binding proteins, unique in their high cysteine (Cys) content. These proteins are widespread in eukaryotes and plants and are also found in prokaryotes [27].

The increased organ/body weight ratio for liver and kidney of Cd treated rats could be attributed to the toxicity of Cd in these organs. Changes in organ/body weight ratio in rats administered Cd have also been observed by many workers. Horiguchi et al. [16] observed hepatosplenomegaly and kidney swelling after subcutaneous administration of Cd in rats. Similarly, a Cd-induced increase in liver and spleen weight has also been observed by Tu et al. [28].

The data in Tables 2 and 3 indicates that Cd inhibited the activity of the oxidative enzymes in the liver and kidney after one and three month exposure. Timbrell [3] had reported that the inhibition of enzymes by Cd could be linked to the displacement of essential metal cofactors from the enzyme active site, or the formation of covalent bonds by cadmium with sulphydryl and other groups essential for the enzyme action. The Cd induced inhibition of the oxidative enzymes observed in this study may thus be by any of these effects. Besides, the inhibiting activity of Cd towards the molybdenum hydroxylases (AO and XO) and the molybdenum-dependent SO may be associated with metal-metal interactions, while the reduction of FAD-activated MO flavoprotein activity may be due to the prooxidative activity of Cd.

MO inhibition is accompanied by marked changes in the sensitivity of the organism to some dietary constituents (e.g. tyramine, tryptophan and other amines and amine precursors) as well as many drugs (e.g. sympathomimetics, opiates, reserpine and caffeine) [29]. Similarly the molybdenum hydroxylases AO and XO both play important roles in the metabolism of many exogenous and endogenous compounds. They exhibit oxidative activity towards various heteroxcyclic compounds and aldehydes and the liver cytosol of various mammals also exhibits a significant reductive activity toward nitro, sulfoxide, N-oxide and other moieties catalyzed by AO [30]. The conventionally accepted role of XO is purine catabolism, in which it catalyzes the oxidation of hypoxanthine to xanthine, then to uric acid [30]. SO, another molybdoprotein, is involved in the oxidation of endogenous sulphite arising from the degradation of sulphur amino acids [31]. Thus, the decreases observed in the activities of these oxidative enzymes would not only affect their contribution towards the detoxification of xenobiotics but may also affect other aspects of metabolism, for instance the decrease in sulphite oxidase (SO) would invariably affect the metabolism of sulphur containing amino acids. This in turn may affect the metabolism of glutathione and proteins such as metallothionein which are essential free radical scavengers; thus the toxicity of Cd is aggravated.

It is noteworthy that the inhibition of MO, AO, SO and XO in both tissues (liver and kidney) of the one and three months exposed rat was dose dependent. A similar effect of Cd on these enzymes has been observed in the catfish, *Clarias gariepinus*, during a recent study in our laboratory (unpublished results). It is, therefore, conceivable that, at higher doses of cadmium, the oxidation processes managed by these enzymes would be compromised.

The decrease in the percentage inhibition of the oxidative enzymes in the liver of the Cd exposed rat over time for each concentration of exposure is also noteworthy. It would not seem reasonable to exclude that this is connected with the induction of MT in the liver. Besides aiding the transport and distribution of Cd, MT also decreases its toxicity [1,32–34]. Experimental evidence indicates that the liver is more efficient in the induction of MT than other organs [35]. The increased activity of the oxidative enzymes in the liver of rats in all treated groups as compared to the kidney is not surprising as this organ is the main site of xenobiotic metabolism.

In conclusion this study indicates that Cd inhibits the activity of oxidative enzymes in the liver and kidney of rats. The inhibition of these enzymes may disturb metabolism of bioactive endogenous substances, exogenous components of food and some xenobiotics.

ACKNOWLEDGEMENTS

The author is very thankful to Dr G.E. Eriyamremu for his editorial assistance during the preparation of the manuscript.

REFERENCES

1. World Health Organisation. *Environmental health criteria, 135, Cadmium — environmental aspects*. Geneva: World Health Organisation; 1992.
2. Jacobson KB, Turner JE. *The interaction of cadmium and certain other metal ions with proteins and nucleic acids*. Toxicology 1980;16(1):1–37.
3. Timbrell J. *Principles of biochemical toxicology*. 3rd ed. London: Taylor & Francis; 2000.

4. Moore MR. *A commentary on the impacts of metals and metalloids in the environment upon the metabolism of drugs and chemicals*. Toxicol Lett 2004;148:153–8.
5. Plewka A, Plewka D, Nowaczyk G, Brzóska MM, Kamiński M, Moniuszko-Jakoniuk J. *Effects of chronic exposure to cadmium on renal cytochrome P450-dependent mono-oxygenase system in rats*. Arch Toxicol 2004;78(4):194–200.
6. Kostapopoulos I, Kalmanti D, Theodoropoulou B, Loumbourdis NS. *Effects of exposure to a mixture of cadmium and chromium on detoxification enzyme (GST, P450-MO) activities in the frog Rana ridibunda*. Ecotoxicology 2005;14(5): 439–47.
7. Henczová M, Beér AK, Komlósi V, Mink J. *Detection of toxic effects of Cd2+ on different fish species via liver cytochrome P450-dependent monooxygenase activities and FTIR spectroscopy*. Anal Bioanal Chem 2006;385(3):652–9.
8. Beedham C. *Molybdenum hydroxylases.* In: Ioanides C, editor. *Enzymes systems that metabolize drugs and other xenobiotics*. New York: John Wiley; 2002. p. 147–87.
9. Rang HP, Dale MM, Ritter JM. *Pharmacology*. 5th ed. London: Harcourt Publishers Limited; 2001.
10. Hille R. *Molybdenum-containing hydroxylases*. Arch Biochem Biophys 2005;433(1):107–16.
11. Tabor CW, Tabor H, Rosenthal SM. *Mitochondrial monoamine oxidase*. Biol Chem 1954;208:615–61.
12. Johns DG. *Human liver aldehyde oxidase: Differential inhibition of oxidation of charged and uncharged substrates*. J Clin Invest 1967;46:1492–505.
13. Stirpe F, Della Corte E. *Regulation of rat liver xanthine oxidase. Conversion in vitro of the enzyme activity from dehydrogenase (type D) to oxidase (type O)*. J Biol Chem 1969;244:3855–63.
14. Macleod RM, Farkas W, Fridovich I, Handler P. *Purification and properties of hepatic sulphite oxidase*. J Biol Chem 1961;236:1841–6.
15. Sokal RR, Rohlf FU. *Principles and practice of statistics in biological research*. San Francisco: Freeman; 1969.
16. Horiguchi H, Sato M, Konno N, Fukushima M. *Long-term cadmium-exposure induces anaemia in rats through hypoinduction of erythropoietin in the kidney*. Arch Toxicol 1996;71:11–9.
17. Asagba SO, Eriyamremu GE. *Oral cadmium exposure alters haematological and liver function parameters of rats fed a Nigerian-like diet*. J Nutr Environ Med 2007;16(1–3): 267–74.
18. Mesonero JE, Rodriquez Yoldi MC, Rodriquez Yoldi MJ. *Effect of cadmium on enzymatic digestion and sugar transport in the small intestine of rabbits*. Biol Trace Elem Res 1993;38(3):217–26.
19. Elsenhans B, Hunder G, Strugala G, Schmann K. *Longitudinal pattern of enzymatic and absorptive functions in the small intestine of rats after short-term exposure to dietary cadmium chloride*. Arch Environ Contam Toxicol 1999;36(3):341–6.
20. Eriyamremu GE, Asagba SO, Onyeneke EC, Adaikpoh MA. *Changes in carboxypeptidase A, dipeptidase and Na^+/K^+-ATPase activities in the intestine of rats orally exposed to different doses of cadmium*. Biometals 2005;18:1–6.
21. Haouem S, Hmad N, Najjar MF, El Hani A, Sakly, R. *Accumulation of cadmium and its effects on liver and kidney functions in rats given diet containing cadmium polluted radish bulb*. Exp Toxicol Pathol 2007;59(1):77–80.
22. Koizumi N, Murata K, Hayashi C, Nishio H, Goji J. *High cadmium accumulation among humans and primates: comparison across various mammalian species — a study from Japan*. Biol Trace Elem Res 2008;121(3):205–14.
23. Kamiyama T, Miyakawa H, Li JP, Akiba T, Liu JH, Liu J, et al. *Effects of one year cadmium exposure on livers and kidneys and their relation to glutathione levels*. Res Com Mol Pathol Pharmacol 1995;88(2):177–86.
24. Borges LP, Brandäo R, Godoi B, Noqueira CW, Zeni G. *Oral administration of diphenyl diselenide protects against cadmium-induced liver damage in rats*. Chem Biol Interact 2008;171(1):15–25.
25. Ohta H, Seki Y, Imamiva S. *Possible role of metallothionein on the gastrointestinal absorption and distribution of cadmium*. Kitasato Arch Exp Med 1993;65:137–45.
26. Chang X, Jin T, Chen L, Nordberg M, Lei L. *Metallothionein 1 isoform mRNA expression in peripheral lymphocytes as biomarker for occupational cadmium exposure*. Exp Biol Med (Maywood) 2009;234(6):666–72.
27. Manuel Y, Thomas Y, Pellegrini O. *Metallothionein and tissue damage*. In: Nordberg GF, Herber RFM, Alessio L, editors. *Cadmium and the environment*. Lyon: International Agency for Research on Cancer; 1992. p. 231–7.

28. Tu YJ, Han XY, Xu ZR, Wang YZ, Li WF. *Effect of cadmium in feed on organs and meat colour of growing pigs*. Vet Res Commun 2007;31(5):621–30.
29. Murphy LD, Kalin NH. *Biological and behavioural consequences of alterations in monoamine oxidase activity*. Schizophr Bull 1980;6(2):355–67.
30. Kitamura S, Sugihara K, Ohta S. *Drug metabolizing ability of molybdenum hydroxylases*. Drug Metab Pharmacokinet 2006;21(2):83–98.
31. Cohen HJ, Drew RT, Johnson JL, Rajugopalan KV. *Molecular basis of the biological function of molybdenum. The relationship between sulfite oxidase and the acute toxicity of bisulfate and SO_2*. Proc Natl Acad Sci USA 1973;70(12):3655–9.
32. Xue W, Liu O, Cai L, Wang Z, Feng W. *Stable overexpression of human metallothionein-IIA in a heart derived cell line confers oxidative protection*. Toxicol Lett 2009;188(1):70–6.
33. Liu J, Qu W, Kadiiska MB. *Role of oxidative stress in cadmium toxicity and carcinogenesis*. Toxicol Appl Pharmacol 2009;238(3):209–14.
34. Klaassen CD, Liu J, Diwan BA. *Metallothionein protection of cadmium toxicity*. Toxicol Appl Pharmacol 2009;238(3): 215–20.
35. Koropatrick J, Cherian MG. *Exposure to different forms of cadmium in mice: difference in metallothionein and alphafetoprotein mRNA induction in liver and kidney*. J Biochem Toxicol 1988;3:159–72.