Effects of Dust Storm Fine Particle-Inhalation on the Respiratory, Cardiovascular, Endocrine, Hematological, and Digestive Systems of Rats

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To the Editor: In China and neighboring countries, there is increasing health concern over the dust storm fine particulate matter (PM) originating in the Gansu Corridor and Mongolian regions of China.[1] PM is a mixture of various particles including crustal material, metals, and bioaerosols. Elevated levels of ambient PM were also reported to be associated with increased cardiopulmonary morbidity and mortality.[2] A recent World Health Organization report indicated that exposure to fine PM air pollution and other pollutants caused approximately seven million deaths in 2012 worldwide, while the Global Burden of Disease Study showed that PM exposure was responsible for 2.9 million deaths and 69.7 million disability-adjusted life-years in 2013.[2] Finally, studies showed that reduced PM concentrations were associated with reduced mortality risk.

Despite these studies, the health effects of dust PM remain largely unknown. Furthermore, the majority of studies have examined the effects of short-term exposure and particle-induced damage to the respiratory and cardiovascular systems.[3] Thus, the long-term effects of PM exposure remain unclear on respiratory and cardiovascular systems and on other organ systems. There are few studies examining the adverse effects of particulate air pollutants on other organs and systems except respiratory and cardiovascular systems.[3] Nevertheless, most studies examining the detrimental effects of PM were performed in the absence of simulation of a real dust storm environment. We previously reported that PM at 9000 μg/m^3 in rats can cause PM deposition in the lung, with the development of lung inflammation and fibrosis.[4] Thus, using this model, this study aimed to examine the effects of dust storm PM, involving simulation of a real dust storm environment using a wind tunnel system, on the respiratory, cardiovascular, endocrine, and digestive systems in rat.

Dust particles were collected from the surface soil in the Alxa Plateau of Inner Mongolia, the source region of dust storms in Northern China. From March to May in 2015 and 2016, 50 sampling points were set in a 100 m^2 area, with no large obstacles or pollution sources near the sites. After collection, the samples were sieved with a 250-mesh screen. Experimental dust samples were prepared in 5 L glass bottles and heated at 360°C for 30 min in an electric heater to remove toxic materials (e.g., microbiological materials, sulfate, and nitrate). The particles in the samples were then measured by a field emission scanning electron microscope equipped with an energy dispersive spectrometer detector. This procedure was repeated three times.

The methodology for exposing rats to concentrated PM was based on our previous study.[4] In brief, ambient particles from the Alxa Plateau of Inner Mongolia were concentrated using a modified wind tunnel and an ultrafine particle concentrator, as reported by Sioutas *et al*. [Supplementary Figure 1].[5] The wind tunnel used virtual impactor technology, and an aerosol containing concentrated particles was directed into the exposure chamber through the bottom of the particle concentrator. The average ground air dust concentration was measured at 9000 μg/m^3 when stable wind-dust clouds were formed in the wind tunnel; this was considered to simulate the average dust concentration observed in strong dust storms.[6]

Six-week-old male Wistar rats (180 ± 20 g) were purchased from the Experimental Animal Center of the Gansu University of Chinese Medicine (Lanzhou, China). Animals were housed in metallic cages with *ad libitum* access to laboratory chow and tap water. Animals were maintained with a 12-h light/dark cycle at 22°C ± 2°C and 55% ± 10% relative humidity. Forty-eight rats were acclimatized for 1 week before the experiment. All animal procedures were approved by the Ethics Committee of the Gansu Provincial Hospital and were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

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Rats were randomly and evenly assigned to the PM exposure group ($n = 24$) and the control group ($n = 24$). The exposure group was exposed to a simulated dust storm environment in a wind tunnel for 5 h each day. The control group was fed outside of the wind tunnel with inhalation of normal air containing. The concentration and time of treatment were based on our earlier studies on the toxic effect of PM exposure on the rat lung, with some modifications. On the 45th, 90th, 135th, and 180th day of exposure, six rats per group were deprived of food for 24 h, and then prepared for following the experimental procedure. The rats were examined for red blood cell and white blood cell counts, increased expression of cytokines, chemokines, hormones, and enzymes capacity in peripheral blood and in serum. Moreover, pathological changes in hearts, livers, spleens, lungs, kidneys, stomach, and thymus were examined on the 180th day.

Blood samples were obtained just after the end of the PM exposure period. Several peripheral blood parameters, including red blood cell and white blood cell counts, and hemoglobin (Hb), were assessed (Sysmex KX-21 autoanalyzer; TOA Medical Electronics Co., Japan).

Serum was separated from whole blood samples (coagulation at room temperature for 10–20 min, centrifugation, and supernatant removal), stored at −80°C, and then recentrifugated before the assay. Serum cytokine levels and enzymes, including interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-6, transforming growth factor (TGF)-β1, superoxide dismutase (SOD), glutathione (GSH), inducible nitric oxide synthase (iNOS), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), triglyceride (TG), thyroid-stimulating hormone (TSH), adrenocorticotropic hormone (ACTH), B-type natriuretic peptide (BNP), renin, angiotensin II (Ang-II), Hb, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), and insulin, were measured using commercial available enzyme-linked immunosorbent assay. Each sample (50 μl) was added to a well and gently mixed, and the samples then incubated for 30 min at 37°C in a water bath. The plate was washed five times with washing buffer, HRP-conjugate reagent (50 μl) added to each well, and the plate then incubated and washed as previously. After chromogen staining, the fluorescence of each well was measured in a spectrofluorometer (Thermo Scientific Lumina, USA) at 450 nm.

Rats were sacrificed on 180th day, and the tissues dissected. The tissues were then fixed overnight, embedded in paraffin, and sectioned for staining with hematoxylin and eosin staining (H and E) or Masson's Trichrome staining to quantify the degree of tissue fibrosis. In brief, slides stained with H and E were examined under high power and scored for a total of 10 random fields per specimen. Digitized images were analyzed by Image Pro-Plus 6.0 software (MEDIA CYBERNETICS, Inc. Maryland, USA). The overall and fibrotic areas of the tissues were outlined, the pixels of total versus fibrotic tissue then summed over each tissue, and a percentage obtained.

All values were expressed as mean ± standard deviation (SD). The student's *t*-test was used to compare quantitative data. All statistical analyses were performed with statistical software (PRISM version 6.0; Graphpad Software, Inc. USA). A value of $P < 0.05$ was accepted as statistically significant.

After PM exposure, there was a significant increase in the number of white blood cells in the peripheral blood compared with the control group. There was also a significant increase in the number of red blood cells at 45 and 180 days of PM exposure. By contrast, there were no significant differences in Hb levels between the exposure and control groups.

Dust storm PM exposure caused a significant increase in serum levels of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6) and profibrotic factors (TGF-β1) at the various exposure durations (45th, 90th, 135th, and 180th day) compared with controls ($P < 0.05$). Serum SOD and GSH levels were significantly decreased in the PM exposure group ($P < 0.05$), while there was a trend towards an increase in iNOS levels ($P < 0.05$) [Supplementary Figure 2].

The serum levels of various hormones and enzymes are shown in Supplementary Figures 3 and 4. Long-term PM exposure caused a significant increase of LDL-C ($P < 0.05$), TC, TG, TSH, ACTH, BNP, renin, Ang-II, ALT, AST, and TB levels, while there were no changes in insulin levels. PM exposure was also associated with a significant decrease in HDL-C ($P < 0.05$) levels compared with controls.

Sandstorm PM, in particular, PM2.5 and PM10, was respirable PMs that could deposit, penetrate, and accumulate in lung tissues. We observed the presence of PM in alveolar macrophages in the PM group, with evidence of a mild inflammatory response and lung fibrosis [Figure 1]. Compared with controls, there was also evidence of renal parenchyma atrophy in the PM-exposed group [Supplementary Figure 5a and 5b]. The spleen was disorganized, with the destruction of the white and red pulp [Supplementary Figure 5d]. However, there were no obvious inflammatory changes or fibrosis in other organs in the PM-exposed group or controls.

The major finding of the present study was that repeated exposure to dust storm fine PM air pollution was associated with an increase in circulating inflammatory cytokines and enzymes, a decrease in antioxidative stress blood profile, and pathological changes in the heart, liver, spleen, stomach, lung, kidney, and thymus. These findings provide a characteristic signature of systemic injury induced by PM exposure and provide new insights into the potential mechanisms by which inhalation of PM increases the

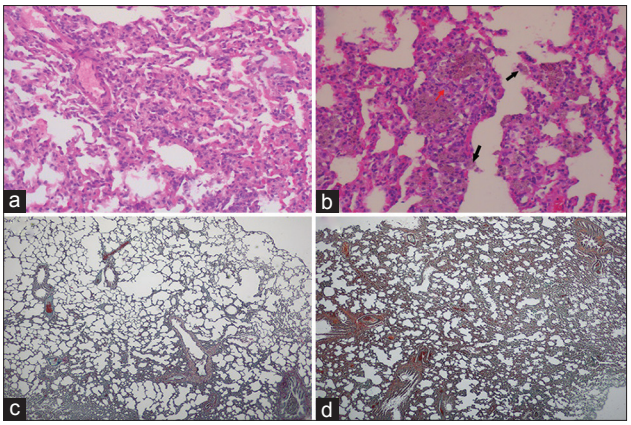

Figure 1: Sand-dust PM inhalation-induced pulmonary lesions in rats stained by H and E and Masson in control ($n = 12$) and exposure groups ($n = 12$). (a) Control group (H and E, ×200). (b) The severity of alveolar thickening appeared greater in PM-exposed rats, and PM-induced chronic phase responses are characterized by interstitial fibrosis and formation of epithelioid granulomas (H and E, ×200). (c) Control group (Masson staining, ×40). (d) Although lung lesions caused by PM (Masson staining, ×40) were widely distributed throughout the lung section on postexposure day 180, the exacerbation response in PM-exposed rats appeared patchy compared to the control group. Red arrow indicates an area where exacerbated fibrosis was apparent; black arrow indicates alveolar macrophage with a few PM particles in the cytoplasm. PM: Particulate matter; H and E: Hematoxylin and eosin.

risk of cardiorespiratory and other multisystem injury, leading to premature mortality.

We observed definitive pulmonary injury, accompanied by increased white blood cell counts in peripheral blood, in animals exposed to long-term sand-dust PM in our dust storm simulation [Figure 1]. Particulates suspended during sand-dust storms can be inhaled directly into the airways of humans and animals, potentially disrupting immune system homeostasis, leading to lung disease. Previous studies have reported an increased percentage of neutrophils and total cells in bronchoalveolar lavage specimens in animals exposed to fine particles. The increase in white blood cell counts in PM-exposed rats in the present study suggest that the recruitment of leukocytes into the blood forms a key component of the initial host systemic response following exposure to PM air pollution. Furthermore, single instillation of small inert particles directly into the lungs was reported to stimulate the bone marrow and reduce the transit time of polymorphonuclear leukocytes through bone marrow in rabbits. An earlier study showed leukocytosis and increased circulating band cells in young military recruits exposed to an acute episode of PM air pollution during an accidental forest fire of Southeast Asia in the summer of 1997, suggesting that acute PM exposure can cause bone marrow stimulation in humans.

However, the pattern of bone marrow stimulation with repeated dust storm PM exposure is distinctly different from that following acute particle exposure. Acute exposure was reported to cause acute leukocytosis from the marrow and an accelerated transit time through all bone marrow pools. By contrast, in the present study, repeated PM exposure was not associated with a persistent increase in white blood cell counts in peripheral blood and had a smaller effect on some other blood parameters Furthermore, elevated red blood cell counts were only elevated on the 90th and 180th days of PM exposure, and there were no changes in platelets or Hb at any time. This distinct bone marrow response following repeated PM exposure may relate, at least in part, to differences in levels of inflammatory mediators [Supplementary Figure 2]. In some findings, an increase in peripheral blood leukocyte count was a predictor of total mortality, independent of cigarette smoking. However, further studies are needed to elucidate the exact mechanisms underlying PM-mediated hematological changes and to confirm the causative relationship of elevated peripheral blood leukocytes with increased mortality.

We also found increased pro-inflammatory cytokines (IL-1β, TNF-α, and IL-6) and the profibrotic factor TGF-β1 in the serum of rats following exposure to PM during a dust storm stimulation. In support, PM exposure was reported to cause a marked increase in IL-6 in rabbits. IL-6 is an important mediator of the acute-phase response and is a potent stimulus for the leukocyte release from the bone marrow. IL-6 can also have profound effects on innate and acquired immune responses. TGF-β1 was suggested as a biomarker for progression of coal workers' pneumoconiosis.[7] Some reports also showed patients with high plasma TNF-α levels have increased the risk of cerebrovascular events at follow-up. Furthermore, exposure to fine PMs was associated with acute coronary events, particularly ST-segment elevation myocardial infarction, in patients with preexisting seriously diseased coronary arteries, but not in those with non-diseased coronary arteries. Thus, the increase in IL-1, IL-6, and TNF-α following PM exposure in the present study may trigger the acute-phase response, which is characterized by an early release of 'alarm' cytokines IL-1 and TNF-α, followed by a second wave of cytokines such as IL-8 and IL-6. Several of these cytokines can stimulate the liver to produce acute phase proteins that increase blood coagulability, which is a major risk factor for acute cardiovascular events in susceptible individuals. Finally, TGF-β1 is the most potent and efficacious pleiotropic cytokine for promoting fibrosis. Overall, these data suggest that mediators produced in the lung enter the circulation and contribute to the systemic response associated with exposure to sandstorm PM. In turn, these circulating cytokines may activate the endothelium, and thus contribute to coronary and carotid artery disease.

Another key finding of the present study was that dust storm PM caused a decrease in antioxidant molecules, including SOD and GSH, and an increase in iNOS levels, suggesting that dust storm fine PM, is a systemic toxic agent that can damage multiple systems through oxidative stress. It is possible that after inhalation of atmospheric dust storm PM pollutants, the lung becomes a primary target for inhaled oxidants generated naturally, thus triggering a multisystem oxidative response. Alternatively, dust storm PM may travel directly from the lung into the circulation. Indeed, traces of ultrafine elemental silver were previously reported in the heart, liver, kidney, spleen, and brain of rats.[8] Interestingly, inhalation of carbon nanoparticles was recently shown to induce severe pulmonary inflammation, with profound systemic effects on extrapulmonary tissues, but which were not observed following intra-arterial carbon nanoparticles infusion (i.e. bypassing of the lungs).[9] Studies also showed that exposure to diesel exhaust environment pollution increases iNOS expression and activity in response to inflammatory cytokines as part of host defense responses and may contribute to vascular dysfunction, atherogenesis, and potential urban air pollution-associated cardiovascular morbidity and mortality.

We also found a significant increase in serum LDL-C, TC, TG, TSH, ACTH, BNP, renin, Ang-II, ALT, AST, TB, and HDL-C levels following dust storm PM exposure, but no differences in insulin content. Serum TG has a prognostic value for predicting the effect of treatment and for assessing coronary heart disease risk, especially when used in combination with HDL-C and LDL-C. Interestingly, HDL-C is associated with concurrent insulin resistance, while lower HDL-C and HDL-C predict earlier and larger initiation of pharmacologic glucose control.[10] Although we found no changes in insulin levels with PM exposure, it mains possible that an interaction between PM and insulin may contribute to susceptibility to type 2 diabetes or other endocrine diseases system in conditions of dust storm fine PM exposure. BNP is an independent prognostic predictor for patients with heart failure, and a strong indicator of mortality in patients with heart failure. In the present study, exposure to sandstorm PM increased BNP expression. As elevated BNP predicts an increased risk for all outcomes in heart failure patients, while decreased BNP predicts decreased risk, then PM exposure may increase the risk of heart disease and (or) heart failure and aggravate heart failure by disturbing the neuroendocrine system and interfering with lipid metabolism.

The main roles of ACTH are to promote glucocorticoid secretion and to maintain the normal shape and function of the adrenal gland. Under physiological conditions, the hypothalamus, pituitary, and adrenal gland are in a relatively dynamic balance, while a disorder in ACTH can cause adrenal atrophy, hyperplasia, or secretion dysfunction. TSH is secreted by the pituitary gland to promote the growth and function of the thyroid gland. Felske *et al*.[11] demonstrated that TSH and insulin can act inversely on differentiated human adipocytes through the modulation of intracellular signaling pathways. Renin production, a critical step in the renin-angiotensin system, induces aldosterone production and determines plasma angiotensin concentration. Subsequently,

overactivation of the renin-angiotensin system may contribute to the development of hypertension. Although Shao *et al*.[12] demonstrated that >50% of intrarenal Ang II levels are derived from *de novo* local formation, part of the Ang II content in the kidney is likely related to Ang II uptake from the circulation. High renin levels can cause high blood pressure and release catecholamine. Furthermore, there is increasing evidence that changes in catecholamine levels are associated with sudden cardiac death, coronary heart disease, and congestive heart failure. Although there is limited evidence that PM exposure can damage the nervous and endocrine systems, an association of ambient air PM concentrations and increased the risk of mortality attributable to diabetes was previously reported.[13] Finally, levels of serum ALT, AST, and bilirubin are widely used to assess liver function, and many studies have reported that elevated ALT is associated with increased liver-related morbidity and mortality. We suggest that sandstorm PM can affect the endocrine system, as well as multiple other systems including the circulation and the digestive system. However, the exact mechanisms underlying this wide-spread systemic injury remain to be determined.

Finally, we found that PM exposure was associated with interstitial fibrosis and formation of epithelioid granulomas in the lungs (Masson's Trichrome staining for collagen fibers; H and E for fibrosis severity) at 180 d of exposure [Figure 1b and 1d]. By contrast, fibrosis was not detected in other organs such as the heart, liver, spleen, stomach, kidneys, and thymus [Supplementary Figure 5]. It is possible that a longer time of exposure to dust PM is required to induce fibrosis in these organs.

In conclusion, we found that dust storm PM is composed of a large number of fine or ultrafine particles, which can cause systemic inflammation and systemic injury to multiple organ systems, including the respiratory, cardiovascular, endocrine, hematological, and digestive systems. Although the exact mechanisms by which dust storm fine PM causes lung inflammation and systemic injury remain unclear, our data suggest a potential role of dust-mediated oxidative stress injury and systemic inflammation. Further awareness of the potential health implications of dust storms is fundamental for its prevention and treatment.

Supplementary information is linked to the online version of the paper on the Chinese Medical Journal website.

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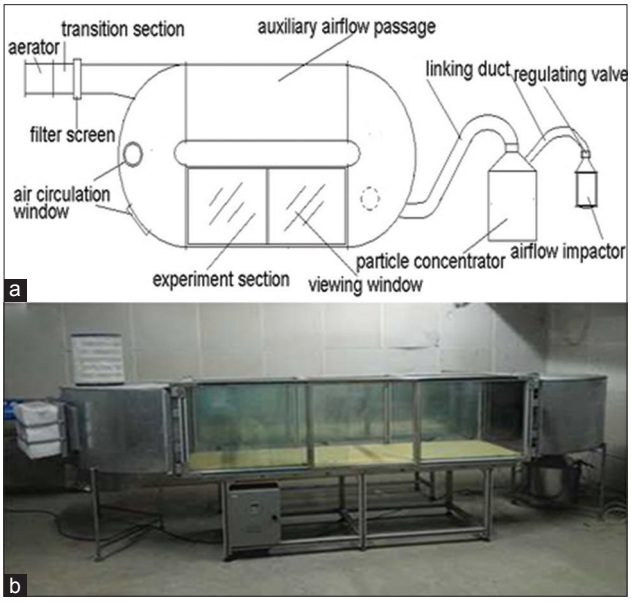
Conflicts of interest

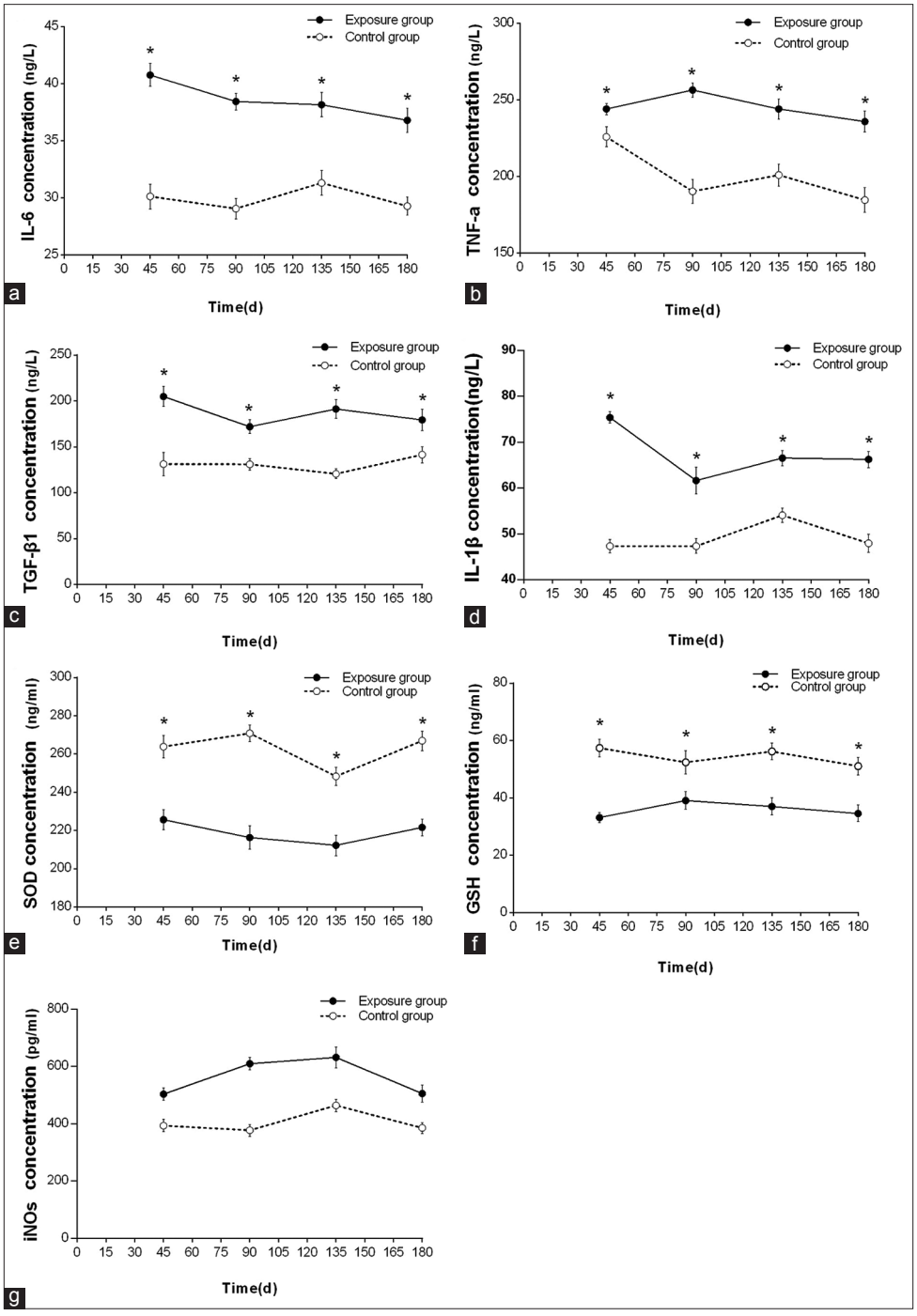
There are no conflicts of interest.

References

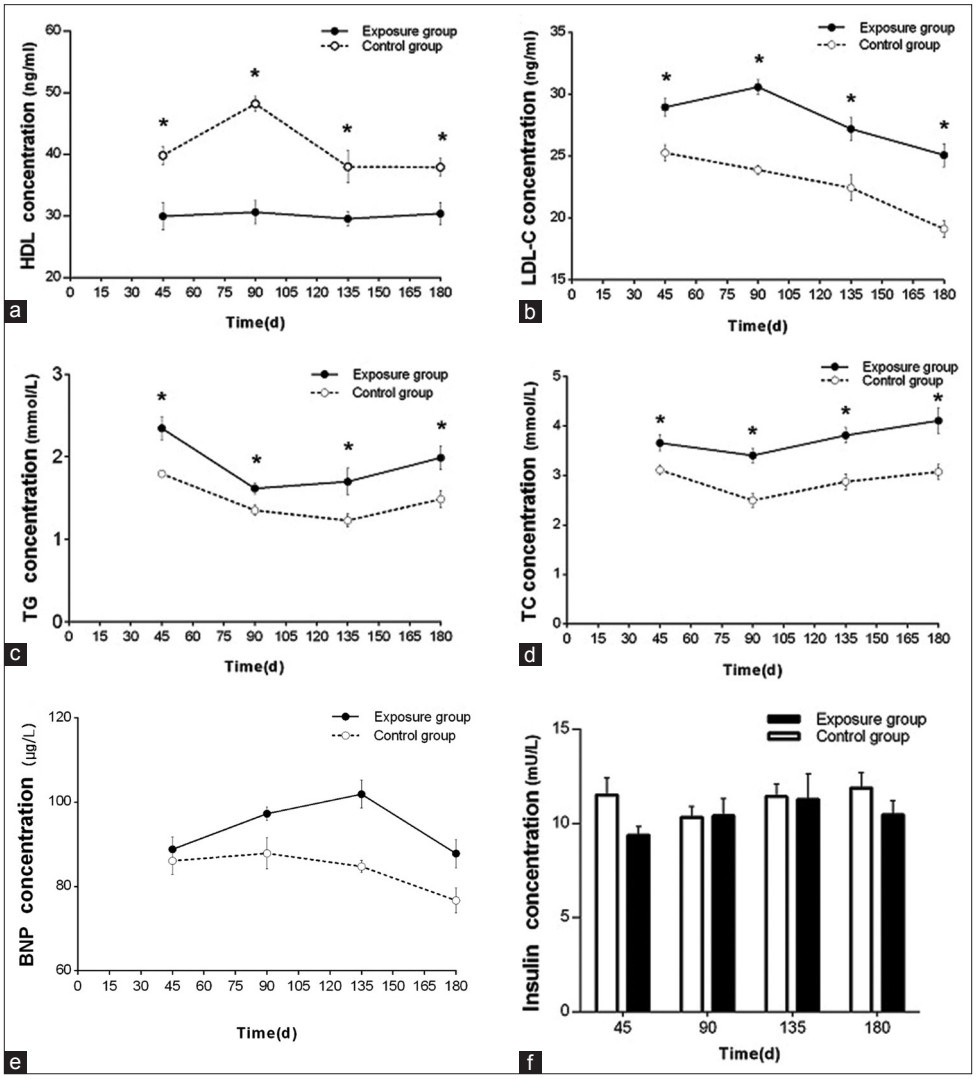
1. Bishop JK, Davis RE, Sherman JT. Robotic observations of dust storm enhancement of carbon biomass in the North Pacific. Science 2002;298:817-21. doi: 10.1126/science.1074961.
2. GBD 2013 Risk Factors Collaborators, Forouzanfar MH, Alexander L, Anderson HR, Bachman VF, Biryukov S, *et al.* Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks in 188 countries, 1990-2013: A systematic analysis for the Global Burden of Disease Study 2013. Lancet 2015;386:2287-323. doi: 10.1016/S0140-6736(15)00128-2.
3. Shah AS, Lee KK, McAllister DA, Hunter A, Nair H, Whiteley W, *et al*. Short term exposure to air pollution and stroke: Systematic review and meta-analysis. BMJ 2015;350:1-10. doi: 10.1136/bmj.h1295.
4. Lei FF, Luo WY, Dong ZB, Sang YZ, Luo LZ, Huang G, *et al*. An investigation of the effects of dust storms on rat lung using HRCT and blood gas analysis. Sci Cold Arid Reg 2016;8:319-24. doi: 10.3724/sp.j.1226.2016.00319.
5. Sioutas C, Kim S, Chang M. Development and evaluation of a prototype ultra-fine particle concentrator. J Aerosol Sci 1999;30:1001-17. doi: 10.1016/S0021-8502(98)00769-1.
6. Lei YC, Chan CC, Wang PY, Lee CT, Cheng TJ. Effects of asian dust event particles on inflammation markers in peripheral blood and bronchoalveolar lavage in pulmonary hypertensive rats. Environ Res 2004;95:71-6. doi: 10.1016/s0013-9351(03)00136-1.
7. Lee JS, Shin JH, Lee KM, Hwang JH, Baek JE, Kim JH, *et al.* Serum levels of TGF-β1 and MCP-1 as biomarkers for progressive coal workers' pneumoconiosis in retired coal workers: A three-year follow-up study. Ind Health 2014;52:129-36. doi: 10.2486/indhealth.2013-0112.
8. Takenaka S, Karg E, Roth C, Schulz H, Ziesenis A, Heinzmann U, *et al.* Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. Environ Health Perspect 2001;109 Suppl 4:547-51. doi: 10.1289/ehp.01109s4547.
9. Ganguly K, Ettehadieh D, Upadhyay S, Takenaka S, Adler T, Karg E, *et al.* Early pulmonary response is critical for extra-pulmonary carbon nanoparticle mediated effects: Comparison of inhalation versus intra-arterial infusion exposures in mice. Part Fibre Toxicol 2017;14:19. doi: 10.1186/s12989-017-0200-x.
10. Waldman B, Jenkins AJ, Davis TM, Taskinen MR, Scott R, O'Connell RL, *et al.* HDL-C and HDL-C/ApoA-I predict long-term progression of glycemia in established Type 2 diabetes. Diabetes Care 2014;37:2351-8. doi: 10.2337/dc13-2738.
11. Felske D, Gagnon A, Sorisky A. Interacting effects of TSH and insulin on human differentiated adipocytes. Horm Metab Res 2015;47:681-5. doi: 10.1055/s-0034-1395673.
12. Shao W, Seth DM, Navar LG. Augmentation of endogenous intrarenal angiotensin II levels in val5-ANG II-infused rats. Am J Physiol Renal Physiol 2009;296:F1067-71. doi: 10.1152/ajprenal.90596.2008
13. Yitshak Sade M, Kloog I, Liberty IF, Schwartz J, Novack V. The association between air pollution exposure and glucose and lipids levels. J Clin Endocrinol Metab 2016;101:2460-7. doi: 10.1210/jc.2016–1378.



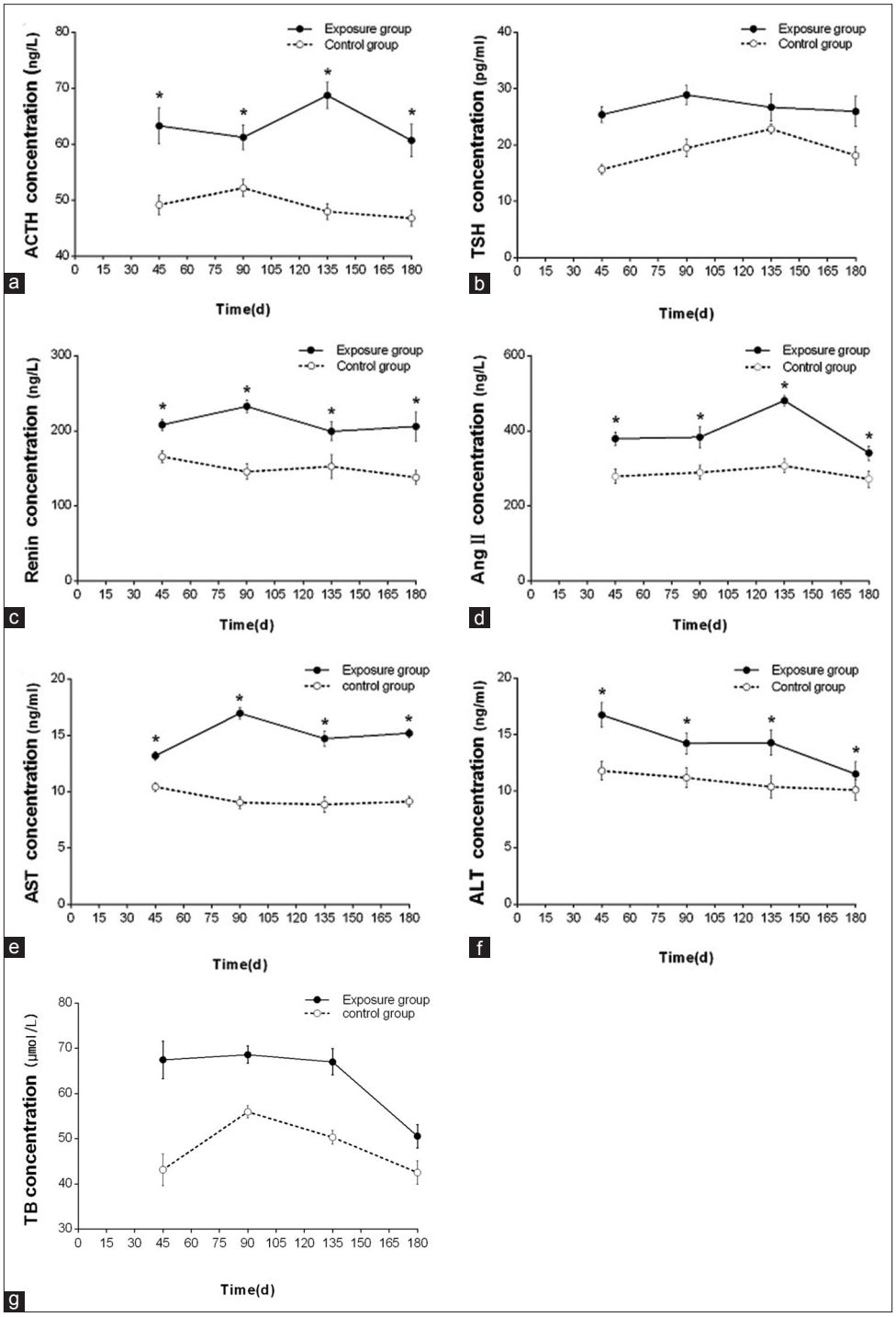
Supplementary Figure 1: Schematic of the prototype of wind tunnel for its characterization (a); Photograph of the experimental set-up (b).



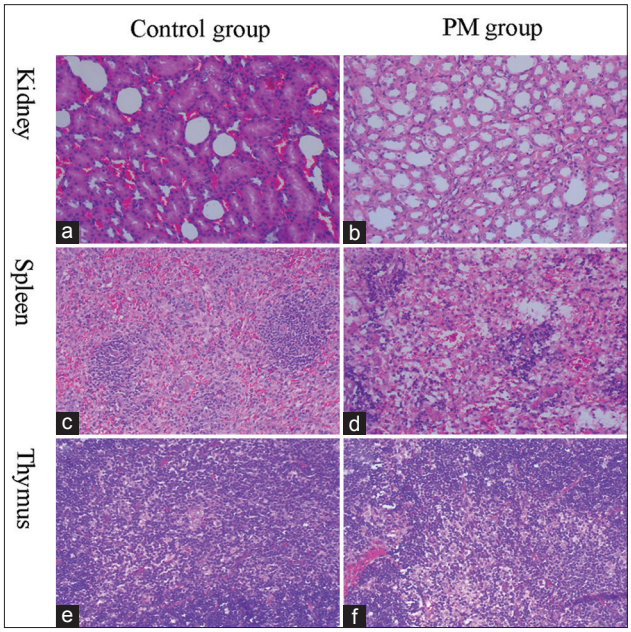
Supplementary Figure 2: Dust storm PM-induced effects of on the contents of IL-6 (a), TNF-α (b), TGF-β1 (c), IL-1β (d), SOD (e), GSH (f), and iNOs (g). *$P < 0.05$, compared to control ($n = 24$). PM: Particulate matter; IL: Interleukin; TNF-α: Tumor necrosis factor-a; TGF-β1: Transforming growth factor; SOD: Superoxide dismutase; GSH: Glutathione; iNOS: Inducible nitric oxide synthase.



Supplementary Figure 3: Dust storm PM-induced effects of on the levels of HDL (a), LDL-C (b), TG (c), TC (d), BNP (e), and insulin (f). *$P < 0.05$, compared to control ($n = 24$). PM: Particulate matter; HDL: High-density lipoprotein; LDL-C: Low-density lipoprotein cholesterol; TC: Total cholesterol; TG: Triglyceride, TSH: Thyroid-stimulating hormone; ACTH: Adrenocorticotropic hormone; BNP: B-type natriuretic peptide.



Supplementary Figure 4: PM exposure-induced effects of on the levels of ACTH (a), TSH (b), renin (c), Ang-II (d), AST (e), ALT (f), and TB (g) in rats. *$P < 0.05$, compared to control ($n = 24$). PM: Particulate matter; TSH: Thyroid stimulating hormone; ACTH: Adrenocorticotropic hormone; Ang-II: Angiotensin II; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; TB: Total bilirubin.



Supplementary Figure 5: Pathology change in kidney, spleen, and thymus in rats on the 180th day (H and E, ×200). The certain atrophy of renal parenchyma was found in the PM-exposed group (b) compared with control group (a). The spleen was disorganized in the exposure group (d) compared with the control group (c). There were no more obvious inflammatory changes and fibrosis between PM-exposed group (f) and the control group (e) in the thymus. PM: Particulate matter; H and E: Hematoxylin and eosin.