

HOSTED BY



ELSEVIER

Contents lists available at ScienceDirect

## Asian Pacific Journal of Tropical Biomedicine

journal homepage: [www.elsevier.com/locate/apjtb](http://www.elsevier.com/locate/apjtb)Original article <http://dx.doi.org/10.1016/j.apjtb.2016.12.012>

## Effects of interaction Cd–Zn on serum-PSA level and prostate histology in rats

Abdelkrim Berroukche<sup>1\*</sup>, Mohamed Terras<sup>1</sup>, Abdelrahmane Labani<sup>1</sup>, Hafsa Dellaoui<sup>2</sup>, Wassila Lansari<sup>2</sup><sup>1</sup>Laboratory of Water Resources and Environment, Biology Department, Faculty of Sciences, Tahar-Moulay University, Saida, 20000, Algeria<sup>2</sup>Laboratory of Bio-Toxicology, Pharmacognosy and Biological Recycling Plant, Biology Department, Faculty of Sciences, Tahar-Moulay University, Saida, 20000, Algeria

## ARTICLE INFO

## Article history:

Received 22 Aug 2016

Received in revised form 8 Oct, 2nd

revised form 12 Oct, 3rd revised form

31 Oct 2016

Accepted 19 Nov 2016

Available online 10 Dec 2016

## Keywords:

Cadmium

Zinc

PSA

Hyperplasia

## ABSTRACT

**Objective:** To assess cadmium sulfate (CdSO<sub>4</sub>) and zinc chloride (ZnCl<sub>2</sub>) antagonist effects on the prostate specific antigen and prostatic cell organization in rats.**Methods:** The study included 40 adult male rats, divided into four groups: Group 1 (CdSO<sub>4</sub>), Group 2 (ZnCl<sub>2</sub>), Group 3 (CdSO<sub>4</sub>–ZnCl<sub>2</sub>) and Group 4 (control). Animals were treated with CdSO<sub>4</sub> and ZnCl<sub>2</sub> at the same dose (15 mg/L) during 30 days.**Results:** It was showed a higher body weight and a lowering serum-prostate specific antigen concentration [(1.8 ± 0.6) ng/mL] in animals treated with CdSO<sub>4</sub>. CdSO<sub>4</sub> induced a cyto-nuclear atypia, proliferative lesions, hyperplasia and precancerous foci in prostate tissue. Toxic effects of ZnCl<sub>2</sub> were not recorded in this study.**Conclusions:** Protective role of zinc was exhibited against toxic effects of cadmium in prostate gland.

## 1. Introduction

The Cadmium (Cd) was in trace in waters. Human activities become a major source of emission of Cd in the atmosphere. Cd is a risk toxic metal for public health [1]. Cd is classified as a type I carcinogen by the International Agency for Research on Cancer and the National Toxicology Program USA [2]. Studies showed Cd induced different cancers in the kidney, lung, testis and prostate tissues. Cd and zinc (Zn) have similar physicochemical properties [3]. In cells, Cd binds to metallothioneins (MTs). These MTs allow Cd's detoxification. Therefore, Cd competes with its antagonist Zn [4]. The biological half-life of Cd is

10–30 years and only 10% of the Cd absorbed are excreted in the urine and feces. Cd toxicity induces massive cell injuries and tissue necrosis as lungs, liver and kidney [5]. Cd, in cells, induces synthesis of reactive oxygen species (ROS). These free radicals cause an oxidative damage in the cell membrane [6]. The mechanisms of Cd carcinogenesis are displayed in a simplified diagram (Figure 1). The cellular effect of Cd, in ionic form as Cd<sup>2+</sup>, could suppress some processes while enhancing others (Figure 1). Zn, as an antioxidant, inhibits free radicals synthesis [7]. Studies reported that Zn is able to interact with metals and decrease their tissue concentrations. Consequently, Zn inhibits an oxidative stress process [8]. Zn allows the stability of cell membrane through the synthesis of MTs and inhibition of the tissue Cd absorption and accumulation [9,10]. On the other hand, studies suggested an association of Cd-exposure with risk of prostate cancer [11]. Serum-prostate specific antigen (PSA) is used as a biological tumor marker in early detection of prostate cancer [12]. In patients with prostate cancer, an increased serum-PSA was observed. Studies have explored an association of PSA with Cd administered in animals [13,14]. This study aimed to assess the cadmium-sulfate (CdSO<sub>4</sub>) toxic effects, simultaneously with zinc-chloride (ZnCl<sub>2</sub>), on the PSA and a cell organization of prostate tissue in rats.

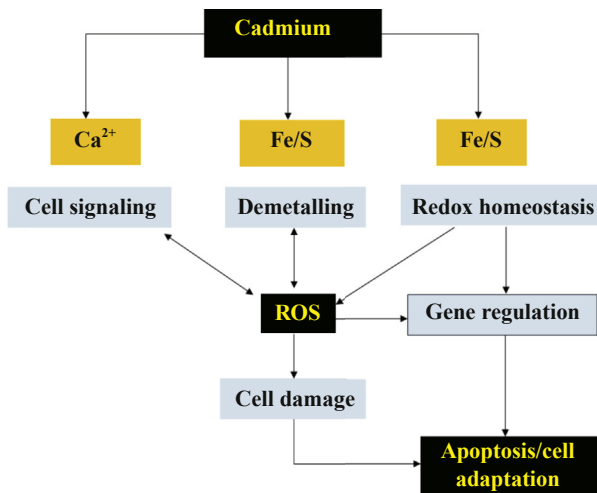
\*Corresponding author: Abdelkrim Berroukche, Laboratory of Water Resources and Environment, Biology Department, Faculty of Sciences, Tahar-Moulay University, Saida, 20000, Algeria.

Tel: +213 798520868

E-mail: [kerroum1967@yahoo.fr](mailto:kerroum1967@yahoo.fr)

All experimental procedures involving animals were conducted in accordance to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and approved by the Animal Ethics Committee of the UFPE (Process No. 012974).

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.



**Figure 1.** Summary of molecular effects discussed to be involved in Cd cell damage and ROS genesis.

## 2. Materials and methods

### 2.1. Animals

Experiments were carried out on 40 adult male Wistar rats at weights ranging from 100 to 300 g. Animals were housed under standard environmental conditions at 22 °C with 12:12 h light–dark cycle and maintained with free access to water *ad libitum* [15]. Their body weight was measured every week. The experimental period was 30 days. The animals were divided into four groups with 10 rats in each group. In Group 1, rats received orally a dose of CdSO<sub>4</sub> at 15 mg/kg body weight per day. In Group 2, rats received orally a dose of ZnCl<sub>2</sub> at 15 mg/kg body weight per day. In Group 3, rats received a mixture (ZnCl<sub>2</sub> and CdSO<sub>4</sub>) in the same experimental conditions. In Group 4, normal rats received a distilled water and standard diet, served as control group.

The experimental protocol was approved by the Animal Ethics Committee of the UFPE (Process No. 012974) in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals [15].

### 2.2. Preparation of CdSO<sub>4</sub> and ZnCl<sub>2</sub> doses

The toxic doses of CdSO<sub>4</sub> and ZnCl<sub>2</sub> were prepared by dissolving 15 mg Cd and Zn in 1000 mL of distilled water, respectively [16].

### 2.3. Blood-PSA assay

At the end of the experiment, animals were anesthetized by the intraperitoneal injection of sodium pentobarbital 40 mg/kg body weight. Blood samples were obtained from the hearts of animals and allowed to clot for 20 min in laboratory temperature and then centrifuged at 3000 r/min for 10 min for serum separation. Serum-PSA levels were measured by mini VIDAS automate analyzer (BioMérieux, Marcy-l'Étoile, France). The method used was an enzyme-linked fluorescent assay which is an enzyme immunoassay ELISA “sandwich” in heterogeneous phase where the molecules of PSA are caught between two monoclonal antibodies of murine nature. The levels of the PSA were obtained in two stages to a final detection by fluorimetry. A

quality control was performed for each used kit VIDAS-PSA to validate the results.

### 2.4. Blood Cd<sup>2+</sup> assay

Blood Cd<sup>2+</sup> assay was carried out by the spectrophotometric analysis. A portion of blood was also collected in ethylene diamine tetraacetic acid tubes to be analyzed by automate instrument named Abacus 4 Hematology Analyzer (Diatron, Budapest, Hungary).

### 2.5. Histological study

Animals were dissected to isolate prostate tissues. After draining the blood, prostate samples were excised, washed with normal saline and processed separately for histological observations. Initially, the materials were fixed in 10% buffered neutral formalin for 48 h and then with bovine serum albumin for 6 h. Paraffin sections were taken at 5 mm thickness, processed in alcohol-xylene series. For light microscopy, semi thin sections of prostate tissue were stained with alum haematoxylin-eosin (H & E) and examined with an Olympus BH-2 light microscope and photographed with Sony DSC-W610 digital camera (Sony Corporation Konan, Minato-ku, Tokyo, Japan).

### 2.6. Statistical analysis

Data are expressed as mean ± SD, with a value of  $P < 0.05$  considered statistically significant. Statistical evaluation was performed by One-way ANOVA followed by the Tukey's *t*-test for multiple comparisons. All analyses were made with the statistical software SigmaPlot (version 11.0).

## 3. Results

As shown in Table 1, animals were monitored and controlled during the experimental period. CdSO<sub>4</sub> induced significant decrease in body weight in animals of Group 1 compared to normal control rats (Group 4). Lowering of body weight was respectively illustrated by the following values (113.00 ± 8.10) g vs. (203.10 ± 30.10) g whereas animals treated with ZnCl<sub>2</sub> (Group 2) and with the CdSO<sub>4</sub>–ZnCl<sub>2</sub> (Group 3) showed a statistically significant increase in body weight compared to experimental animals administrated with CdSO<sub>4</sub> (Group 1), and their body weights were respectively (125.40 ± 26.30) g and (172.20 ± 15.30) g vs. (113.00 ± 8.10) g.

A rising of prostate gland weight (Group 1) explained the higher Cd levels in the prostatic cells whereas in animals treated with Zn (Groups 2 and 3), prostate gland weight increased significantly (Table 1). These data elucidated an antagonist function of Zn that eliminated Cd and detoxified the prostate tissue from this toxic heavy metal. Although a slight increase of prostate gland weight was observed in animals of Group 1 [(3.10 ± 1.80) g], it remained statistically insignificant ( $P > 0.05$ ) to other groups of animals, including Group 2 [(1.20 ± 0.10) g] and Group 3 [(0.90 ± 0.01) g] (Table 1).

Blood-PSA concentrations were nearly identical, in animals treated with CdSO<sub>4</sub> after 10 and 15 days, while after 20 days it showed a significant increase of blood-PSA in the same animals (Group 1). The blood-PSA concentrations were

( $17.10 \times 10^{-2} \pm 2.60$ ) ng/mL (Group 1) vs. ( $1.80 \times 10^{-2} \pm 0.60$ ) ng/mL (Group 2) and ( $6.80 \times 10^{-2} \pm 1.00$ ) ng/mL (Group 3) (Table 1).

(Figure 2B). Prostate atypical hyperplasia was the result of an excessive proliferation and an irregular differentiation associated to a loss of epithelium integrity, stroma and changes in nuclei

**Table 1**

Anthropometric and biological parameters in rats exposed to CdSO<sub>4</sub> and ZnCl<sub>2</sub> and in normal control rats.

Groups	Body weight (g)	Prostate weight (g)	PSA ( $\times 10^{-2}$ ng/mL)	Cd <sup>2+</sup> ( $\times 10^{-2}$ µg/L)
Group 1	113.00 $\pm$ 8.10 <sup>a</sup>	3.10 $\pm$ 1.80	17.10 $\pm$ 2.60	879.80 $\pm$ 152.10 <sup>b</sup>
Group 2	125.40 $\pm$ 26.30	1.20 $\pm$ 0.10	1.80 $\pm$ 0.60 <sup>b</sup>	74.00 $\pm$ 4.50
Group 3	172.20 $\pm$ 15.30	0.90 $\pm$ 0.01	6.80 $\pm$ 1.00	142.60 $\pm$ 50.30
Group 4	203.10 $\pm$ 30.10	0.40 $\pm$ 0.00	0.40 $\pm$ 0.30 <sup>b</sup>	3.20 $\pm$ 2.40
P-value	< 0.001	> 0.05	< 0.001	< 0.001

Values are expressed as mean  $\pm$  SD. <sup>a</sup> : Significant difference at  $P < 0.05$ ; <sup>b</sup> : Highly significant difference at  $P < 0.001$ .

Animals, with higher PSA ( $17.10 \times 10^{-2}$  ng/mL), showed a higher blood-Cd<sup>2+</sup> concentration ( $879.80 \times 10^{-2}$  µg/L) (Table 1), but the statistically significant difference of the distribution of the blood-Cd<sup>2+</sup> concentrations was reported among Groups 2 and 3 (Table 1).

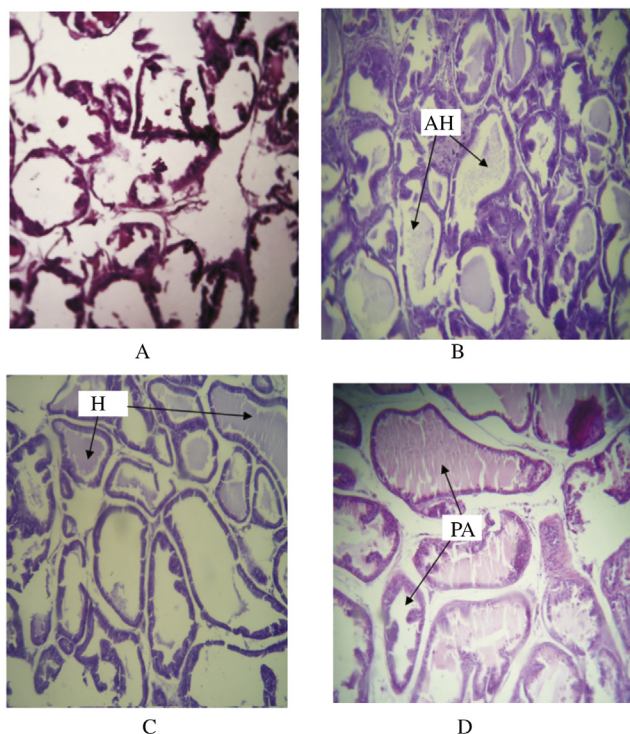
Histological study in control group showed a normal prostate tissue, with acini in spherical forms and a single layer of epithelial cells in prismatic size (Figure 2A). The prostate glands, in animals of Group 1, had an atypical hyperplastic structure. It was also observed large glands with fine and pronounced papillary folds. A large prismatic epithelium, projecting into the gland lumen, corresponded to an atypical hyperplasia

forms. It was also reported large prostate glands with pseudo-papillary multi-layered epithelia. Prostate glands, in animals treated with only ZnCl<sub>2</sub> and with the mixture (CdSO<sub>4</sub> + ZnCl<sub>2</sub>), had normal cells with organized structure and an accentuated glandular hyperplasia (Figure 2C, D).

#### 4. Discussion

Studies suggested that Cd is involved in the carcinogenesis process of different tissues including prostate, liver and pancreas [17]. In this study, a lowering body weight of CdSO<sub>4</sub> treated animals is similar to the results reported by Bosland [18]. Although this study showed the Cd toxic effects in prostate tissues of animals, a rigorous caution has been considered in the analysis of the results about their extrapolation to human organism because the anatomy and histology of the prostate in both human and rat (vertebrate mammals) are considerably different. In other studies, the microscopy of murine prostate tissue could not detect impending neoplastic changes after CdSO<sub>4</sub> subcutaneous administration during an experimental period of two years [19]. Another study, conducted on rats and mice orally administered with CdSO<sub>4</sub> during two years, revealed similar results [20]. Furthermore, Olisekodiaka *et al.* reported a prostate adenocarcinoma in CdCl<sub>2</sub> intra prostatic injection rats at the dose used (80–400 mg/kg) [21]. Studies have supported the hypothesis that Cd, by orally way, has been associated with prostate cancer in humans [22]. In rat, Cd, orally administered, induced proliferative lesions and cell injuries at the prostatic ventral and lateral lobes. These lesions showed multiple foci in prostate lateral lobe, similar to those found in this study. Results of this study attest the carcinogenic potential of Cd on prostate lateral lobe. It was suggested that the prostate lateral lobe of rat has structural and functional properties nearly similar to a human prostate. In this study, it has been revealed that the blood-PSA concentration was higher in animals administered with CdSO<sub>4</sub> whereas this tumoral marker was lower in non-treated rats. This result incriminates Cd as a risk factor in the genesis and progression of prostate tumors.

Zn is another well-known antioxidant. Although it's an essential trace-element, it also plays important roles in testosterone production and spermatogenesis [23]. Furthermore, Zn reduces toxic effects of Cd and its antioxidant mechanism is not yet elucidated [24]. Nevertheless, molecular mechanisms were proposed for Zn protective activity. Zn stabilizes cell membranes and protects lipid from the free radical peroxidation [25]. Consequently, Zn induces hepatocellular MT production,



**Figure 2.** Effects of CdSO<sub>4</sub>–ZnCl<sub>2</sub> on prostate tissue architecture compared with normal control rats.

A: Normal prostate of control rat. Differentiated prostatic ventral lobe (H & E, 10  $\times$  40). B: Micrographic slide of prostate tissue of rat exposed to CdSO<sub>4</sub>. Atypical hyperplasia (AH) (colored with H & E and observed at magnification 10  $\times$  40). C: Micrographic slide of prostate tissue of rat exposed to ZnCl<sub>2</sub>. More or less accentuated hyperplasia (H) (with H & E, 10  $\times$  40). D: Micrographic slide of prostate of rat exposed to CdSO<sub>4</sub>–ZnCl<sub>2</sub>. Prostatic acini (PA) with normal size and inter-glandular areas (H & E, 10  $\times$  40).

which is involved in protecting tissues against Cd toxicity [26]. Blood Cd and Zn concentrations increase proportionally with the age [27–29].

Zn and Cd had exhibited significant antagonist effects in prostate tissue. However, further studies are required to elucidate the mechanism of this antagonism.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Acknowledgments

We express thanks to Dr Z. Haddi of Biological-Medical Analysis Laboratory, for providing facilities to carry out the serum-PSA assay.

### References

- [1] Chandurvelan R, Marsden ID, Gaw S, Glover CN. Acute and sub-chronic effects of sub-lethal cadmium exposure on energy metabolism in the freshwater shrimp, *Paratya curvirostris*. *Ecotoxicol Environ Saf* 2017; **135**: 60-7.
- [2] Hermand V, Julio E, Dorlhac de Borne F, Punshon T, Ricachenevsky FK, Bellec A, et al. Inactivation of two newly identified tobacco heavy metal ATPases leads to reduced Zn and Cd accumulation in shoots and reduced pollen germination. *Metallomics* 2014; **6**(8): 1427-40.
- [3] Wang YW, Yang K, Tang H, Chen D, Bai YL. Toxicity assessment of repeated intravenous injections of arginine-glycine-aspartic acid peptide conjugated CdSeTe/ZnS quantum dots in mice. *Int J Nanomedicine* 2014; **17**(9): 4809-17.
- [4] Nazima B, Manoharan V, Miltonprabu S. Grape seed proanthocyanidins ameliorates cadmium-induced renal injury and oxidative stress in experimental rats through the up-regulation of nuclear related factor 2 and antioxidant responsive elements. *Biochem Cell Biol* 2015; **93**(3): 210-26.
- [5] Nasiadek M, Skrzypińska-Gawrysiak M, Daragó A, Zwierzyńska E, Kilanowicz A. Involvement of oxidative stress in the mechanism of cadmium-induced toxicity on rat uterus. *Environ Toxicol Pharmacol* 2014; **38**(2): 364-73.
- [6] Xu X, Liu C, Zhao X, Li R, Deng W. Involvement of an antioxidant defense system in the adaptive response to cadmium in maize seedlings (*Zea mays* L.). *Bull Environ Contam Toxicol* 2014; **93**(5): 618-24.
- [7] Vergilio Cdos S, de Melo EJ. Autophagy, apoptosis and organelle features during cell exposure to cadmium. *Biocell* 2013; **37**(2): 45-54.
- [8] Kheradmand F, Nourmohammadi I, Ahmadi-Faghieh MA, Firoozrai M, Modarressi MH. Zinc and low-dose of cadmium protect sertoli cells against toxic-dose of cadmium: the role of metallothionein. *Iran J Reprod Med* 2013; **11**(6): 487-94.
- [9] Salińska A, Włostowski T, Maciak S, Łaskiewicz-Tiszczenko B, Kozłowski P. Combined effect of dietary cadmium and benzo(a) pyrene on metallothionein induction and apoptosis in the liver and kidneys of bank voles. *Biol Trace Elem Res* 2012; **147**(1–3): 189-94.
- [10] Kwong RW, Niyogi S. Cadmium transport in isolated enterocytes of freshwater rainbow trout: interactions with zinc and iron, effects of complexation with cysteine, and an ATPase-coupled efflux. *Comp Biochem Physiol C Toxicol Pharmacol* 2012; **155**(2): 238-46.
- [11] Chen C, Xun P, Nishijo M, Carter S, He K. Cadmium exposure and risk of prostate cancer: a meta-analysis of cohort and case-control studies among the general and occupational populations. *Sci Rep* 2016; **13**(6): 25814.
- [12] Tetreault A, Crook JM, Hamm J, Pickles T, Keyes M, McKenzie M, et al. Long-term prostate-specific antigen stability and predictive factors of failure after permanent seed prostate brachytherapy. *Int J Radiat Oncol Biol Phys* 2016; **96**(suppl 2): S137.
- [13] Andreucci A, Mocevic E, Jönsson BA, Giwercman A, Giwercman YL, Toft G, et al. Cadmium may impair prostate function as measured by prostate specific antigen in semen: a cross-sectional study among European and Inuit men. *Reprod Toxicol* 2015; **53**: 33-8.
- [14] Neslund-Dudas C, Kandegedara A, Kryvenko ON, Gupta N, Rogers C, Rybicki BA, et al. Prostate tissue metal levels and prostate cancer recurrence in smokers. *Biol Trace Elem Res* 2014; **157**(2): 107-12.
- [15] Carbone L, Austin J. Pain and laboratory animals: publication practices for better data reproducibility and better animal welfare. *PLoS One* 2016; **11**(5): e0155001.
- [16] Alvarez SM, Gómez NN, Scardapane L, Zirulnik F, Martínez D, Giménez MS. Morphological changes and oxidative stress in rat prostate exposed to a non-carcinogenic dose of cadmium. *Toxicol Lett* 2004; **153**: 365-76.
- [17] Chandler JD, Wongtrakool C, Banton SA, Li S, Orr ML, Barr DB, et al. Low-dose oral cadmium increases airway reactivity and lung neuronal gene expression in mice. *Physiol Rep* 2016; <http://dx.doi.org/10.14814/phy2.12821>.
- [18] Bosland MC. A perspective on the role of estrogen in hormone-induced prostate carcinogenesis. *Cancer Lett* 2013; **334**(1): 28-33.
- [19] Luevano J, Damodaran C. A review of molecular events of cadmium-induced carcinogenesis. *J Environ Pathol Toxicol Oncol* 2014; **33**(3): 183-94.
- [20] Prajapati A, Rao A, Patel J, Gupta S, Gupta S. A single low dose of cadmium exposure induces benign prostate hyperplasia like condition in rat: a novel benign prostate hyperplasia rodent model. *Exp Biol Med (Maywood)* 2014; **239**(7): 829-41.
- [21] Olisekodiaka MJ, Igbeneghu CA, Onuegbu AJ, Oduru R, Lawal AO. Lipid, lipoproteins, total antioxidant status and organ changes in rats administered high doses of cadmium chloride. *Med Princ Pract* 2012; **21**(2): 156-9.
- [22] Hartwig A. Cadmium and cancer. *Met Ions Life Sci* 2013; **11**: 491-507.
- [23] Chemek M, Mimouna SB, Boughammoura S, Delbès G, Messaoudi I. Protective role of zinc against the toxicity induced by exposure to cadmium during gestation and lactation on testis development. *Reprod Toxicol* 2016; **63**: 151-60.
- [24] Yu YY, Chen SJ, Chen M, Tian LX, Niu J, Liu YJ, et al. Effect of cadmium-polluted diet on growth, salinity stress, hepatotoxicity of juvenile Pacific white shrimp (*Litopenaeus vannamei*): protective effect of Zn(II)-curcumin. *Ecotoxicol Environ Saf* 2016; **125**: 176-83.
- [25] Toušová Z, Kuta J, Hynek D, Adam V, Kizek R, Bláha L, et al. Metallothionein modulation in relation to cadmium bioaccumulation and age-dependent sensitivity of *Chironomus riparius* larvae. *Environ Sci Pollut Res Int* 2016; **23**(11): 10504-13.
- [26] Safwen K, Selima S, Mohamed E, Ferid L, Pascal C, Mohamed A, et al. Protective effect of grape seed and skin extract on cerebral ischemia in rat: implication of transition metals. *Int J Stroke* 2015; **10**(3): 415-24.
- [27] Gumulec J, Masarik M, Adam V, Eckschlager T, Provaznik I, Kizek R. Serum and tissue zinc in epithelial malignancies: a meta-analysis. *PLoS One* 2014; **9**(6): e99790.
- [28] Jihen el H, Fatima H, Nouha A, Baati T, Imed M, Abdelhamid K. Cadmium retention increase: a probable key mechanism of the protective effect of zinc on cadmium-induced toxicity in the kidney. *Toxicol Lett* 2010; **196**(2): 104-9.
- [29] Nzengue Y, Steiman R, Rachidi W, Favier A, Guiraud P. Oxidative stress induced by cadmium in the C6 cell line: role of copper and zinc. *Biol Trace Elem Res* 2012; **146**(3): 410-9.