Effect of Chromium, Cadmium and Arsenic on Growth and Morphology of HeLa Cells

Aftab Ahmad*, Bushra Muneer and Abdul Rauf Shakoori

School of Biological Sciences, University of the Punjab, New Campus, Lahore 54590, Pakistan

Abstract: Rapid industrialization and anthropogenic activities are main causes of environmental pollution and level of heavy metals is on the increase in biosphere. These heavy metals have deleterious effects on human health and cause many abnormalities. In the present study, we investigated the effects of arsenic, chromium and cadmium on the growth and morphology of HeLa cell. The total protein profile of control as well as treated cells was checked by SDS-PAGE. Chromium was used to induce the expression of metallothionein protein and expression of protein was detected by SDS-PAGE. There was reduction in proliferation of cells in chromium, cadmium and arsenic containing medium. Cell necrosis was observed with the increase in the concentration of chromium and at 0.10 µg/mL concentration of chromium complete cell lysis was observed. There was change in morphology of cells with increase in concentration of cadmium and at 1.0 µg/ml cells became round. Arsenic also proved to be deleterious for the growth of HeLa cells and there was change in morphology of cells at 1.0 µg/ml but it was not as toxic as chromium and cadmium. There was no difference in protein profile of control and chromium treated cells except lower in concentration of protein due to less number of cells. Metallothionein were not observed in treated cells by SDS-PAGE. Heavy metal have very deleterious effects on human cells and with increase in metal concentration there was change in morphology of cells and also great reduction in proliferation.

Keywords: HeLa cells, Heavy Metals. Apoptosis, Metallothionein.

INTRODUCTION

Due to rapid increase in world population and urbanization, environment related problems are becoming severe. The bioavailability of heavy metals is one of the major environmental problems. Heavy metals are highly bioactive and toxic elements. Human health and food safety are threatened by elevation in level of heavy metals in soil and drinking water [1, 2]. Heavy metals effect the living system as teratogen, mutagen and carcinogen as well as they effect the respiratory, immune and digestive system [3, 4]. Water reservoir and large area of cultivated land is contaminated by cadmium, chromium and arsenic due to application of pesticides and chemical fertilizers, precipitation from heavy coal combustion, smelter waste etc. [5, 6].

Chromium exists in two stable oxidation states that are more likely encountered in biological system 7]. Chromium (III) compounds are insoluble and non-toxic. They are important for body in metabolism of sugar and fats [8]. Chromium (VI) compounds are highly soluble and very toxic because these are potent oxidizing compounds [9]. Cadmium has very diverse toxic effect on human body. It has very high half life in human body (20-30 years) and it is secreted in very low amount (1-2 µg/day). It is mainly accumulated in soft tissues of the body (liver and Kidney) rather than in bones [10]. In addition to its toxic effect, cadmium is also classified as human carcinogen [11]. Arsenic is one of the first substances known to be human carcinogen as a link was found between using a medicine containing arsenic and skin cancer [12]. Recently it is reported that increasing exposure to arsenic result in great mortality mainly due to skin cancer [13].

Metallothionein (MT) are stress protein of small size. These are cysteine rich heavy metal binding protein, involved in array of protective stress response. MT function is revealed during exposure to environmental insults and mainly due to exposure of cells to heavy metals. Mammalian MT provides protection against heavy metal toxicity and oxidative stress. Cell which contain more MT are protected against heavy metal toxicity and oxidative stress while those with lower expression leads to more sensitivity to heavy metal toxicity [14, 15].

As the level of heavy metals especially chromium, cadmium and arsenic is increasing so it is very important to know the effect of these heavy metals on growth and morphology of human cells and to figure out toxic level of these metal. In the present study we investigated the effect of chromium, cadmium and arsenic on HeLa cells. Comparison was also made in protein profile of chromium treated and un-treated cells and in one experiment we also studied the induction of expression of MT by chromium.

*Address corresponding to this author at the School of Biological Sciences, University of the Punjab, Lahore 54590, Pakistan; Tel: 92-300-7402202;
E-mail: aftabac@yahoo.com

ISSN: 1814-8085 / E-ISSN: 1927-5129/12

MATERIALS AND METHODS

HeLa Cell Culture

DMEM Media (Gibco) was supplemented with 10% fetal bovine serum (FBS) (PAA), and 100 U/ml penicillin, and 100 µg/ml streptomycin (ICN). The HeLa cells were maintained in above culture media, with incubation at 37^{o}C with 5% CO_2, and culture passaging after three days.

Heavy Metals

Chromium (potassium chromate), Cadmium (cadmium acetate) and Arsenic (Sodium arsenate) was used for this study. Stock solutions were prepared and filter sterilized with 0.2 µm filter (Orange Scientific) and stored at room temperature.

Heavy Metal Toxicity Evaluation

HeLa cells were grown in DMEM medium supplemented with 10% FBS and 100 U/ml penicillin, and 100 µg/ml streptomycin for 2 days at 37^{o}C with 5% CO_2. Cells were washed twice by Phosphate Buffered Saline (PBS) and then detached by adding 1 ml Trypsin-EDTA. Cells were counted by hemocytometer. 5 x 10^4, 3 x 10^4 and 3 x 10^4 Hela cells were added for chromium, cadmium and arsenic toxicity respectively in sterilized 24 well plastic plate (NUNC) in 1 ml medium. After incubating the cells for 24 h, cells were treated with 0.02, 0.04, 0.06, 0.08 and 0.10 µg/ml chromium and 0.2, 0.4, 0.6, 0.8 and 1.0 µg/ml of cadmium and arsenic respectively for 4 days.

Cell Morphology and Number

After each day morphology of the cells were observed and images were taken by inverted microscope (Olympus, IX51). The numbers of cells were counted by hemocytometer each day and recorded.

Protein Analysis

7.5 x 10^4 HeLa cells were added in each well of 6 well plate (NUNC) in 2 ml medium. Cells were incubated for 24 h and then cells were treated with 0, 1, 2, 3, 4 and 5µg/ml concentration of chromium for total 4 days. After each day, cells were washed twice with PBS and detached from plate by Trypsin-EDTA treatment. Cells were centrifuged at 12,000 rpm for 2 min and then washed with 0.5 ml of PBS by repeating the centrifugation step. The pellet was re-suspended in100 µl of lysis buffer (2M Urea, 2% SDS, 10mM DTT, 10% Glycerol, 10mM Tris-HCL (pH 6.8), Bromophenol blue 1 mg, dH_2O 3ml and 1% PMSF) for cell lysis. The solution was heated in boiling water for 4 min and finally centrifuged at 12,000 rpm for 1 min. 5 µl of solution was loaded from each sample on 15% SDS-PAGE to get protein profile. The procedure was repeated each day for 4 days.

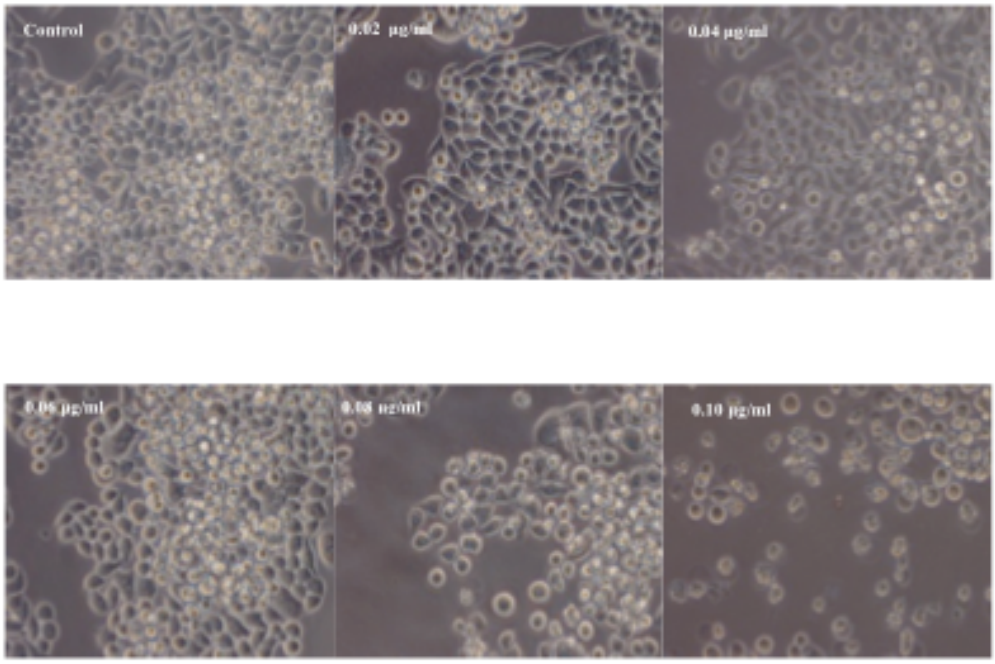

Figure 1: Effect of chromium on HeLa cells. HeLa cells were treated with 0.02, 0.04, 0.06, 0.08 and 0.10 µg/ml chromium. The treated chromium concentration is given on each figure. These images are after 4th day of treatment with chromium.

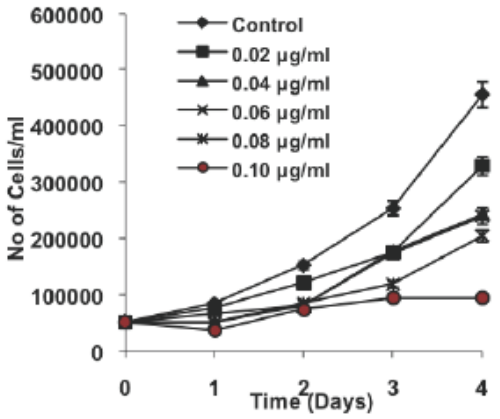

Figure 2: Graphical representation of effect of chromium on proliferation of HeLa cells.

Metallothionein Induction Experiment

6x 10^5 Cells was added in two flasks, one was labeled as control and other as treated and incubated for 24 h. In treated labeled flask added 0.1 µg/ml chromium for metallothionein induction and incubated the flask again for 6 h. HeLa cells were washed twice with PBS-Tween (1000 ml PBS + 1ml of Tween) followed by addition of 1 ml lysis buffer for 5 min. the solution was taken in microfuge tube and heated in boiling water for 4 min and then centrifuged at 12,000 rpm for 1 min. 10 µl solution was loaded on 15% SDS PAGE.

RESULTS

Effect of Chromium on Growth and Morphology of Hela Cells

The morphology of the HeLa cells was totally changed with increasing the concentration of chromium resulted in necrosis and apoptosis. At 0.08 µg/ml concentration cells started becoming round in shape and at 0.1 µg/ml there was clear change in morphology of cells as well as complete deterioration in cells shape (Figure **1**) There was also great reduction in the number of cells with increased concentration of chromium (Figure **2**).

Effect of Cadmium on Growth and Morphology of HeLa Cells

There was a clear change in morphology and reduction in proliferation of cells with increase in concentration of cadmium. Shrinkage in cell size, change in cells shape to round and cell lysis was observed at 1.0 µg/ml concentration of cadmium (Figure **3**). The rate of proliferation of cells greatly decreased with increase in cadmium concentration (Figure **4**).

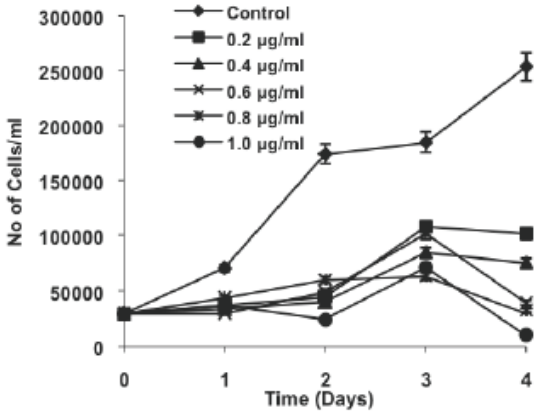

Figure 4: Graphical respresentation of effect of cadmium on proliferation of HeLa cells.

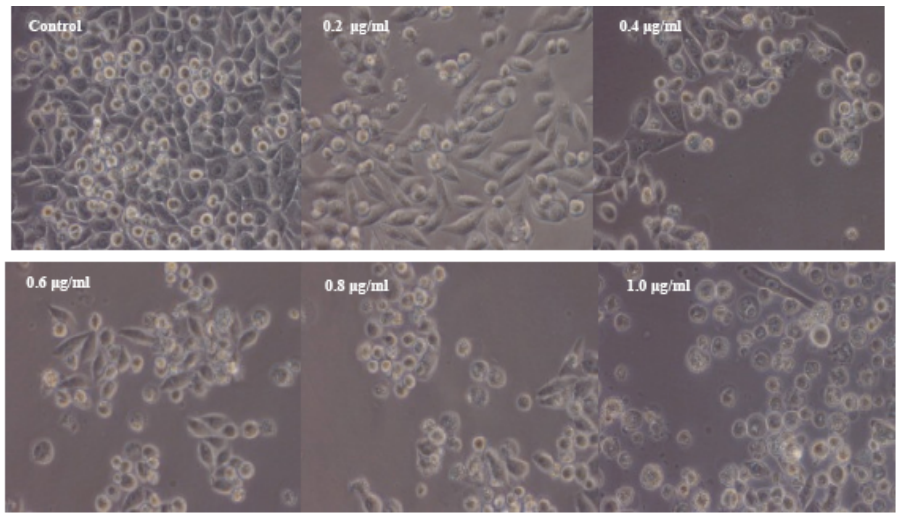

Figure 3: Effect of cadmium on HeLa cells. HeLa cells were treated with 0.2, 0.4, 0.6, 0.8 and 1.0 µg/ml cadmium. The treated cadmium concentration is given on each figure. The above images are after 4th day of treatment with cadmium.

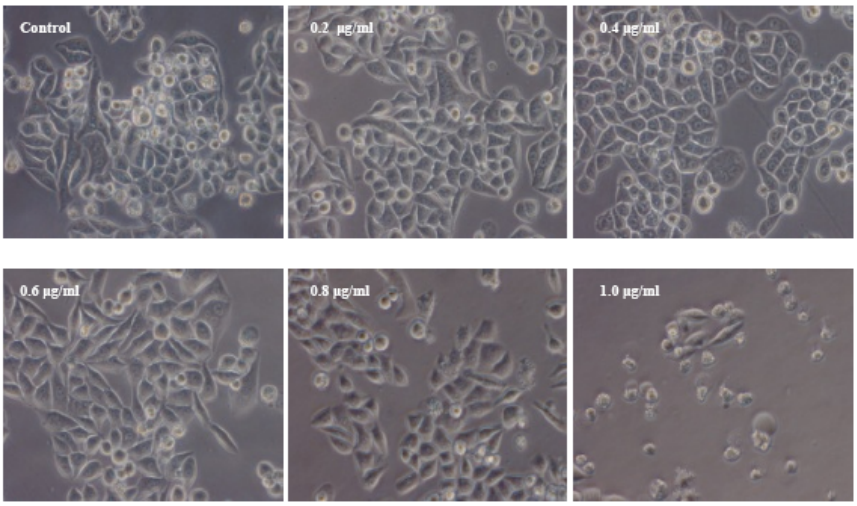

Figure 5: Effect of arsenic on HeLa cells. HeLa cells were treated with 0.2, 0.4, 0.6, 0.8 and 1.0 µg/ml arsenic. The treated arsenic concentration is given on each figure. The images are after 4th day of treatment with arsenic.

Effect of Arsenic on Growth and Morphology of HeLa Cells

There was decrease in number and change in morphology of HeLa cells with increase in concentration of arsenic. Arsenic at higher concentration results in shrinkage of cells size and change in shape of cells (Figure **5**). There was also decrease in proliferation of cells but effect of arsenic was not as significant as other metals (Figure **6**).

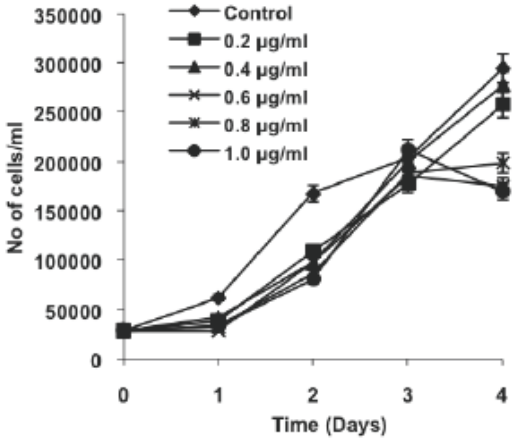

Figure 6: Graphical representation of effect of arsenic on HeLa cells.

Protein Analysis

It is clear from the figure that with increase in metal concentration there was decrease in total protein that is in correlation with the number of metal treated and untreated cells as equal volume of sample was loaded on SDS-PAGE. There was no change in protein profile was observed for treated and untreated sample except few bands. There was an extra band in control sample that was not observed in metal treated samples (Figure **7**).

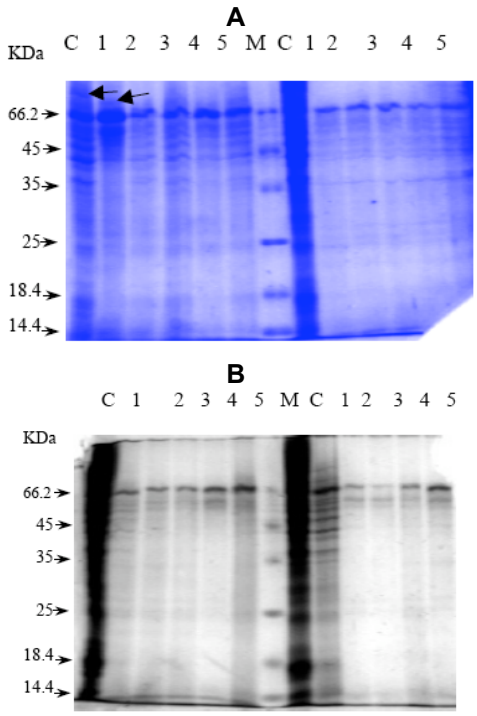

Figure 7: 15% SDS-PAGE of chromium treated HeLa Cells. 1, 2, 3, 4 and 5 are the concentration of chromium in ug/ml. C is untreated and M protein marker. A. On the left side of marker are day1 processed samples and on right day 2 processed samples. B. On the left side of marker are day3 processed samples and on right day4 processed samples.

Metallothionein Induction Experiment

No metallothionein (MT) band was detected in treated cells in MT protein size range (3.5 to 14KDa).

The protein profile was same in both treated (chromium exposed) and control samples (Figure **8**).

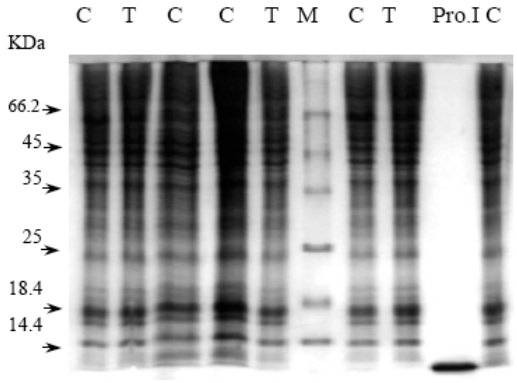

Figure 8: 15% SDS-PAGE of HeLa Cells treated with chromium (100 ng/ml). C (Control) and T (Treated) samples. M is protein Marker and Pro.I is Pro-Insulin band of 8.8KDa to locate the position of Metallothioneine. Same amount of samples were loaded in each lane. The experiment was done in triplicate.

DISCUSSION

Researchers investigated the effect of various heavy metals on plants [16], animals [17], and Human [18], but it is easy to study the effect of heavy metals directly on cells in culture, as it is easy to grow the cells and all the parameters can be controlled. Researchers used various primary [19] and established cell lines [20] to check the effect of various environmental pollutant on growth and morphology of cells as well as molecular mechanism of heavy metal toxicity [21]. In the present study we observed the effect of heavy metals on HeLa cells.

In case of effect of chromium on growth and morphology of HeLa Cells, the number of cells increased from $5.17x10^4$ to $4.56x10^5$ in control but there was great reduction in number of treated cells especially at 0.10 µg/ml concentration of chromium. The cells were almost normal in different chromium concentration but at 0.10 µg/ml concentration the cells size greatly reduced and did not adhere to the surface properly, in addition cell necrosis and lysis was also observed at this concentration. The results clearly indicated that 0.10 µg/ml chromium concentration is very toxic for HeLa cells.

When we observed the effect of cadmium on growth of HeLa Cells, there was clear difference of normal cells with treated cells at higher metal concentrations (0.8 and 1 µg/ml), as normal cells were in adherent form and in normal shape while cadmium treated cells squeeze in size and the effect of cadmium was more drastic after four days of exposure so increase in concentration and time of exposure greatly reduced the proliferation of cells in the presence of cadmium. Cadmium not only reduced the proliferation of cells but also effected the normal morphology of cells.

In case of arsenic the effect was a little bit different and we observed that morphologically there was very little effect of arsenic on growth of HeLa cells as the treated cells were almost in normal shape as that of control cells, but the cell number was reduced in treated cells as compared to untreated cells although it was not very significant. Only at fourth day and at 1.0 µg/ml arsenic concentration the effect of arsenic on shape of cells can be observed as the cells greatly squeezed in size as compared to normal cells.

As we observed in case of treated cells there was decrease in total number of cells. We also run SDS-PAGE of chromium treated cells at various concentrations and it was clearly observed that there was decrease in total protein contents as the bands of metal treated cells were not as prominent as in control cells. It is clear from the figure 7 and 8 that there was increase in amount of protein with the increase in days of growth but there was gradual decrease in the total protein contents with increase in metal concentration and number of days. There was no change in protein profile of treated and untreated cells except few bands that are indicated by arrow. So there is need to conduct 2D gel to have a good comparison of protein profile of treated and untreated cells.

Cadmium and zinc are the main inducer of metallothionein (MT) [22]. All the chemicals that increase the oxidative stress increase the expression of MT [23]. Expression of MT decrease in malignant cells as was observed in prostate cancer and hepatocellular carcinoma [24, 25] as compared to normal cells.

Experiment was done to check induction of expression of MT in chromium treated cells or not. The size of MT ranges from 3.5 to 14 KDa. No MT band was observed in any of the lane on SDS-PAGE. Instead there is extra protein band in control lane 2 and 3 that was not present in treated. It means that with the effect of metal there is down regulation in the expression of 10KDa protein which require further analysis. As HeLa Cells are cancerous cells so it could be reason of no detectable expression of MT, or if we have used cadmium or zinc instead of chromium then we might have detected the expression of MT. there is still need of further investigation in this regard.

In the present study the results indicated that heavy metals are toxic for human cells in even nano gram quantities and chromium is the most toxic heavy metal for HeLa cells followed by cadmium and arsenic. There was great reduction in proliferation of cells in the presence of heavy metal and cell morphology was totally changed at higher metal concentrations and increase in metal exposure time. There was no change in protein profile was observed in treated and un-treated cells by SDS-PAGE as well as no MT protein was observed in chromium treated cells.

REFERENCES

[1] Kang JQ, Jin YL. Study progress of adverse effects of arsenic on health. J Hygien Res 2004; 33: 372–6.

[2] Geng CN, Zhu YG, Liu WJ, Smith SE. Arsenate uptake by seedlings of two rice (Oryza sativa L.) genotypes grown in solution culture. Aq Bot 2005; 83: 321–31. http://dx.doi.org/10.1016/j.aquabot.2005.07.003

[3] Zhou QX, Song YF. Principles and Methods of Contaminated Soil Remediation, Science Press, Beijing, China, 2004.

[4] Liao XY, Chen TB, Xie H, Liu, YR. Soil As contamination and its risk assessment in areas near the industrial districts of Chenzhou City, southern China. Environ Int 2005; 31: 791–8. http://dx.doi.org/10.1016/j.envint.2005.05.030

[5] Xie RK, Seip HM, Wibetoe G, Nori S. McLeod CM. Heavy coal combustion as the dominant source of particulate pollution in Taiyuan, China, corroborated by high concentrations of arsenic and selenium in PM10. Sci Tot Environ 2006; 370: 409–15. http://dx.doi.org/10.1016/j.scitotenv.2006.07.004

[6] Verma VK, Singh YP, Rai JPN. Biogas production from plant biomass used for phytoremediation of industrial wastes. Biore Tech 2007; 98: 1664–9. http://dx.doi.org/10.1016/j.biortech.2006.05.038

[7] Costa M. Toxicity and carcinogenicity of Cr(VI) in animal models and humans. Crit Rev Toxicol 1997; 27: 431–42. http://dx.doi.org/10.3109/10408449709078442

[8] Anderson RA. Chromium as an essential nutrient for humans. RegulToxicol Pharmacol 1997; 26: 35–41. http://dx.doi.org/10.1006/rtph.1997.1136

[9] Cohen MD, Kargacin B, Klein CB. Costa M. Mechanisms of chromium carcinogenicity and toxicity. Crit Rev Toxicol 1993; 23: 255–81. http://dx.doi.org/10.3109/10408449309105012

[10] Harbison RD. Cadmium. In Hamilton and Hardy's Industrial Toxicology (R. D. Harbison, Ed.), 5th ed., 1998; pp. 47–50. Mosby, St. Louis, MO.

[11] IARC. Beryllium, cadmium, mercury, and exposures in the glass manufacturing industry. In IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals in Humans, 1993; Vol. 58. World Health Organization, International Agency for Research on Cancer, Lyon.

[12] Hutchinson J. On some examples of arsenic-keratoses of the skin and arsenic cancer. Trans Pathol Soc London 1988; 39: 352–63.

[13] Finkelman RB, Belkin HE, Zheng B. Health impacts of domestic coal use in China. Proc Natl Acad Sci USA 1999; 96: 3427–31. http://dx.doi.org/10.1073/pnas.96.7.3427

[14] Liu J, Liu YP, Hartley D, Klaassen CD, Shehin-Johnson SE, Lucas A, Cohen SD. Metallothionein-I/II knockout mice are sensitive to acetaminophen-induced hepatotoxicity. J Pharmacol Exp Ther 1999; 289: 580–6.

[15] Pitt BR, Schwarz M, Woo ES, Yee E, Wasserloos K, Tran S, Weng WL, Mannix RJ, Watkins SA, Tyurina YY, Tyurin VA, Kagan VE, Lazo JS. Overexpression of metallothionein decreases sensitivity of pulmonary endothelial cells to oxidant injury. Am J Physiol 1997; 273: 856–65.

[16] Chatterjee J, Chatterjee C. 2000. Phytotoxicity of cobalt, chromium, and copper in cauliflower. Environ Pollut 109: 69-74. http://dx.doi.org/10.1016/S0269-7491(99)00238-9

[17] Grace ND, Lee J. Effect Of Co, Cu, Fe, Mn, Mo, Se And Zn Supplementation On The Elemental Content Of Soft Tissues And Bone In Sheep Grazing Ryegrass/White Clover Pasture. New Zealand J Agr 1990; 33: 635-47.

[18] Ikeda M, Zhang ZW, Higashikawa W. Background exposure of general women populations in japon to cadmium in the environment and possible health effects. Toxicol Let 1999; 108: 161-6. http://dx.doi.org/10.1016/S0378-4274(99)00084-3

[19] Endo T, Sakata M, Shaikh ZA. Mercury uptake by primary cultures of rat renal cortical epithelial cells. Toxicol Appl Pharmacol 1995; 132: 36–43. http://dx.doi.org/10.1006/taap.1995.1084

[20] Douglas BT; Bradley JC; Diane OJ, Cynthia SS, Gregg ED, Moiz MM, Robert E. Effects of Arsenic, Cadmium, Chromium, and Lead on Gene Expression Regulated by a Battery of 13 Different Promoters in Recombinant HepG2 Cells. Toxicol and Appl Pharmacol 2000; 168: 79-90. http://dx.doi.org/10.1006/taap.2000.9014

[21] Beyersmann D, Hechtenberg S. Cadmium, gene regulation, and cellular signalling in mammalian cells. Toxicol Appl Pharmacol 1997; 144: 247–61. http://dx.doi.org/10.1006/taap.1997.8125

[22] Thomas JP, Bachowski GL, Girotti AW. Inhibition of cell membrane lipid peroxidation by cadmium- and zincmetallothionein. Biochim Biophys Acta 1986; 884: 448–61. http://dx.doi.org/10.1016/0304-4165(86)90195-9

[23] Bauman JW, Liu YP, Klaassen, CD. Increase in metallothionein production by chemicals that induce oxidative stress. Toxicol Appl Pharmacol 1991; 110: 347–54. http://dx.doi.org/10.1016/S0041-008X(05)80017-1

[24] Zhang XH, Jin L, Sakamoto H, Takenaka I. Immunohistochemical localization of metallothionein in human prostate cancer. J Urol 1996; 156: 1679-81. http://dx.doi.org/10.1016/S0022-5347(01)65481-8

[25] Datta J, Majumder S, Kutay H, Motiwala T, Frankel W, Costa R, Cha HC, MacDougald OA, Jacob ST, Ghoshal K. Metallothionein expression is suppressed in primary human hepatocellular carcinomas and is mediated through inactivation of CCAAT/enhancer binding protein alpha by phosphatidylinositol 3-kinase signaling cascade. Cancer Res. 2007; 67: 2736-46. http://dx.doi.org/10.1158/0008-5472.CAN-06-4433

Received on 10-01-2012 Accepted on 28-01-2011 Published on 13-02-2012

DOI: http://dx.doi.org/10.6000/1927-5129.2012.08.01.17