

Received: 2016.12.23  
Accepted: 2017.01.10  
Published: 2017.08.12

# Cardiotoxicity and Mechanism of Particulate Matter 2.5 (PM2.5) Exposure in Offspring Rats During Pregnancy

Authors' Contribution:  
Study Design A  
Data Collection B  
Statistical Analysis C  
Data Interpretation D  
Manuscript Preparation E  
Literature Search F  
Funds Collection G

BCEF **Hongling Wang**  
CD **Xiangwen Peng\***  
D **Fenglin Cao\***  
EF **Ying Wang\***  
E **Huijie Shi\***  
F **Shuai Lin#**  
E **Weijie Zhong#**  
ACDEFG **Jingxia Sun**

Department of Obstetrics, First Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang, P.R. China

\* These authors contributed equally to this study

# These authors contributed equally to the study

**Corresponding Author:** Jingxia Sun, e-mail: [sunjxfahmu128@163.com](mailto:sunjxfahmu128@163.com)

**Source of support:** This research was supported by the Heilongjiang Provincial Education Department Project (12521300)

**Background:** The aim of this study was to investigate the cardiotoxicity and mechanism of particulate matter 2.5 (PM2.5) exposure on offspring rats during pregnancy.

**Material/Methods:** Wistar rats were used to establish a PM2.5 exposure animal model during pregnancy, and they were divided into a control group, a low-dose group, a middle-dose group, and a high-dose group according to PM2.5 exposure dose. The pathological changes of heart tissue, the rate of myocardial cell apoptosis, the levels of LDH, AST, and CM-KB in serum, and the difference in mitochondrial fusion genes (OPA1 and Mfn1) and mitochondrial genes (Drp1 and Fis1) were compared among groups.

**Results:** The arrangement of myocardial fibers in offspring mice of PM2.5 exposure groups became disordered, the shape of some cardiomyocytes became irregular, and some staining darker nuclei appeared. The apoptotic rates of myocardium in offspring rats exposed to PM2.5 were (12.61±0.93)% in the low-dose group, (25.14±1.53)% in the middle-dose group, and (30.13±1.50)% in the high-dose group, which were all significantly higher than in the control group (9.12±0.80)% (P<0.05). The levels of LDH, AST, and CM-KB and the expression of OPA1, Mfn1, Drp1, and Fis1 in offspring mice of PM2.5 exposure groups increased with the increase of PM2.5 exposure dose, and were significantly higher than that of the control group (P<0.05).

**Conclusions:** The mitochondria of the offspring mice were damaged due to the abnormal expression of mitochondrial fusion/splicing gene by PM2.5 exposure during pregnancy, and the hearts of offspring mice were damaged due to damaged mitochondria.

**MeSH Keywords:** **Aspartate Aminotransferase, Mitochondrial • Pregnancy, Animal • Rats**

**Full-text PDF:** <https://www.medscimonit.com/abstract/index/idArt/903006>

 2090

 2

 4

 23



## Background

Fine particulate matter 2.5 (PM2.5) is a collective term referring to atmospheric fine particles [1]. PM2.5 is regarded as one of the most important air pollutants in many cities of China [2]. As air pollution caused by PM2.5 is intensifying in recent years, there has been a rising incidence of cardiovascular [3] and respiratory diseases [4]. One study [5] has shown that PM2.5 enters the human lungs via respiration and adheres to the lung epithelium. Part of the PM2.5 inhaled cannot be removed by the lymphatic system and enters the blood circulation via diffusion. Then, the inhaled PM2.5 goes into the heart, kidneys, and other organs. Choi et al. [6] pointed out that several main ingredients of PM2.5, including polycyclic aromatic hydrocarbons, can enter the fetus via the placenta and air-blood barrier. The tender and delicate fetal organs at the early stage of embryonic development are very sensitive to the environment, and PM2.5 may damage the newborn's health. It has been indicated by many epidemiological studies [7–11] that exposure to PM2.5 during pregnancy can lead to low birth weight, birth defects, premature delivery, bronchopulmonary dysplasia, and fetal death. Therefore, PM2.5 exposure not only increases the risk of cardiovascular and respiratory diseases in adults, but also increases the risk of cardiac injury and cardiovascular diseases in the offspring of pregnant women. We built an animal model of PM2.5 exposure during pregnancy and compared the degree of cardiac injury and the expression of mitochondrial fusion/fission genes in offspring rats receiving different treatments. The purpose of the study was to investigate the effect of PM2.5 exposure during pregnancy on the cardiac health of offspring rats and to discuss the potential working mechanism.

## Material and Methods

### Experimental animals

Sixty Wistar rats aged 10–11 weeks were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. There were 40 male rats (300–320 g) and 20 female rats (250–270 g). After 1-week acclimatization, 2 male rats and 1 female rat were placed into 1 cage. The smears of vaginal secretions from the female rats were observed on the next day. The female rats were considered pregnant if sperm was present. Then, the pregnant female rats were reared in a separate cage. The non-pregnant female rats were reared with male rats in the same cage until pregnancy.

### Materials

The materials used in the experiments were: BTPM-AS1 atmospheric particle sampler (Bettersize Instruments Ltd, China),

Minute™ total protein extraction kit (SD-001, Inventbiotech, USA), animal tissue total RNA extraction kit (DP431, Tiangen Biotech Co., Ltd, China), the reverse transcription kit (K1622, Fermentas, Lithuania), and OPA1, Mfn1, Drp1, Fis1,  $\beta$ -actin, and goat anti-rabbit secondary antibody (sc-30572, sc-50330, sc-21804, sc-98900, sc-7201, sc-3846, Santa Cruz Biotechnology, USA).

### Experiment

#### Collection and treatment of PM2.5 samples

PM2.5 collection was performed at Harbin from September 2014 to March 2016, using the BTPM-AS1 atmospheric particle sampler. The glass fibers used for adsorbing PM2.5 were cut into 1.5×1.5-cm strips and immersed in pure water. After ultrasonic vibration and freeze drying, the PM2.5 sample was preserved at –20°C. For the formal experiment, the sample was diluted with 0.9% normal saline into a PM2.5 suspension of a specific concentration and preserved at 4°C.

#### Construction of the PM2.5 exposure model in pregnant rats

Twenty pregnant female rats were randomly divided into a control group (0.9% normal saline), a low-dose group (PM2.5=0.375 mg/kg), a medium-dose group (PM2.5=1.5 mg/kg), and a high-dose group (PM2.5=6.0 mg/kg). Each group had 5 female rats. PM2.5 exposure was simulated as follows: at 10 d, 12 d, 14 d, and 16 d of pregnancy, the female rats were anesthetized and immobilized on a wooden plank. The PM2.5 suspension or normal saline of equal concentration was administered dropwise into the trachea using a syringe.

#### Morphology observation of the cardiac tissues in offspring rats

After natural delivery, 10 offspring rats were randomly selected from each group and sacrificed within 24 h after birth to harvest the cardiac tissues. The cardiac tissues were washed with normal saline and fixed in 10% formalin for over 24 h. The specimens were dehydrated, transparentized, paraffin-embedded, sliced, and subjected to HE staining. The cardiac tissues were observed under a microscope.

#### Detection of cardiocyte apoptosis rates in offspring rats

The cardiocyte apoptosis rate was detected by using the TUNEL assay. First, the specimens were dewaxed and dehydrated conventionally, and incubated with protease K for 0.5 h at room temperature for 15 min and then at 37°C for 15 min. The specimens were further incubated with the TUNEL reaction mixture at 37°C for 1 h, transforming agent-POD at 37°C for 0.5 h, DAB substrate at room temperature for 10 min, and

**Table 1.** PCR primers designed for mitochondrial fusion/fissure genes.

Gene	Primer sequence (5'-3')	Length (bp)
OPA1	F: GCCAGTGGTATGTGGCTGAA	510
	R: GGATGTTTTGTATTGCTTCTCAG	
Mfn1	F: ACTGACACAGCCAGTAAAATGT	468
	R: CCCTCCCATCTACCACTCA	
Drp1	F: TCTGTTTTCAGAGCAGCGGA	292
	R: GCATACTACTTCTCACAGGACTTCT	
Fis1	F: CGCAGAGTTGGGGTTGTTTG	478
	R: CCGAGGCTGCACCTTTCTT	
β-actin	F: AAGTACTCCGTGGATCGG	615
	R: TCAAGTTGGGGACAAAAAG	

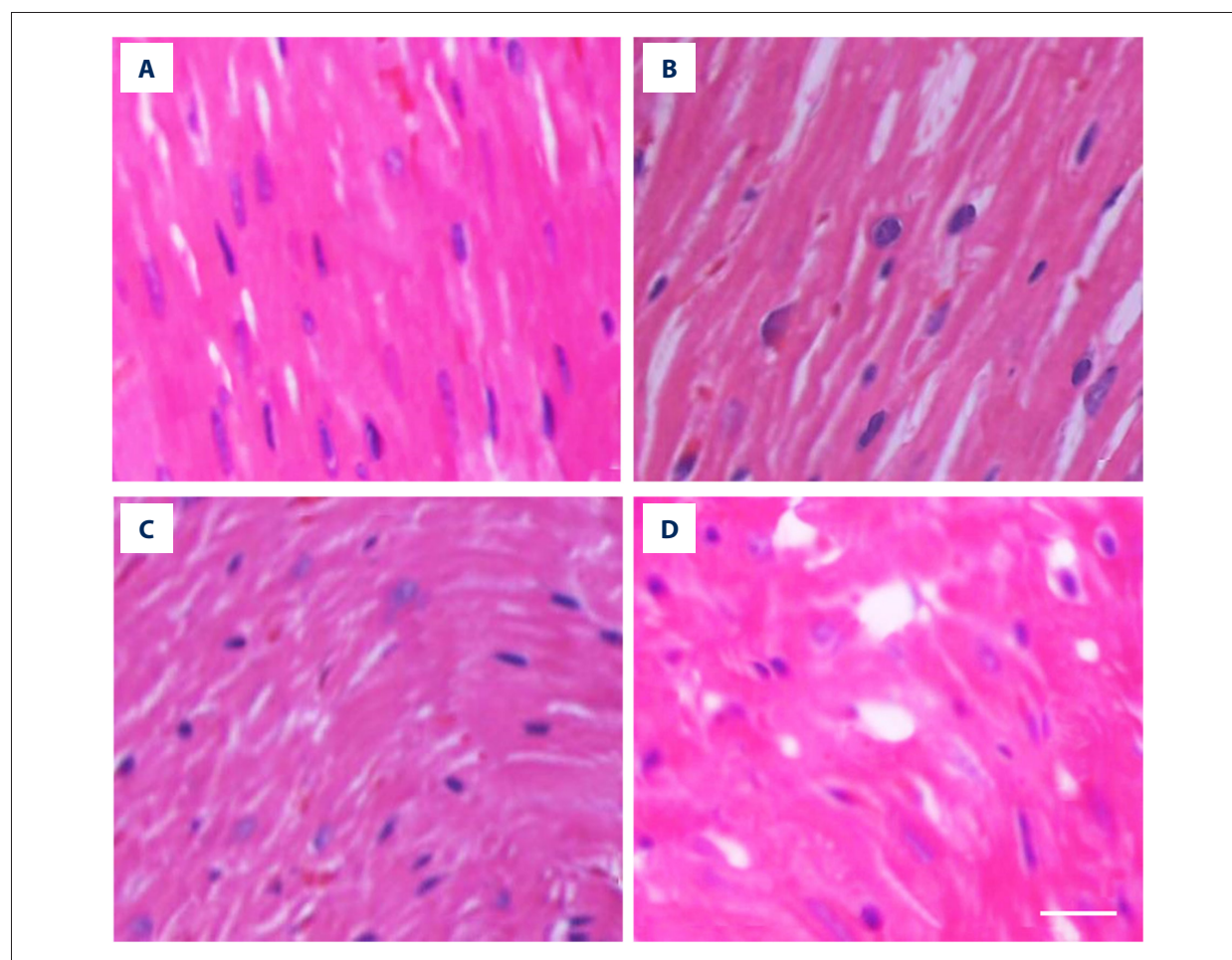
counterstained with hematoxylin. The slides were mounted and analyzed with image analysis software.

#### *Determination of serum levels of LDH, AST, and CK-MB in offspring rats*

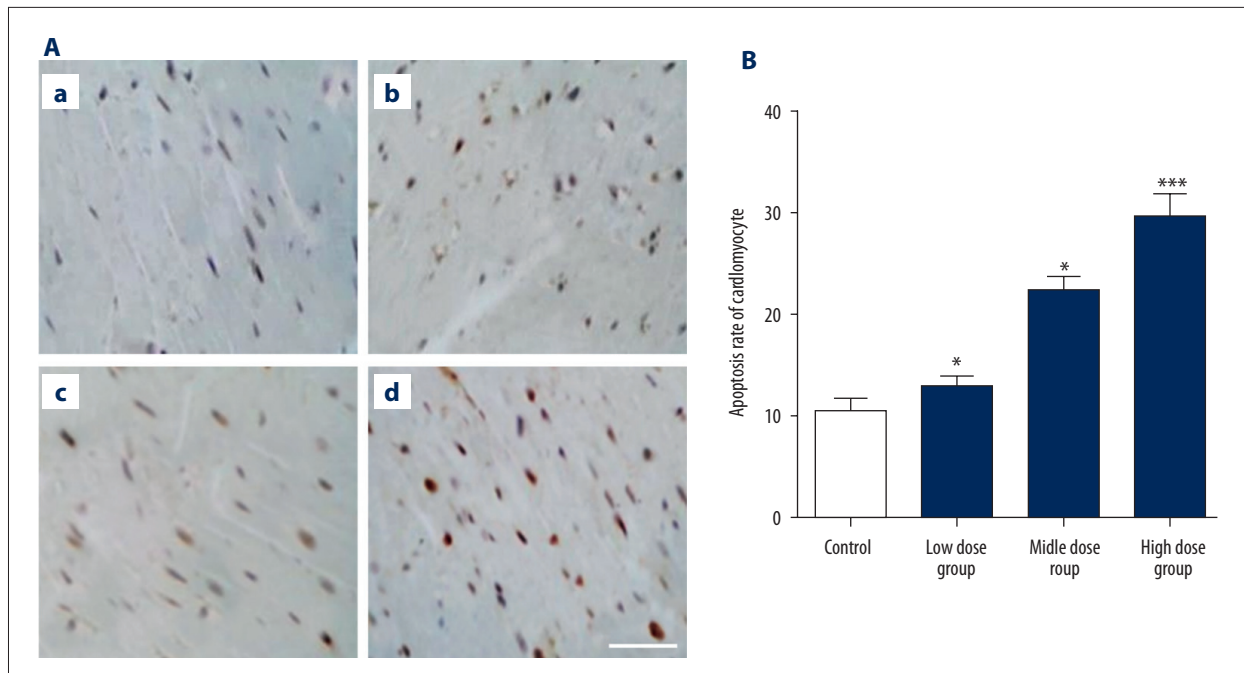
After natural delivery, 10 offspring rats were randomly selected from each group and reared in a SPF-grade animal laboratory for 1 week. Then, the rats were anesthetized and received cardiac puncture. The blood samples were centrifuged at 1000 rpm for 10 min, and the supernatant (serum) was collected and cryopreserved. The serum levels of LDH, AST, and CK-MB were detected by using a fully automatic biochemistry analyzer.

#### *Detecting the expression of mitochondrial fusion/fissure genes in offspring rats*

After natural delivery, 10 offspring rats were randomly selected from each group and sacrificed within 24 h after birth to



**Figure 1.** HE staining of cardiac tissues in offspring rats. (A) Control group. (B) Low-dose group. (C) Middle-dose group. (D) High-dose group. Bar: 50 μm.



**Figure 2.** The cardiocyte apoptosis rate in each group. (A) a: the control group; b: the low-dose group; c: the middle-dose group; d: the high-dose group. (B) Apoptosis rate of myocardial cells. Bar: 50  $\mu$ m.

harvest the cardiac tissues. The cardiac tissues were washed with normal saline. Total RNA was extracted from the cardiac tissues using the animal tissue total RNA extraction kit. cDNA was synthesized using the reverse transcription kit, which was followed by semi-quantitative PCR. Then, 10  $\mu$ l of the product was used for nucleic acid gel electrophoresis. The gels were imaged using the Bio-Rad gel imager and photographed, and then analyzed with Quantity One software. The results are expressed as relative expression levels of the target genes with  $\beta$ -actin as the internal reference. The primers were designed for the mitochondrial fusion genes (OPA1 and Mfn1), mitochondrial fission genes (Drp1 and Fis1), and  $\beta$ -actin according to the sequences in the NCBI website. Table 1 shows the primer sequences.

Total protein was extracted from the cardiac tissues using the total protein extraction kit. The total protein concentration was determined with the BCA kit, and 100  $\mu$ g of the protein was used for SDS-PAGE. The separated proteins were wet-transferred to the membranes, which were then sealed, incubated with primary and secondary antibodies, and color development substrate. The band brightness was analyzed using Image-J software.

### Statistical analysis

The results are expressed as mean  $\pm$  standard deviation. SPSS 19.0 was used for statistical analysis. The independent-samples

*t* test was used for intergroup comparisons.  $P < 0.05$  was considered a significant difference.

## Results

### HE staining of the cardiac tissues in offspring rats

The myocardial fibers in offspring rats from the control group were very neatly arranged and the nuclei were uniformly stained. The sarcoplasm was intact and had an oval shape (Figure 1A). In the low-dose group, the myocardial fibers were neatly arranged, but some cardiocytes were of an irregular morphology and a few nuclei were deeply stained (Figure 1B). The morphological characteristics of the cardiac tissues were similar in the medium- and high-dose groups; both had a disordered arrangement of myocardial fibers, with many irregularly shaped cardiocytes and deeply stained nuclei (Figure 1C, 1D).

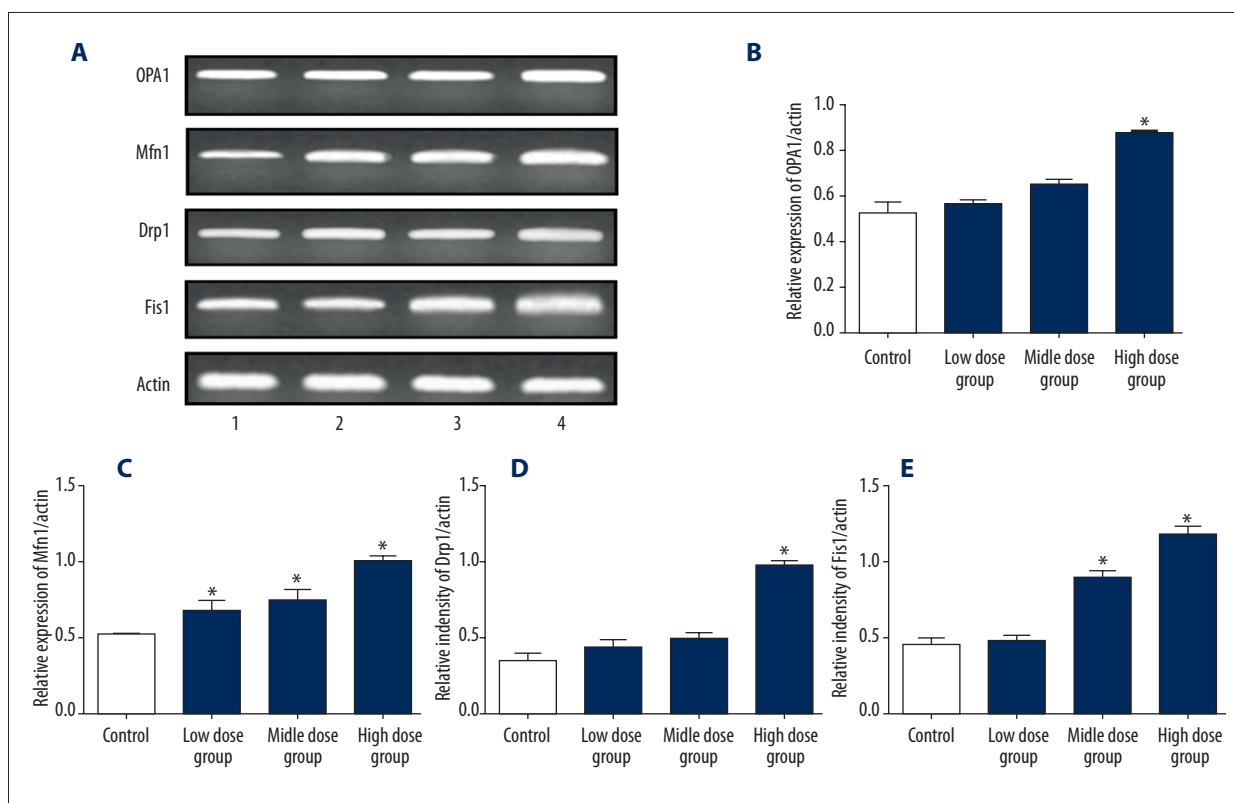
### The cardiocyte apoptosis rate in offspring rats

The cardiocyte apoptosis rates in the offspring rats were  $12.61 \pm 0.93\%$ ,  $25.14 \pm 1.53\%$ , and  $30.13 \pm 1.50\%$  in the low-, medium-, and high-dose groups, respectively; all of them were significantly higher than that of the control group ( $9.12 \pm 0.80\%$ ) (Figure 2).

**Table 2.** Comparison of serum levels of LDH, AST and CK-MB in offspring rats.

Group	N	LDH (U/L)	AST (U/L)	CK-MB (U/L)
Control group	10	65.28±4.79	22.36±2.66	114.53±11.14
Low-dose group	10	156.49±11.19*	28.63±1.79*	152.06±12.14*
Medium-dose group	10	181.77±9.81*	31.38±2.14*	210.87±16.34*
High-dose group	10	198.45±7.57*	33.67±2.43*	240.43±19.13*

\* Compared with the control group.



**Figure 3.** Effects of different doses of PM2.5 exposure on mRNA expression of mitochondrial fusion/fission genes in offspring rats. (A) The mRNA expression of mitochondrial fusion/fission genes. (B) Relative intensity of OPA1/actin. (C) Relative intensity of Mfn1/actin. (D) Relative intensity of Drp1/actin. (E) Relative intensity of Fis1/actin. \*  $P < 0.05$  indicates significant difference as compared with the control group.

### Serum levels of LDH, AST, and CK-MB in offspring rats

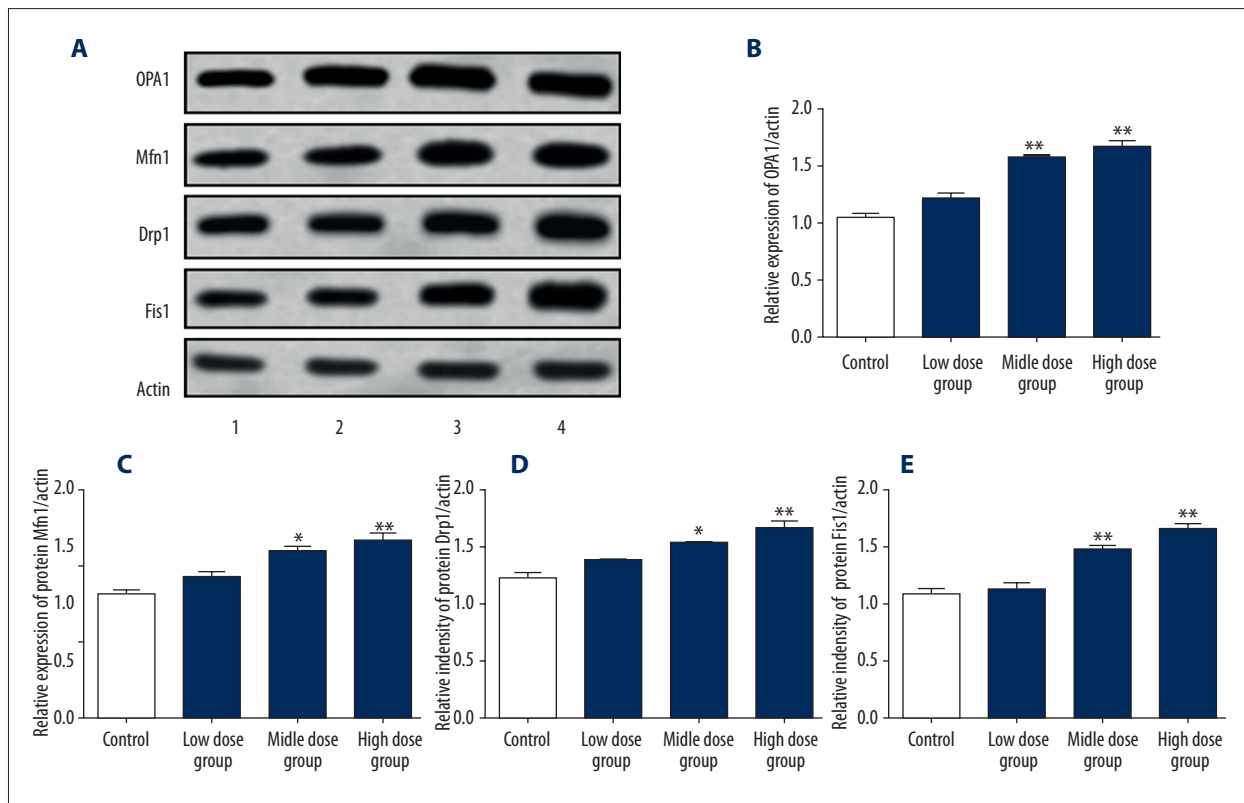
There was a dramatic rise in the serum levels of LDH, AST, and CK-MB in offspring rats in all treatment groups as compared with the control group ( $P < 0.05$ ). The expression levels of each gene increased in a dose-dependent manner (Table 2).

### Expression of mitochondrial fusion/fission genes and proteins

The expression of mitochondrial fusion genes OPA1, Drp1, and Fis1 was not significantly different between the low-dose

group and the control group ( $P > 0.05$ ); however, there was a statistical difference in the mRNA expression of Mfn1 between the low-dose group and the control group ( $P < 0.05$ ), but there were no significant differences from the expression of the corresponding proteins.

The expressions of mitochondrial fusion/fission genes Mfn1 ( $P < 0.05$ ) and Fis1 ( $P < 0.01$ ) were significantly higher in the medium-dose group than those in the control group. OPA1 and Drp1 were not significantly different between the medium-dose group and the control group ( $P > 0.05$ ). The expressions of OPA1, Mfn1, Drp1, and Fis1 were significantly higher in the



**Figure 4.** Effects of different doses of PM2.5 exposure on mRNA expression of mitochondrial fusion/fission genes in offspring rats. (A) The protein expression of mitochondrial fusion/fission genes. (B) Relative intensity of protein OPA1/actin. (C) Relative intensity of protein Mfn1/actin. (D) Relative intensity of protein Drp1/actin. (E) Relative intensity of protein Fis1/actin. \*  $P < 0.05$  indicates significant difference as compared with the control group; \*\*  $P < 0.01$  indicates significant difference as compared with the control group.

medium-dose group than in the control group ( $P < 0.01$ ,  $P < 0.05$ ,  $P < 0.05$ , and  $P < 0.01$ , respectively).

In the high-dose groups, for genes OPA1, Mfn1, Drp1, and Fis1 and the correspondence proteins OPA1, Mfn1, Drp1 and Fis1, there were significant differences compared with the control groups (Figures 3, 4).

## Discussion

PM2.5 refers to any tiny particles or droplets in the air that are 2.5 microns or less in width and are capable of suspending in the air for a long period of time. The PM2.5 level can be used to determine the degree of air pollution. After being inhaled into the lungs, PM2.5 can further go into the human blood circulation via diffusion and reach nearly every organ of the body, causing cardiovascular diseases [3], bronchitis [12], asthma [12], and hypertension [13].

The fetus absorbs nutrients and oxygen via the umbilical cord connected to the placenta. The PM2.5 entering the maternal

blood circulation can also enter the fetus. Hooven et al. [9] showed that PM2.5 exposure during pregnancy was negatively correlated with the newborn's weight in the second and third month after birth and positively correlated with the risk of premature delivery. There is a growing body of epidemiological evidence showing that because the fetal organs are highly sensitive to the environment, long-term exposure to polluted air can lead to birth defects, bronchopulmonary dysplasia, and cardiovascular diseases; cardiovascular diseases are the most common among the affected fetuses [14,15]. Only 10–25% of congenital heart diseases are caused by genetic factors alone, while the majority are associated with external factors [16]. We found that the offspring rats whose mothers were exposed to PM2.5 during pregnancy showed disordered arrangement of myocardial fibers, irregular morphology of some cardiocytes, and some deeply stained nuclei; the mitochondrial cristae were obscured and showed edema. Moreover, as a result of PM2.5 exposure during pregnancy, there was a significant increase in the cardiocyte apoptosis rate and serum levels of LDH, AST, and CK-MB in the offspring rats as compared with the control rats. These results indicate cardiotoxicity in offspring rats due to PM2.5 exposure during pregnancy.

It is generally believed that oxidative stress response, inflammatory response, and cell apoptosis mechanism play important roles in cardiotoxicity associated with PM2.5 exposure [17]. The most important oxidative stress response in cardiotoxicity associated with PM2.5 exposure is the hyperactivity of oxidative phosphorylation of cardiocytes, which eventually leads to mitochondrial injury of cardiocytes and cardiocyte death [18]. Inflammatory response and oxidative stress response are closely correlated with respect to their roles in cardiac injury caused by PM2.5. Both responses can generate many free radicals, which in turn aggravate the inflammatory response and oxidative response. It should be noted that free radicals are closely related to mitochondrial injury [19]. The proteins or their complexes that regulate cell apoptosis can only serve their function via cytochrome c after entering the mitochondrial membrane [20]. It is apparent that mitochondria play a crucial role in the cardiotoxicity caused by PM2.5. To fulfill their function, the mitochondria must undergo constant fission and fusion. If there are any abnormalities in mitochondrial fission and fusion, diseases may occur [21]. OPA1 and Mfn 1 and 2 jointly regulate mitochondrial fusion; Drp1 and Fis1 mediate mitochondrial fission [22]. The normal expression of the mitochondrial fusion/fission genes is the prerequisite for normal mitochondrial functioning. Otherwise,

there will be mitochondrial fragmentation, which leads to impaired mitochondrial function [23].

We also found that the expression levels of mitochondrial fusion genes (OPA1 and Mfn1) and fission genes (Drp1 and Fis1) in the offspring rats increased with increasing dose of PM2.5 exposure during pregnancy. The difference reached a significant level as compared with the control group. This indicates mitochondrial injury in offspring rats, which is related to abnormal expression of mitochondrial fusion/fission genes due to PM2.5 exposure during pregnancy.

## Conclusions

PM2.5 exposure during pregnancy can cause mitochondrial injury of cardiocytes in offspring rats by inducing abnormal mitochondrial fusion/fission of the cardiocytes, which further leads to cardiac injury in offspring rats.

## Conflict of Interest

None.

## References:

- Li Y, Tao J, Zhang L et al: High contributions of secondary inorganic aerosols to PM2.5 under polluted levels at a regional station in Northern China. *Int J Environ Res Public Health*, 2016; 13: 1202–17
- Li J, Jin M, Xu Z: Spatiotemporal variability of remotely sensed PM2.5 concentrations in China from 1998 to 2014 based on a bayesian hierarchy model. *Int J Environ Res Public Health*, 2016; 13: 772–89
- Luo K, Li W, Zhang R et al: Ambient fine particulate matter exposure and risk of cardiovascular mortality: Adjustment of the meteorological factors. *Int J Environ Res Public Health*, 2016; 13: 1082–99
- Bonyadi Z, Ehrampouh MH, Ghaneian MT et al: Cardiovascular, respiratory, and total mortality attributed to PM2.5 in Mashhad, Iran. *Environ Monit Assess*, 2016; 188(10): 570
- Liu C, Xu X, Bai Y et al: Air pollution-mediated susceptibility to inflammation and insulin resistance: Influence of CCR2 pathways in mice. *Environ Health Perspect*, 2014; 122: 17–26
- Choi H, Wang L, Lin X et al: Fetal window of vulnerability to airborne polycyclic aromatic hydrocarbons on proportional intrauterine growth restriction. *PLoS One*, 2012; 7: 24
- Rossner P Jr, Tabashidze N, Dostal M et al: Genetic, biochemical, and environmental factors associated with pregnancy outcomes in newborns from the Czech Republic. *Environ Health Perspect*, 2011; 119: 265–71
- Rudra CB, Williams MA, Sheppard L et al: Ambient carbon monoxide and fine particulate matter in relation to preeclampsia and preterm delivery in western Washington State. *Environ Health Perspect*, 2011; 119: 886–92
- van den Hooven EH, Pierik FH, de Kluizenaar Y et al: Air pollution exposure during pregnancy, ultrasound measures of fetal growth, and adverse birth outcomes: A prospective cohort study. *Environ Health Perspect*, 2012; 120: 150–56
- Atkinson RW, Carey IM, Kent AJ et al: Long-term exposure to outdoor air pollution and incidence of cardiovascular diseases. *Epidemiology*, 2013; 24: 44–53
- Liu Y, Wang L, Wang F et al: Effect of fine particulate matter (PM2.5) on rat placenta pathology and perinatal outcomes. *Med Sci Monit*, 2016; 22: 3274–80
- Liu C, Huang F, Yang Z et al: [A Meta analysis on the associations between air pollution and respiratory mortality in China]. *Zhonghua Liu Xing Bing Xue Za Zhi*, 2015; 36: 889–95 [in Chinese]
- Xu WX, Chen RJ, Kan HD: [The impact of ambient fine particulate matter on the blood pressure of an urban population in Shanghai, China: A panel study]. *Zhonghua Yu Fang Yi Xue Za Zhi*, 2016; 50: 716–20 [in Chinese]
- van der Bom T, Zomer AC, Zwinderman AH et al: The changing epidemiology of congenital heart disease. *Nat Rev Cardiol*, 2011; 8: 50–60
- Dadvand P, Rankin J, Rushton S et al: Association between maternal exposure to ambient air pollution and congenital heart disease: A register-based spatiotemporal analysis. *Am J Epidemiol*, 2011; 173: 171–82
- Lage K, Greenway SC, Rosenfeld JA et al: Genetic and environmental risk factors in congenital heart disease functionally converge in protein networks driving heart development. *Proc Natl Acad Sci USA*, 2012; 109: 14035–40
- Martinelli N, Olivieri O, Girelli D: Air particulate matter and cardiovascular disease: A narrative review. *Eur J Intern Med*, 2013; 24: 295–302
- Pei Y, Jiang R, Zou Y et al: Effects of fine particulate matter (PM2.5) on systemic oxidative stress and cardiac function in ApoE(–/–) mice. *Int J Environ Res Public Health*, 2016; 13: 484–99
- Wang G, Zhen L, Lu P et al: [Effects of ozone and fine particulate matter (PM2.5) on rat cardiac autonomic nervous system and systemic inflammation]. *Wei Sheng Yan Jiu*, 2013; 42: 554–60
- Wang Q, Zhang L, Yuan X et al: The Relationship between the Bcl-2/Bax proteins and the mitochondria-mediated apoptosis pathway in the differentiation of adipose-derived stromal cells into neurons. *PLoS One*, 2016; 11: e0163327
- Zorzano A, Liesa M, Sebastian D et al: Mitochondrial fusion proteins: Dual regulators of morphology and metabolism. *Semin Cell Dev Biol*, 2010; 21: 566–74
- Yang X, Shi Q, Sun J et al: Aberrant alterations of mitochondrial factors Drp1 and Opa1 in the brains of scrapie experiment rodents. *J Mol Neurosci*, 2016; 6: 6
- Westermann B: Mitochondrial fusion and fission in cell life and death. *Nat Rev Mol Cell Biol*, 2010; 11: 872–84