Effects of Chronic Exposure to Particulate Air Pollution on Cardiovascular System: An Experimental Study Using Mice Models

A K Fauzie¹,²,* and G V Venkataramana¹

¹ Department of Studies in Environmental Science, University of Mysore, Manasagangotri, Mysuru – 570006, Karnataka, India
² Department of Environment and Cleaning Service, Regencial Government of Karawang, Karawang – 41311, West Java, Indonesia

*aziskemalfauzie@gmail.com

Abstract. Our study investigated the chronic effects of short-term and medium-term exposure to urban particulate matter (PM) on cardiovascular system using murine models. Three-week-old Swiss albino mice (Mus musculus) were exposed to vehicular PM for five days (5D) and three months (3M), and some of them were kept in laboratory as control. Blood samples were analyzed using an automated hematological analyzer and cardiac tissues followed histopathological analysis to determine myocardial infarction as well as fibrosis and elastosis of coronary arteries. Results showed depletions ($p < 0.1$) in packed cell volume, red blood cells, and neutrophils after 3M of PM exposure. The density of neutrophils infiltrated in the myocardium was increased after 5D and 3M of PM exposure ($p < 0.05$). Higher thickness of arteriolar walls were found in almost all sized arterioles after 3M of PM exposure ($p < 0.05$) indicating higher content of collagen and elastic fibers, but no evidence of such after 5D of PM exposure. All parameters were compared to the control condition. We conclude that exposure to PM air pollution significantly altered cardiovascular system in animal models signaling that their blood and organs were badly injured.

1. Introduction

Air pollution is one of the important problems threatening human health from all over the world for many centuries. There are five major air pollutants namely carbon monoxide, nitrogen dioxide, sulfur dioxide, ground-level ozone, and particulate matter (PM). The latter gets the highest concern nowadays due to the higher degree of urbanization and motorized transportation. There are plenty epidemiological studies related to the effect of long-term exposure to air pollution on chronic morbidity and mortality. Evidences in short-term exposure were also found for adverse effect of air pollution on public health outcomes.

Biomarkers are being used increasingly to evaluate the health status of individuals in relation to pollution [1], including blood sampling. Hematology is considered a sensitive early indicator of the toxic effects of pollutants [2]. Some studies have reported hematological changes in mammals, e.g. rodents, exposed to air pollution [3]. However, relationship between PM air pollution and blood indices still results in controversies. While some studies reported the association between PM exposure and blood cell, some others did not confirm such association [4].
The recent emerging topic intensely discussed worldwide is the effect of air pollution, notably PM, on cardiopulmonary system [5]. Large number of evidence on public health outcomes attributable to air pollution have been reported such as cardiovascular disease [6], increased hospital admission [7], pre- and postnatal morbidity and mortality [8]. However, mechanism by which the PM promotes these effects is still not well-understood.

The common but robust and plausible technique to investigate the effect of PM exposure on cardiovascular system is by biopsy study coupled with morphometric analysis of the affected organs, particularly heart, to observe any changes in the structural state of myocardial tissue [9,10] and coronary arteries [11,12]. Interestingly, this combined study remains scarce and still comes out with some uncertainties and non-significant findings. Thus, the present study was proposed to provide recent evidence on histopathological changes in cardiovascular system focused on the diagnosis of myocardial infarction and structural alterations of coronary arterial wall. We also provide relevant validation on the effect of exposure to suspended particulate matter on both hematological parameters. The experiment employed Swiss albino mice (Mus musculus) as study models and carried out in two different exposure duration of short-term (five days) and medium-term (three months).

2. Materials and Methods

2.1. Study site and air pollution monitoring
The exposure site is located precisely in the side of Irwin Road, a highly narrow, busy, and congested road in Mysore city, Karnataka, India. The site is about 500 m from sub-urban bus stand and about 700 m from city bus stand. The control site was used for data comparison and is located inside the campus of University of Mysore. There are no industries, waste dumping site or significant biomass burning sources in the surroundings. The distance between exposure and control site is about 3.5 km.

The concentration of airborne suspended particulate matter (SPM) was determined gravimetrically using vacuum air pump at a flow rate of 25 L/min. NO2 level was determined using a digital Aeroqual active sampler. Meteorological parameters were measured using a weather center, AcuRite model 00615. Site traffic conditions were also identified by actual road traffic census to give a slight picture on the magnitude of vehicular emission sources in the study area. Emission load generated by vehicles in each site was estimated from their actual traffic volume using formula given elsewhere [13].

2.2. Animal exposure
Three-weeks-old CD-1 Swiss albino mice (Mus musculus) were obtained from the animal health care laboratory, Department of Zoology, University of Mysore. The animals were maintained at 12 to 12 hour light cycle. Food and water were available ad libitum. Animals were divided into groups and some of them were exposed to vehicular PM for 5 days and 3 months in the urban traffic area (each 5 to 6 hours per day). Another two comparable groups of mice were kept in university’s laboratory and served as control. All laboratory animals received humane care in compliance with the guide published by the National Academy of Sciences [14]. This study was approved by the Institutional Animal Ethics Committee of University of Mysore No. UOM/IAEC/25/2018.

The experimental set up consisted of cubical plastic inhalation chamber that was attached to vacuum pump containing auto adjustable speed of one inlet and outlet pipes with two types of exchangeable filters. The filters which prevented the admission of ambient particles (designated as pollutants) were removed, so the atmospheric air can enter the inhalation chamber without filtering system. The exposure was conducted in late winter to midsummer (February to April).

2.3. Hematological analysis
After termination of exposure period animals were anaesthetized using chloroform and then sacrificed. Blood samples were drawn using cardiac puncture and collected in anti-clotting tubes containing EDTA for hematological studies. Blood parameters such as hemoglobin (Hb), hematocrit or packed cell volume (PCV), erythrocytes or red blood cell (RBC) count, leukocytes or white blood cell (WBC)
total count, neutrophil, eosinophil, basophil, monocyte and lymphocyte differential counts, and thrombocytes or platelet (PLT) count were assessed by using an automated hematological analyzer, Sysmex XP-100.

2.4. Histopathological analysis
The chest of the animals was opened and the heart was dissected and fixed in 10% formalin. Afterwards, the tissue was processed to 70%, 80%, 90%, and absolute alcohol, cleared by xylene, and then transferred to molten paraffin wax for block preparation. The sections of 5 micron thickness were prepared using rotary microtome, patched in microscopic slides using egg albumin, cleared by xylene, and stained [15]. Hematoxylin and eosin staining was employed in our study not only to identify collagen but also to differentiate cell nuclei and cytoplasm when conducting myocardial neutrophil count. Hematoxylin stains chromatin a blue color. Eosin colors nuclei red and imparts varying shades of red to pink to the cytoplasm [16].

2.5. Morphometric analysis
Histological slides were observed under a light microscope connected to a camera coupled with a compatible computer. The captured images of interest were processed to obtain morphometric data using image processing software, Digimizer 4. Serial heart sections were analyzed for any diagnosis in myocardial infarction. The area under infarct region was measured based on the microscope specifications. The number of neutrophils infiltrated the myocardium was counted and expressed as tissue neutrophil count per square millimeter of myocardial tissue.

The equivalent thickness of coronary arteriolar wall indicates the collagenous and elastic fiber content in the coronary vessels. Values for individual artery lumen, tunica adventitia (externa), and tunica intima-media were obtained by tracing the perimeter of interest. The area of the lumen was determined as area inside tunica intima-media (Figure 1). The tunica intima and media are measured together as one set in this study, because intima was too thin to be morphometrically analyzed as an isolated tunica [12]. According to the lumen area, coronary arterioles were categorized as small- (S), medium- (M), and large- (L) sized vessels of area <1000, 1000 to 3000, and >3000 μm², respectively. The arterial wall thickness information is useful to diagnose any possible resistance artery narrowing and stiffening that may become prominent factors in the increased vasoconstriction or reduced vasodilatation [11].

Figure 1. Schematic transverse-sectional diagram of coronary arteriole

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\begin{align*}
A_a &= \text{area inside tunica adventitia} \\
P_a &= \text{perimeter of tunica adventitia} \\
A_{im} &= \text{area inside tunica intima-media (lumen area)} \\
P_{im} &= \text{perimeter of tunica intima-media} \\
R_a &= \text{equivalent adventitial radius} = 2A_a / P_a \\
R_{im} &= \text{equivalent lumen radius} = 2A_{im} / P_{im} \\
T_w &= \text{equivalent arteriolar wall thickness} = R_a - R_{im}
\end{align*}
\]

2.6. Statistical analysis
Data were expressed as mean ± SEM. Comparison between groups was done using Student’s t-test. The calculated p values were reported in their real values, unless they follow this following category: \( p > 0.1 \) (insignificant), \( p < 0.1 \) (considerably significant), \( p < 0.05 \) (significant), and \( p < 0.01 \) (highly significant). Linear regression analysis and Pearson’s correlation have been prepared to identify the association between different variables. SPSS version 20 was employed for this statistical analysis.
3. Results and Discussion

3.1. Air pollution and traffic data

The air pollution and vehicular status are significantly different between exposure site and control site as identified in Table 1. We believed that the selected sites are suitable for our comparative experiment in animal exposure to PM air pollution due to these significant differences in air pollution data between both sites. On the other hand, we found no differences in all weather parameters measured at both locations.

Table 1. Actual conditions of the exposure and control site (n = 4, sampled in four seasons)

| Parameters                      | Exposure site          | Control site         | p value |
|---------------------------------|------------------------|----------------------|---------|
| Ambient air quality:            |                        |                      |         |
| SPM, mg/m³                      | 14.05 ± 0.86           | 1.71 ± 0.13          | 0.001   |
| NO₂, ppm                        | 0.11 ± 0.01            | 0.04 ± 0.01          | < 0.001 |
| Traffic volume, vehicles/h      | 3352 ± 98              | 181 ± 12             | < 0.001 |
| Vehicular emission load, kg/h   | 455.35 ± 9.59          | 3.93 ± 0.18          | < 0.001 |
| Weather variables:              |                        |                      |         |
| Outdoor temperature, °C         | 31.64 ± 2.28           | 31.76 ± 1.41         | 0.965   |
| Outdoor humidity, %             | 50.56 ± 7.55           | 47.32 ± 6.45         | 0.756   |
| Atmospheric pressure, kPa       | 101.31 ± 0.08          | 101.16 ± 0.01        | 0.120   |
| Heat index, °C                  | 33.02 ± 3.08           | 32.84 ± 1.47         | 0.960   |
| Wind speed, kph                 | 2.88 ± 0.86            | 3.19 ± 0.28          | 0.741   |

3.2. Changes in hematological parameters

Blood cell counts provide basic evaluation on the health condition of an individual, though the responses may vary depending on age, gender, and genetic background [17]. But in general, any changes in white blood cell count can be a resistance index of an individual to some diseases, whereas red blood cell count, hematocrit and hemoglobin concentrations can provide information in the capacity index of the blood to transfer oxygen [2].

The present study has presented the results in the impact of exposure to PM for 5 days and 3 months on Hb and PCV concentration, RBC, WBC, and PLT counts (Table 2). It showed insignificant differences in Hb concentration, WBC and PLT counts for both 5-day and 3-month PM exposure periods. PCV concentration and RBC count showed insignificant difference for the 5-day group but showed considerably significant lower in the 3-month group compared to their control (p < 0.1). Our result agrees with the earlier findings suggested by numerous researchers [3,18,19,20,21].

Table 2. Changes in hematological and biochemical parameters in mice after PM exposure

| Parameters                      | 5 days (n = 7) | 3 months (n = 6) | p value |
|---------------------------------|---------------|-----------------|---------|
| Blood cell profile:             |               |                 |         |
| Hemoglobin, g/dL                | 5.84±0.34     | 4.60±1.06       | 0.512   |
| Packed cell volume, %           | 18.76±0.90    | 13.60±2.20      | 0.292   |
| RBC count, ×10⁶/mm³             | 4.05±0.18     | 2.92±0.68       | 0.398   |
| WBC count, ×10⁷/mm³             | 3.90±0.37     | 7.40±3.92       | 0.599   |
| Platelet count, ×10⁹/mm³        | 3.84±0.77     | 5.08±2.79       | 0.778   |
| WBC differential count:         |               |                 |         |
| Neutrophils, %                  | 14.20±1.11    | 17.00±3.27      | 0.610   |
| Eosinophils, %                  |               |                 |         |
| Lymphocytes, %                  |               |                 |         |
| Monocytes, %                    |               |                 |         |

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Eosinophils, % 1.80±0.48 1.00±0.00 0.257 2.00±0.00 2.67±0.33 0.184
Monocytes, % 2.80±0.95 3.50±1.22 0.653 3.67±1.20 4.67±0.67 0.507
Lymphocytes, % 81.20±1.89 78.50±2.04 0.376 80.67±0.67 84.33±2.40 0.216

As part of hematological study, attempt was also taken to observe the impact of PM exposure on differential counts of leucocytes including neutrophils, eosinophils, monocytes, and lymphocytes. There was a statistically significant decrease (p < 0.05) in neutrophils after 3 months of PM exposure, but no significant difference after 5 days. This supports the finding of other reports [10,22]. Other parameters in WBC subtypes showed no differences for both 5 days and 3 months of PM exposure when compared to their control. Changes in hematological indices induced by PM exposure may be associated with the pathogenesis of congestive heart failure and myocardial ischemic heart diseases reported by several epidemiological studies [11].

3.3. Myocardial neutrophil infiltration
Animals exposed to PM developed an acute myocardial inflammation, characterized by the recruitment of neutrophils to the cardiac tissue, usually at the border between necrotic myocardium and viable myocardium [23]. A tissue neutrophil count was made on minimum eight myocardium images per group. The myocardial neutrophil count expressed as neutrophils per square millimeter of tissue was significantly higher at both 5-day and 3-month PM-exposed mice compared to those in the control group (p < 0.05, Figure 2a). Our observation is in agreement with previous observation from other investigators [9,10] that in treated animals, the myocardial area at risk due to PM exposure had a higher number of tissue neutrophils as compared with the myocardium of control animals.

Linear regression analysis revealed a considerable positive association between WBC count in blood and leukocyte count in myocardial tissue (p < 0.05, Figure 2b), and between blood neutrophil and tissue neutrophil (p < 0.1). This indicated the high flux of blood neutrophils infiltrated the myocardium of the treated animals. Reports from other studies found a significant positive correlation between tissue neutrophil count and myocardial infarct size of the PM-treated animals which indicated a high flux of neutrophil migration from the peripheral blood into the cardiac tissue [9,10].

![Figure 2](image-url)

Figure 2. (a) Animals exposed to PM showed a significantly higher neutrophil infiltration in their cardiac tissues compared to the control. (b) Blood leukocyte count had significant correlation with tissue leukocyte count (r = 0.606, p = 0.028; control n = 8, treatment n = 5).

3.4. Structural alteration in coronary arteries
The result found that arteriolar vessel expressed a relatively higher thickness in the tunica adventitia and tunica intima-media layers of 3-month PM-exposed group when compared to the unexposed ones (p = 0.005), but no significant difference (p = 0.121) was observed in the coronary arterioles of 5-day
PM-exposed group and its control (Figure 3a). Higher thickness in arteriolar wall vessel indicated higher collagen and elastic fiber content in the coronary arteries.

According to the classification of vascular vessel size, all size of the arterioles of the 5-day and 3-month PM-exposed group expressed the increment of arteriolar wall thickness significantly ($p < 0.001$) compared to the control group (Figure 3b). Similar experimental study conducted earlier also came out with almost similar findings in the increment of arterial wall thickness among different size of coronary arteries [12]. They found that elastic fibers augmented in both tunica adventitia and intima-media layers of almost all sized arteries of ventricles, while collagen increased predominantly in medium- and large-sized arteries.

![Graph](image)

Figure 3. (a) Coronary arteriolar wall thickness showed higher in animals exposed to PM air pollution for 3 months when compared to the unexposed ones, but no difference of such was found in animals exposed to PM for 5 days. (b) This significant difference in the thickness of coronary arteriolar wall was observed in all sized vessels of the PM-exposed group.

4. Conclusion
The present study highlighted the important evidences in the chronic effects of PM toxicity on the cardiovascular system using small mammals as objects of study. The experiment was carried out by placing the animals in a dusty urban roadway environment to allow direct exposure to vehicular particulate pollution. The airborne PM was able to induce few changes in blood parameters, including packed cell volume, RBC, and neutrophil counts. Cardiac alterations become strongly evident for myocardial infarction event in mice models expressed by high number of neutrophils infiltrated the cardiac tissue. Moreover, structural alterations in coronary arteries, particularly the significant artery stiffening and coronary vasoconstriction, provide additional evidence for early cardiac ischemic injury which may promote further acute cardiovascular morbidity. The observed arterial wall changes are significantly evident in animals exposed to PM in medium-term duration (in our case is three months) rather than in short-term duration. Overall, the observed hematological and histopathological changes in cardiovascular system indicated the toxic effects of ambient particles that have been chronically inhaled by the study animals.

References
[1] Peakall D 1992 Animal biomarkers as pollution indicators (London: Chapman and Hall)
[2] Tête N, Afonso E, Bouguerra G and Scheifler R 2015 Blood parameters as biomarkers of cadmium and lead exposure and effects in wild wood mice (Apodemus sylvaticus) living along a pollution gradient Chemosphere 138 940–6
[3] Bersenyi A, Fekete S G, Szocs Z and Berta E 2003 Effect of ingested heavy metals (Cd, Pb, and Hg) on hematology and serum biochemistry in rabbits Acta Vet. Hung. 51(3) 297–304
Poursafa P, Kelishadi R, Amin Amini, Abasgholi Amini, Amin M M, Lahijanzadeh M and Moadaresi M 2011 Association of air pollution and hematologic parameters in children and adolescents J. Pediatr. (Rio J.) 87(4) 350–6

WHO (World Health Organization) 2006 Air quality guidelines Global update 2005 (Copenhagen: WHO Regional Office for Europe)

Emmerechts J, Jacobs L and Hoylaerts M F 2011 Air pollution and cardiovascular disease The impact of air pollution on health, economy, environment and agricultural sources ed M Khalaf (Rijeka: InTech Europe) chapter 4 pp 69–92

Chang C C, Tsai S S, Ho S C and Yang C Y 2005 Air pollution and hospital admissions for cardiovascular disease in Taipei, Taiwan Environ. Res. 98 114–9

Rodrigues N R D, Veras M M, Negri E M, Zanchi A C T, Rhoden C R, Saldiva P H N, Dolhnikoff M and Caldini E G 2009 Effect of pre- and postnatal exposure to urban air pollution on myocardial lipid peroxidation levels in adult mice Inhal. Toxicol. 21(13) 1129–37

Hazarika S, van Scott M R and Lust R M 2004 Myocardial ischemia-reperfusion injury is enhanced in a model of systemic allergy and asthma Am. J. Physiol. Heart Circ. Physiol. 286 H1720–5

Cozzi E, Hazarika S, Stallings H W III, Cascio W E, Devlin R B, Lust R M, Wingard C J and van Scott M R 2006 Ultrafine particulate matter exposure augments ischemia-reperfusion injury in mice Am. J. Physiol. Heart Circ. Physiol. 291 H894–903

Rivero D H R F, Soares S R C, Filho G L, Saiki M, Godleski J J, Antonangelo L, Dolhnikoff M and Saldiva P H N 2005 Acute cardiopulmonary alterations induced by fine particulate matter of São Paulo, Brazil Toxicol. Sci. 85 898–905

Akinaga L M Y, Lichtenfels A J, Oliveira R C, Caldini E G, Dolhnikoff M, Silva L F F, Bueno H M D S, Pereira L A A, Saldiva P H R N and Garcia M L B 2009 Effects of chronic exposure to air pollution from Sao Paulo city on coronary of Swiss mice, from birth to adulthood Toxicol. Pathol. 37 306–14

Venkataramana G V, Fauzie A K and Naveen S 2018 The status of air pollution attributable to automobile emissions in Mysuru: Implications for urban transport planning Clim. Chang. 4(16) 715–22

NAS (National Academy of Sciences) 2011 Guide for the care and use of laboratory animals 8th ed (Washington DC: The National Academies Press)

Kiernan J A 2001 Histological and histochemical methods 3rd ed (Oxford: Oxford University Press)

Gill G W 2010 H&E Staining: Oversight and Insights Special Stains and H&E ed G L Kumar and J A Kiernan (California: Dako North America) pp 119-130

Biser J, Vogel L, Berger J, Hjelle B and Loew S S 2004 Effects of heavy metals on immunocompetence of white-footed mice (Peromyscus leucopus) J. Wildl. Dis. 40(2) 173–84

Seaton A, Soutar A, Crawford V, Elton R, McNerlan S, Cherrie J, Watt M, Agius R and Stout R 1999 Particulate air pollution and the blood Thorax 54 1027–32

Olajire A A and Azeez L 2012 Effects of Solanum macrocarpon (African eggplant) on haematological parameters of wistar rats exposed to urban air pollution Adv. Environ. Res. 1(2) 109–23

Cheraghi J, Hosseini E, Hoshmandfar R and Sahraei R 2013 Hematologic parameters study of male and female rats administrated with different concentrations of silver nanoparticles Int. J. Agri. Crop Sci. 5(7) 789–96

Das P and Chatterjee P 2015 Aerobic capacity and haematological response to exercise: A study on school-going regularly exercising boys in two different air pollution zones J. Exerc. Sci. Fit. 13 99–103

Kooter I M, Boere A J F, Fokkens P H B, Leseman D L A C, Dormans J A M A and Cassee F R
2006 Response of spontaneously hypertensive rats to inhalation of fine and ultrafine particles from traffic: experimental controlled study *Part. Fibre Toxicol.*, 3 7

[23] Burke A P and Butany J 2015 *Pathology of acute myocardial infarction* (Medscape)