## Original Article

# The Cellular Toxicity of $PM_{2.5}$ Emitted from Coal Combustion in Human Umbilical Vein Endothelial Cells*

WANG Fei Fei[1,2], GENG Chun Mei[2], HAO Wei Dong[1,#], ZHAO Yong Dong[3,2], LI Qin[2], WANG Hong Mei[2], and QIAN Yan[2]

*1. Department of Toxicology, School of Public Health, Peking University, Beijing 100191, China; 2. State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing 100012, China; 3. Department of Toxicology, Baotou Medical College of Public Health, Baotou 014040, Inner Mongolia, China*

**Abstract**

**Objective** To explore the relationship between different components of fine particulate matter ($PM_{2.5}$) emitted from coal combustion and their cytotoxic effect in the vascular endothelial cells.

**Methods** Coal-fired $PM_{2.5}$ was sampled using a fixed-source dilution channel and flow sampler. The sample components were analyzed by ion chromatography and inductively coupled plasma atomic emission spectroscopy (ICP-AES). The $PM_{2.5}$ suspension was extracted using an ultrasonic water-bath method and then human umbilical vein endothelial cells (*EA.hy926*) were treated with various concentrations of the $PM_{2.5}$ suspension. Cell proliferation, oxidative DNA damage, and global DNA methylation levels were used to measure the cellular toxicity of $PM_{2.5}$ emitted from coal combustion.

**Results** Compared to other types of coal-fired $PM_{2.5}$ preparations, the $PM_{2.5}$ suspension from Yinchuan coal had the highest cytotoxicity. $PM_{2.5}$ suspension from Datong coal had the highest toxic effect while that from Yinchuan coal had the lowest. Exposure to coal-fired $PM_{2.5}$ from Jingxi coal resulted in lower 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels. At the same dose, $PM_{2.5}$ emitted from coal combustion could produce more severe DNA impairment compared to that produced by carbon black. Cell survival rate was negatively correlated with chloride and potassium ions content. The 5-methylcytosine (5-mC) level was positively correlated with Mn and negatively correlated with Zn levels. The 8-OHdG% level was positively correlated with both Mn and Fe.

**Conclusion** $PM_{2.5}$ emitted from coal combustion can decrease cell viability, increase global DNA methylation, and cause oxidative DNA damage in *EA.hy926* cells. Metal components may be important factors that influence cellular toxicity.

**Key words:** $PM_{2.5}$; Coal combustion; Vascular endothelial cell; Cytotoxicity; DNA methylation

*Biomed Environ Sci, 2016; 29(2): 107-116* *doi: 10.3967/bes2016.012* *ISSN: 0895-3988*

*www.besjournal.com (full text)* *CN: 11-2816/Q* 

## INTRODUCTION

In 2004, the American Heart Association (AHA) published its first scientific statement concluding that exposure to particulate matter (PM) air pollution contributes to cardiovascular morbidity and mortality[1]. Increasing evidence has shown that the overall absolute risk for mortality attributable to PM air pollution is much higher for cardiovascular-caused than for

*This study was supported by the National Science Foundation for Distinguished Young Scholars of China (Grant No.21507122).

#Correspondence should be addressed to HAO Wei Dong, Tel/Fax: 86-101-82802352, E-mail: whao@bjmu.edu.cn

Biographical note of the first author: WANG Fei Fei, female, born in 1978, PhD, majoring in environmental toxicology.

respiratory-caused[2-3]. Heart rate, blood pressure, myocardial infarction, and atherosclerosis are the main health outcome posed by PM exposure[4]. Research has shown that the main pathway by which PM contributes to increased cardiac risk is by promoting atherosclerotic progression via vascular endothelial cell injury[5-10]. Studies have shown that emission from coal burning is mainly responsible for fine particle pollution in China[11-14]. Thus, this study was aimed at exploring the relationship of different components of $PM_{2.5}$ emitted from coal combustion and the cytotoxic effect in the vascular endothelial cells by evaluating cell proliferation, oxidative DNA damage, and global DNA methylation levels.

## MATERIALS AND METHODS

### *Coal Samples and Technical Analysis*

Raw coal from four typical coal fields (Yinchuan, Datong, Jingxi, and Zhijin) in China was purchased from state-owned coal mines[15]. Volatile matter, one of the main indexes in the technical analysis of coal, was determined on a dry basis[16].

### *Sample Collection of Coal-fired $PM_{2.5}$*

$PM_{2.5}$ emitted from coal combustion was sampled by the dilution tunnel system, which was designed and utilized to measure and analyze the emission status and characteristics of particulate matters emitted from stationary sources[16-17]. The dilution tunnel system had a dynamic dilutor to introduce flue gas into the smog chambers to avoid the loss of particles. Dilution and sampling continued until the combustion finished and the coal samples were broken into pieces, after which they were ignited in the stove.

### *Emission Characteristics of Coal-fired $PM_{2.5}$*

Particle size distribution and concentration were measured using Model 3090 EEPS™ (TSI, USA). Water-soluble inorganic ions and metal elements were analyzed by ion chromatograph (761 Compact IC, Metrohm, Switzerland) and mass spectrometer (DRC-e, PerkinElmer, USA).

### *Sample Extraction*

The $PM_{2.5}$ filters were extracted with ultra-pure water in an ultrasonic bath. After ultrasonic elution and freeze-drying, coal-fired $PM_{2.5}$ suspension was prepared and stored at -20 ˚C until it was prepared for cell exposure. The effective particle weight after the extraction divided by the total weight of the particles on the filter was determined to calculate the extraction efficiency.

### *Cell Line and Cell Culture*

Human umbilical vein endothelial cell line (*EA.hy926*) was purchased from ATCC (CRL-2922, Manassas, Virginia, USA). When 80% confluency was achieved, the cells were washed with phosphate-buffered saline (PBS), treated with 0.25% parenzyme (Sigma, USA), and split in a 1:3 ratio of culture to Dulbecco's Modified Eagle's Medium (DMEM) plus 10% fetal bovine serum (FBS). The cells were maintained at 37 °C and 5% $CO_2$[18].

### *Experimental Groups*

The experimental design had six separate treatments consisting of the following: a solvent control group (PBS); $PM_{2.5}$ emitted from Yinchuan coal (YC); $PM_{2.5}$ emitted from Datong coal (DT); $PM_{2.5}$ emitted from Jingxi coal (JX); $PM_{2.5}$ emitted from Zhijin coal (ZJ); and $PM_{2.5}$ carbon black control group (CB, Degussa, Germany). *EA.hy926* cells were treated at concentrations of 0 (PBS), 10, 25, and 50 μg/mL for 24 h.

### *Cell Proliferation Assay*

Cell viability was determined by MTS assay (CellTiter 96® AQueous One Solution Cell Proliferation Assay kit, Promega, USA) according to the manufacturer's instruction[19]. The MTS reaction was measured using a microplate reader to measure the absorbance at 490 nm.

### *DNA Extraction*

DNA was purified using Cell/Tissue DNA Extraction Kit (A&D Technology Corporation, Beijing, China) according to the manufacturer's instruction. DNA concentration and purity was determined by comparing the ratio of optical density measurements at 260 and 280 nm.

### *Determination of Global DNA Methylation*

Global DNA methylation was determined using the Methyl Flash Methylated DNA Quantification Kit (Epigentek, New York, USA) according to the manufacturer's instruction[20]. The kit measures the methyl-cytosine content as a percentage of total cytosine content.

### *Quantitative Determination of Oxidative DNA Damage*

The percentage of 8-OHdG was measured with an EpiQuik 8-OHdG DNA Damage Quantification Direct Kit (Epigentek, New York, USA) according to the manufacturer's instructions[21]. The results are expressed as quantification (%) relative to a positive control provided in the kit and normalized to the input DNA (ng) by using the formula recommended in the instructions.

### *Statistical Analysis*

Statistical analysis was performed using SPSS version 17.0. Results are expressed as mean±standard deviation of the mean (SD) values. Data analysis was performed using unpaired Student's *t*-test and one-way analysis of variance. A stepwise multiple linear regression analysis model was used to check the relationship between variables. A *P* value less than 0.05 was considered statistically significant.

## RESULTS

### *Volatile Matter of Coal Samples*

As shown in Table 1, volatile matter of the coal samples was in the order of DT>YC>ZJ>JX.

### *Characteristics of Coal-fired $PM_{2.5}$*

During the combustion process, the particle concentration was 0-1.5×$10^6$ $cm^{-3}$ and decreased after 1000 s. As shown in Figure 1, the results of ion and metal composition analyses showed that the overall changing trend of content was consistent, although the content of each ion and metal had some differences. $SO_4^{2-}$ was the most abundant ion emitted from coal combustion with $K^+$ and $Cl^-$ being the next most abundant ions emitted. Inaddition to common elements (K, Na, Fe, Zn, Ca, and Al) emitted during the coal burning process, trace heavy metals (As, Co, Cr, Ni, and Pb) were also emitted.

### *Extraction Efficiency*

As shown in Table 2, the extraction efficiency of coal-fired $PM_{2.5}$ was in the order of JX>YC >DT>ZJ.

### *Cell Proliferation*

As shown in Figure 2A-2F, $PM_{2.5}$ emitted from coal combustion and carbon black show a dose-response relationship and could inhibit *EA.hy926* cell proliferation. Furthermore, a significant decrease in cell viability was observed in the middle-and high-dose groups. As shown in Figure 2G, $PM_{2.5}$ suspension from Yinchuan coal had the highest cytotoxicity of all the coal-fired $PM_{2.5}$ preparations, and there was no significant difference in the cytotoxicity of $PM_{2.5}$ preparations of coal obtained from other sources. Figure 2H shows that carbon black had higher cytotoxicity than $PM_{2.5}$ emitted from coal combustion in the high-dose group.

### *Global DNA Methylation*

As shown in Figure 3A-3F, in cells treated with carbon black and $PM_{2.5}$ emitted from coal combustion, the global DNA methylation level increased in a dose-dependent manner. Compared with the PBS control group, there were significant differences in all treatment groups. As shown in Figure 3G, the $PM_{2.5}$ suspension from Datong coal had the highest toxic effect and that from Yinchuan coal had the lowest toxic effect in the coal-fired $PM_{2.5}$ groups. At the same dosage, the 5-mC% content in the carbon black group was lower than that in the coal combustion $PM_{2.5}$ groups (Figure 3H).

**Table 1.** Volatile Matter and Grades of Coal Samples

| Samples | Volatile Matter Yield (%) | Grades | Grade Ranges |
|---|---|---|---|
| ZJ | 5.76 | Super low volatile coal (SLV) | ≤10.00 |
| JX | 4.44 | Super low volatile coal (SLV) | ≤10.00 |
| YC | 19.74 | Low volatile coal (LV) | 10-20 |
| DT | 32.07 | Middle high volatile coal (MLV) | 28-37 |

**Table 2.** Extraction Efficiency of Coal-fired $PM_{2.5}$

| Samples | Before (mg) | After (mg) | Extraction Rate (%) |
|---|---|---|---|
| YC | 0.0666 | 0.0420 | 64.10 |
| ZJ | 0.1052 | 0.0535 | 51.00 |
| JX | 0.0616 | 0.0430 | 70.00 |
| DT | 0.0325 | 0.0190 | 58.46 |

***Oxidative DNA Damage***

All samples of coal-fired $PM_{2.5}$ and carbon black could cause DNA damage in a dose-dependent manner and the corresponding groups showed significant differences compared with the control group (Figure 4A-4F). As shown in Figure 4G, $PM_{2.5}$ emitted from Jingxi coal induced lower 8-OHdG% levels compared to those in the other treatment groups at high doses. At the same dose, $PM_{2.5}$ emitted from coal combustion could produce more severe DNA impairment compared to that produced by carbon black (Figure 4H).

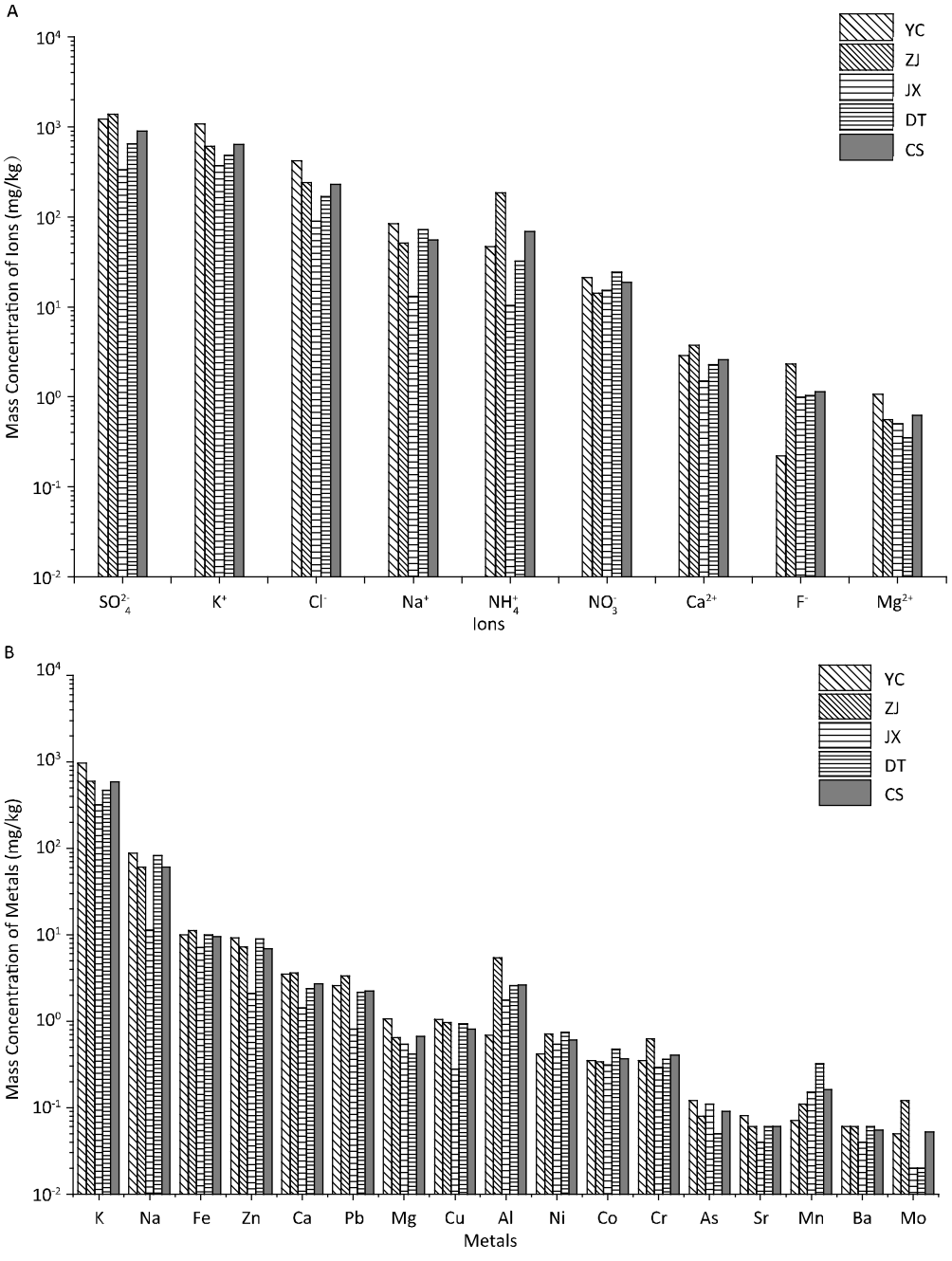


**Figure 1.** Characteristics of coal-fired $PM_{2.5}$. Water-soluble inorganic ions are shown in (A). Metal elementsare shown in (B). CS represents all coal samples.

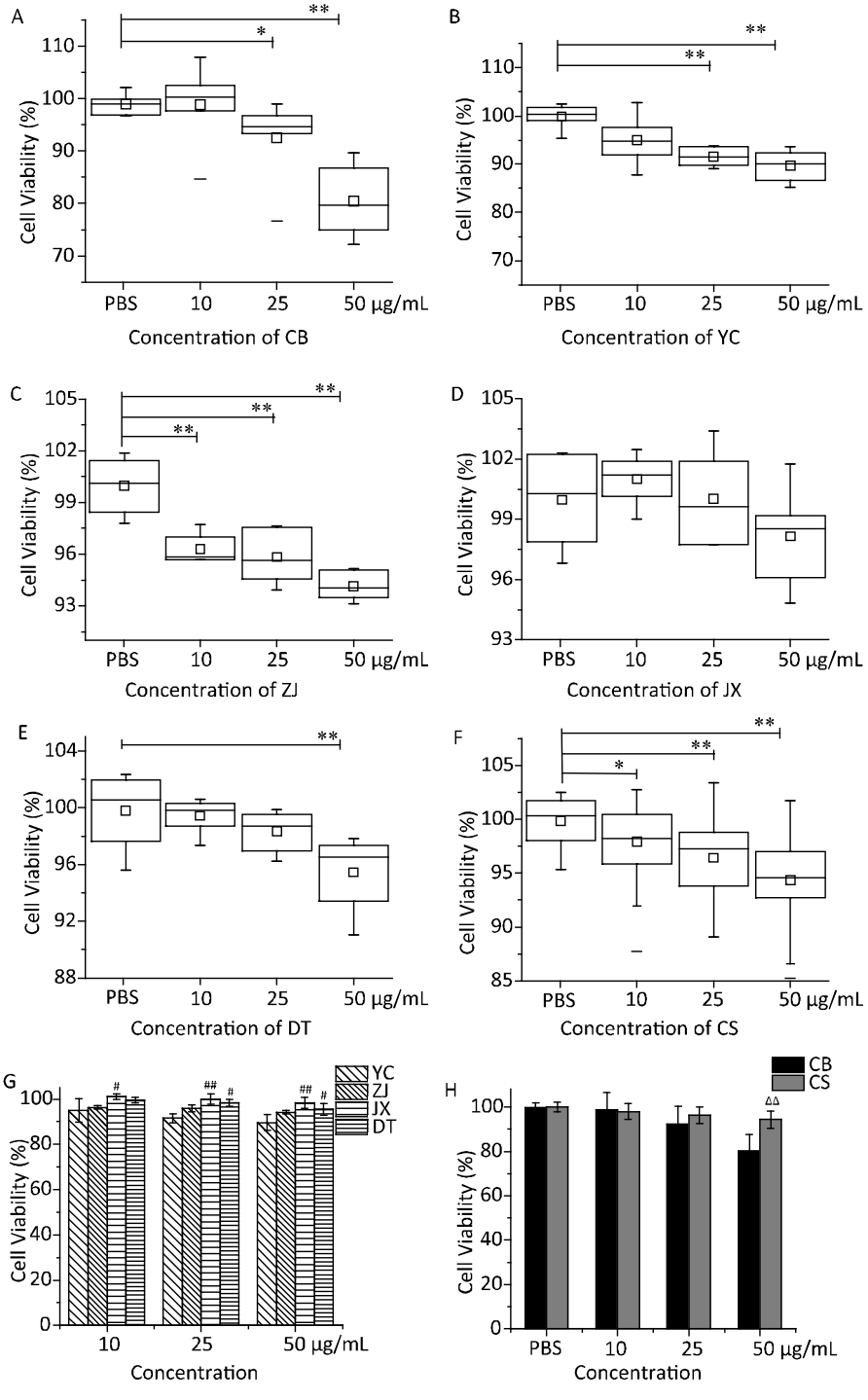


**Figure 2.** Determination of cell proliferation by MTS assay. The viability of cells treated with coal-fired $PM_{2.5}$, CB, and CS is shown in (A-F), respectively. The comparison between coal samples is shown in (G). The comparison between CB and coalis shown in (H). Data are presented as mean±SD values ($n$=6). Asterisksindicate significant differences compared to controls ($^{*}P$≤0.05; $^{**}P$≤0.01). Triangles indicate significant differences compared to CB ($^{\Delta\Delta}P$≤0.01). Hash signindicates significant differences compared to YC ($^{\#}P$≤0.05; $^{\#\#}P$≤0.01). CB, carbon black; CS, all coal samples.

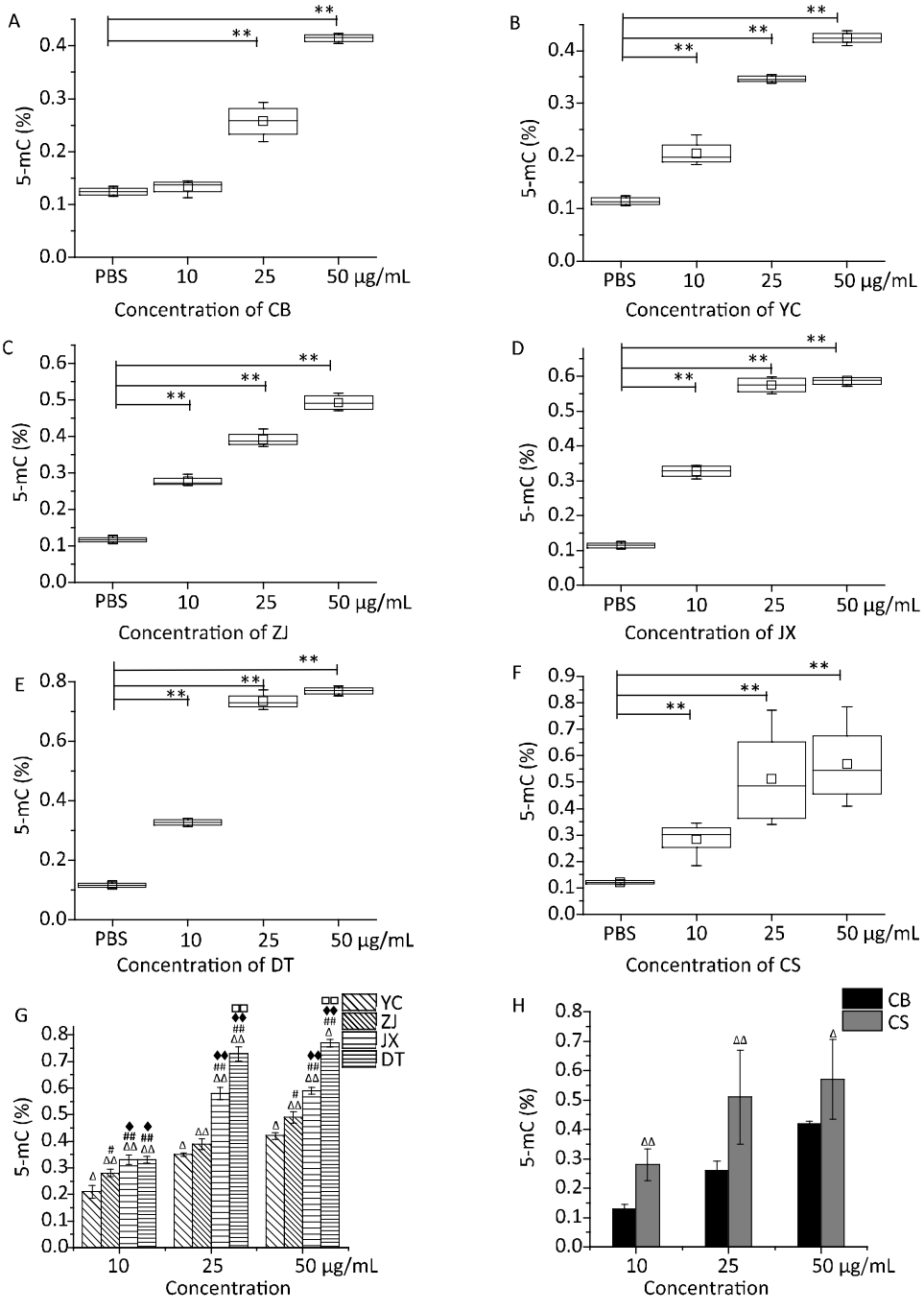


**Figure 3.** Determination of global DNA methylation. The 5-mC content of cells treated with coal-fired $PM_{2.5}$, CB, and CS is shown in (A-F). The comparison between coal samples is shown in (G). The comparison between CB and coal is shown in (H). Data are presented as the mean±SD values ($n$=4). Asterisks indicate significant differences compared to controls ($^{*}P$≤0.05; $^{**}P$≤0.01). Triangles indicate significant differences compared to CB ($^{\Delta}P$≤0.05; $^{\Delta\Delta}P$≤0.01). Hash signindicates significant differences compared to YC ($^{\#}P$≤0.05; $^{\#\#}P$≤0.01). Squares indicate significant differences to compared to JX ($^{\square}P$≤0.05; $^{\square\square}P$≤0.01). Black diamonds indicate significant differences compared to ZJ ($^{\blacklozenge}P$≤0.05; $^{\blacklozenge\blacklozenge}P$≤0.01). CB, carbon black; CS, all coal samples.

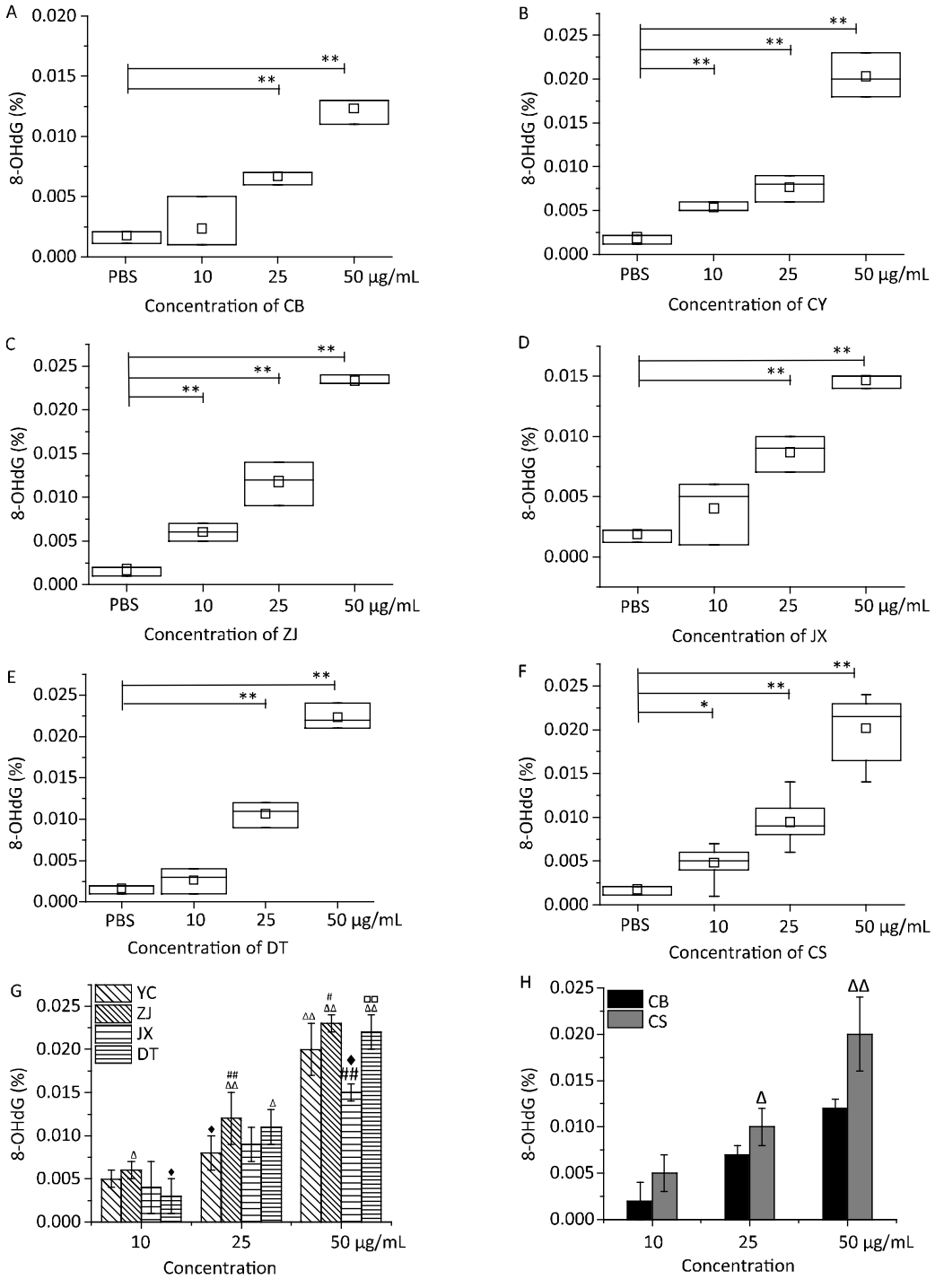


**Figure 4.** Determination of DNA damage. The 8-OHdG% in cells treated with coal-fired $PM_{2.5}$, CB, and CS, are shown in (A-F). The comparison between coal samples is shown in (G). The comparison between CB and coal is shown in (H). Data are presented as the mean±SD values ($n$=3). Asterisks indicate significant differences compared to controls ($^{*}P$≤0.05; $^{**}P$≤0.01). Triangles indicate significant differences compared to CB ($^{\Delta}P$≤0.05; $^{\Delta\Delta}P$≤0.01). Hash signs indicate significant differences compared to YC ($^{\#}P$≤0.05; $^{\#\#}P$ ≤0.01). Squares indicate significant differences compared to JX ($^{\square}P$≤0.05; $^{\square\square}P$≤0.01). Black diamonds indicate significant differences compared to ZJ ($^{\blacklozenge}P$≤ 0.05; $^{\blacklozenge\blacklozenge}P$≤ 0.01). CB, carbon black; CS, all coal samples.

***Relationships between Composition and Toxic Effects***

Stepwise multiple linear regression was performed using cell survival rate, 5-mC%, and 8-OHdG% as the dependent variables and compositions as the independent variables. As shown in Table 3, cell survival rate was negatively correlated with chloride and potassium ions content, and positively correlated with arsenic content. Significant correlations were not found between ions and 5-mC%. Table 3 shows that the 5-mC% level was positively correlated with Mn and negatively correlated with Zn. The 8-OHdG% level had no correlation with ions, but had a positive correlation with Mn and Fe (Table 3).

## DISCUSSION

The volatile matter of coal, correlated with the content of organic matter and coal quality, is the main classification index of coal. Depending on volatile matter, the degree of coalification could be determined. The higher the degree of coal metamorphism and the incombustible mineral content, the lower the volatile matter content[22-23]. The results showed that the volatile matter contents of Datong and Yinchuan coal were higher than those of Jingxi and Zhijin coal. Compared with other kinds of coal-fired $PM_{2.5}$ preparations, $PM_{2.5}$ suspension from Yinchuan coal had the highest cytotoxicity, which was in accordance with the result of volatile matter. Additionally, coal-fired $PM_{2.5}$ from Jingxi coal induced lower 8-OHdG% levels and that from Datong and Yinchuan coal produced more severe DNA impairment, which was in accordance with the volatile matter content. For global DNA methylation, $PM_{2.5}$ suspension from Datong coal had the highest toxic effect, which was also in agreement with the volatile matter content. However, $PM_{2.5}$ emitted from Yinchuan coal had the lowest 5-mC% levels. This result was inconsistent with the results of volatile matter analysis and connected with Mn content, which was positively correlated with the 5-mC% levels in this study. There could be many other reasons for this finding, which need to be further explored.

Several toxicology experiment studies have shown that $PM_{2.5}$ has a dose-dependent inhibitory effect on the growth of vascular endothelial cells[9,24-25]. We also found that $PM_{2.5}$ emitted from coal combustion and carbon black could inhibit *EA.hy926* cell proliferation with a dose-response relationship in this study. Studies have shown that respiratory and cardiovascular system diseases caused by fine particle matter could be related to enrichment of trace metals on the surface. Components of $PM_{2.5}$ such as Al, Ca, Fe, Pb, Mn, K, and V have associated with atherogenic index of plasma, and may contribute to the development of atherosclerosis[26]. Specific metal components of $PM_{2.5}$ (Ni, Cu, Se, and As) may be responsible for the elevated levels of systemic inflammatory markers, such as C-reactive protein and interleukin 6. These results suggest that specific metals may be important components responsible for $PM_{2.5}$-induced cardiovascular effects[27]. The study showed that cell survival rate was negatively correlated with $K^+$ and positively correlated with As. Studies on As found that low-dose exposure could induce the hormesis of proliferation in HaCaT and HELF cells with the dose-effect relation of an inverted U curve[28-29].

Particulate matter can influence gene expression by changing the level of DNA methylation, which leads to the development of a disease[30]. In the current study, it is still unclear whether airborne particulate matters induced down-regulation or up-regulation of the global DNA methylation[31-35]. The

**Table 3.** Regression Analysis of Compositions and Cell Toxic Effects

| *Y* | *X* | Variable | *β* | Equation |
|---|---|---|---|---|
| Cell survival rate | ions | $Cl^-$ | -0.024 | $Y$=99.951-0.024$Cl^-$ |
| | metals | $K^+$ | -0.013 | $Y$=101.111-0.013$K^+$+11.673As |
| | | As | 11.673 | |
| 5-mC% | metals | Mn | 11.369 | $Y$=0.397+11.369Mn-0.007Zn |
| | | Zn | -0.007 | |
| 8-OhdG% | metals | Mn | 0.008 | $Y$=0.002Fe+0.008Mn |
| | | Fe | 0.002 | |

***Note.*** *Y* represents dependent variables. *X* represents independent variables. *β* represents partial regression coefficient ($P$<0.05). According to α=0.10, variables were selected.

global DNA methylation level was measured using MethylFlash Methylated DNA 5-mC Quantification Kit, which is a cost-effective way to accurately measure levels of 5-mC. Using the same method to evaluate the effect of $PM_{10}$ on global DNA methylation in RAW264.7, it was found that exposure to 20 and 50 μg/mL $PM_{10}$ resulted in higher global 5-mC levels compared to those in the control group. However, the global 5-mC levels decreased exposure to 100 μg/mL and increased exposure to 200 μg/mL[36]. Consistent with the results, the global DNA methylation level increased significantly when cells were exposed to 10, 20, and 50 μg/mL $PM_{2.5}$ emitted from coal combustion and carbon black. The results showed that the 5-mC% levels were correlated with Mn and Zn. Similarly, the study suggested that metals can alter the DNA methylation profile[37].

A 2013 assessment by WHO's International Agency for Research on Cancer (IARC) concluded that outdoor air pollution is carcinogenic to humans, with the particulate matter component of air pollution most closely associated with increased cancer incidence[38]. Both *in vitro* and *in vivo* experimental studies have shown that exposure to particulate matter could induce genotoxicity. The results of this study showed that coal fired $PM_{2.5}$ and carbon black could cause DNA damage in a dose-dependent manner. Additionally, the 8-OHdG% levels were higher than those induced by carbon black and had a positive correlation with Mn and Fe. Research has shown that Polycyclic Aromatic Hydrocarbons (PAHs) and metals were biologically active constituents of $PM_{2.5}$ with regard to the induction of oxidative DNA damage[39-40]. Levels of 8-OHdG were correlated with cadmium and chromium levels[41-42]. However, other studies have investigated the effects of toxic heavy metals (As, Cd, Cr, Ni, and Pb) on oxidative stress and found only As and Ni to show a marginal or a significant positive linear dose-dependent effect on 8-OHdG[40].

Received: September 16, 2015;
Accepted: December 17, 2015

## REFERENCES

1. Brook RD, Franklin B, Cascio W, et al. Air pollution and cardiovascular disease: a statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. Circulation, 2004; 109, 2655-71.
2. Brook RD, Rajagopalan S, Pope CA, et al. Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. Circulation, 2010; 121, 2331-78.
3. Sun Q, Hong X, Wold LE. Cardiovascular effects of ambient particulate air pollution exposure. Circulation, 2010; 121, 2755-65.
4. Jia X, Guo X, Li H, et al. Characteristics and popular topics of latest researches into the effects of air particulate matter on cardiovascular system by bibliometric analysis. Inhal Toxicol, 2013; 25, 211-8.
5. Kang YJ, Li Y, Zhou Z, et al. Elevation of serum endothelins and cardiotoxicity induced by particulate matter ($PM_{2.5}$) in rats with acute myocardial infarction. Cardiovasc Toxicol, 2002; 2, 253-61.
6. Hansen CS, Sheykhzade M, Moller P, et al. Diesel exhaust particles induce endothelial dysfunction in apoE-/- mice. Toxicol Appl Pharmacol, 2007; 219, 24-32.
7. Ito T, Suzuki T, Tamura K, et al. Examination of mRNA expression in rat hearts and lungs for analysis of effects of exposure to concentrated ambient particles on cardiovascular function. Toxicology, 2008; 243, 271-83.
8. Miyata R, Hiraiwa K, Cheng JC, et al. Statins attenuate the development of atherosclerosis and endothelial dysfunction induced by exposure to urban particulate matter ($PM_{10}$). Toxicol Appl Pharmacol, 2013; 272, 1-11.
9. Wei H, Wei D, Yi S, et al. Oxidative stress induced by urban fine particles in cultured *EA.hy926* cells. Hum Exp Toxicol, 2011; 30, 579-90.
10. Ying Z, Xu X, Chen M, et al. A synergistic vascular effect of airborne particulate matter and nickel in a mouse model. Toxicol Sci, 2013; 135, 72-80.
11. Andersson A, Deng J, Du K, et al. Regionally-varying combustion sources of the January 2013 severe haze events over eastern China. Environ Sci Technol, 2015; 49, 2038-43.
12. Cheng NL, Li YT, Meng F, et al. Analytical Studies of PM2.5 Pollution and Source identification in China in 2013. Journal of Anhui Agri Sci, 2014; 42, 4721-4.
13. Hao XD, Liu F. Panel data analysis on the relationship between PM (2.5) pollution and coal consumption in China. Productivity Research, 2013; 2, 118-9, 27.
14. Wang P, Cao JJ, Shen ZX, et al. Spatial and seasonal variations of PM2.5 mass and species during 2010 in Xi'an, China. Sci Total Environ, 2015; 508, 477-87.
15. Bai XF. The distributions, modes of occurrenee and volatility of trace elements in coals of China: China Coal Research Institute; 2003.
16. Geng C, Wang K, Wang W, et al. Smog chamber study on the evolution of fume from residential coal combustion. Journal of Environmental Sciences, 2012; 24, 169-76.
17. Liu XY. The characteristics of particulate matters emitted from tpicle stationary source. Chinese Research Academy of Environmental Sciences, 2007.
18. Li D, Wang C, Li N, et al. Propofol selectively inhibits nuclear factor-kappaB activity by suppressing p38 mitogen-activated protein kinase signaling in human *EA.hy926* endothelial cells during intermittent hypoxia/reoxygenation. Mol Med Rep, 2014; 9, 1460-6.
19. Ramachandran V, Arumugam T, Wang H, et al. Anterior gradient 2 is expressed and secreted during the development

of pancreatic cancer and promotes cancer cell survival. Cancer Res, 2008; 68, 7811-8.
20. Fernandez-Roig S, Lai SC, Murphy MM, et al. Vitamin B12 deficiency in the brain leads to DNA hypomethylation in the TCblR/CD320 knockout mouse. Nutr Metab (Lond), 2012; 9, 41-4.
21. Nocchi L, Daly DM, Chapple C, et al. Induction of oxidative stress causes functional alterations in mouse urothelium via a TRPM8- mediated mechanism: implications for aging. Aging cell, 2014; 13, 540-50.
22. Zhao L, Song LM, Liu Z, et al. Investigation on volatiles and elements release characters of pulverized coal. Journal of Nort h China Electric Power, 2007; 34, 83-7.
23. Li SG, Hu WW, Lin HF, et al. Experimental Study on Influence of Coal Ash Content and Volatile Matter upon Its Methane Adsorption Capability. Mining Safety & Environmental Protection, 2015; 42, 16-8.
24. Zhao J, Xie Y, Jiang R, et al. Effects of atorvastatin on fine particle-induced inflammatory response, oxidative stress and endothelial function in human umbilical vein endothelial cells. Hum Exp Toxicol, 2011; 30, 1828-39.
25. Mo Y, Wan R, Chien S, et al. Activation of endothelial cells after exposure to ambient ultrafine particles: the role of NADPH oxidase. Toxicol Appl Pharmacol, 2009; 236, 183-93.
26. Fan J. Effects of metal components of fine particulate matter ($PM_{2.5}$) on atherogenic index of plasma: TianJin Medical University; 2014.
27. Niu J, Liberda EN, Qu S, et al. The role of metal components in the cardiovascular effects of PM2.5. PLoS One, 2013; 8, e83782.
28. Liu SY, Li X. Effect of low dose arsenic exposure on the proliferation and apoptosis of HaCat cells. Chin J Ctrl Endem Dis, 2013; 1-3. (In Chinese)
29. Yang P, Liu QZ, Li AP, et al. The relationship betwetm hormesis of proliferation and oxidative stress induced by sodium arsenite in human embryo lung fibroblasts. Chin J Ind Hyg Occup Dis, 2006; 24, 103-5.
30. Wang T, Garcia J, GN, Zhang W. Epigenetic Regulation in Particulate Matter-Mediated Cardiopulmonary Toxicities: A Systems Biology Perspective. Current pharmacogenomics and personalized medicine, 2012; 10, 314-21.
31. Bind MA, Baccarelli A, Zanobetti A, et al. Air pollution and markers of coagulation, inflammation, and endothelial function: associations and epigene-environment interactions in an elderly cohort. Epidemiology, 2012; 23, 332-40.
32. Madrigano J, Baccarelli A, Mittleman MA, et al. Prolonged exposure to particulate pollution, genes associated with glutathione pathways, and DNA methylation in a cohort of older men. Environ Health Perspect, 2011; 119, 977-82.
33. Tarantini L, Bonzini M, Apostoli P, et al. Effects of particulate matter on genomic DNA methylation content and iNOS promoter methylation. Environ Health Perspect, 2009; 117, 217-22.
34. Baccarelli A, Wright RO, Bollati V, et al. Rapid DNA methylation changes after exposure to traffic particles. Am J Respir Crit Care Med, 2009; 179, 572-8.
35. Lund G, Andersson L, Lauria M, et al. DNA methylation polymorphisms precede any histological sign of atherosclerosis in mice lacking apolipoprotein E. J Biol Chem, 2004; 279, 29147-54.
36. Miousse IR, Chalbot M-CG, Aykin-Burns N, et al. Epigenetic alterations induced by ambient particulate matter in mouse macrophages. Environmental and Molecular Mutagenesis, 2014; 55, 428-35.
37. Brocato J, Costa M. Basic mechanics of DNA methylation and the unique landscape of the DNA methylome in metal-induced carcinogenesis. Crit Rev Toxicol, 2013; 43, 493-514.
38. WHO. Air pollution and cancer: International Agency for Research on Cancer; 2013.
39. Wei Y, Han IK, Shao M, et al. $PM_{2.5}$ constituents and oxidative DNA damage in humans. Environ Sci Technol, 2009; 43, 4757-62.
40. Wang T, Feng W, Kuang D, et al. The effects of heavy metals and their interactions with polycyclic aromatic hydrocarbons on the oxidative stress among coke-oven workers. Environ Res, 2015; 140, 405-13.
41. Pizzino G, Bitto A, Interdonato M, et al. Oxidative stress and DNA repair and detoxification gene expression in adolescents exposed to heavy metals living in the Milazzo-Valle del Mela area (Sicily, Italy). Redox Biol, 2014; 2, 686-93.
42. Zhao L, Song Y, Pu J, et al. Effects of repeated Cr(VI) intratracheal instillation on club (Clara) cells and activation of nuclear factor-kappa B pathway via oxidative stress. Toxicol Lett, 2014; 231, 72-81.