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Research Article
Effects of Lunar Soil Simulant on Systemic Oxidative Stress and Immune Response in Acute Rat Lung Injury

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Abstract

Background and Objective: Lunar dust is a kind of fine particles exists on the surface of the moon and it has become one of the most important challenges in future space missions. Lunar dust is toxic but the understanding of lunar dust toxicity is very poor. The aim of this study was to investigate the acute lung injury induced by lunar soil simulant and the mechanism of toxicity. **Materials and Methods:** Wistar rats were randomly divided into four groups including saline control group (tracheal perfusion in a 1 mL volume), two Chinese Academy of Sciences-1(CAS-1) lunar soil simulant groups (tracheal perfusion with 7 and 0.7 mg, respectively in a 1 mL volume) and $PM_{2.5}$ group (tracheal perfusion with 0.7 mg in a 1 mL volume). The levels of tumor necrosis factor alpha, interleukin 6, immunoglobulin A and immunoglobulin G in the bronchoalveolar lavage fluid were examined and the changes of malondialdehyde, glutathione peroxidase and superoxide dismutase in the lung tissue were later measured after 4 and 24 h. **Results:** The determination of the cytokines, immunoglobulin and the oxidative stress index in lung tissue showed that the significant immune and oxidative stress response in the respiratory system were caused by lunar soil simulant and the immune and oxidative stress response promoted each other, resulting in toxic effect on pulmonary parenchymal cells and membrane of rats. **Conclusion:** The overall results showed that the immune response and oxidative stress reaction induced by lunar soil simulant were significant in the respiratory system. They promote each other, so as to cause the toxic effects on the lung parenchyma cells and membrane tissues of rats.

Key words: Lunar soil simulant, acute injury, inflammation, immune response, oxidative stress reaction

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In recent years, with the view of "return to the moon", how to ensure the health and safety of astronauts is an important problem plaguing us, in the lunar missions. Lunar dust is one of the main challenges facing various problems in future space missions. Due to the lunar gravity environment and the near vacuum conditions, it is easily disturbed by human activity and asunder[1].

So far, the main risk of lunar dust comes from inhalable particles. Lunar dust contains a large number of respirable particles (<10 μm) and about 10% of the dust particles are inhalable particles. Lunar dust contains soluble and insoluble particles. When the dust enters the body, the insoluble particles would be removed by macrophages, mucociliary or lymphatic system, while soluble particles still remained in the pulmonary interstitial cells[2]. Even if the astronauts wear protective clothing, attached dust can easily be brought back to their living and working places. The super fine dust with sharp edges will lead to a large number of health problems when they are inhaled into the lungs. They affect respiratory and cardiovascular systems and there is elevated risk of airway inflammation transforming into cancer. NASA and Russia had carried on some preliminary research on the biological toxicity of the dust especially pulmonary toxicity. Researches showed that lunar dust can cause animal lung fibrosis and other symptoms of pneumonia. With the extension of exposure time in the lunar dust, acute inflammation of the mice lung may turn into chronic inflammation[3-8]. *In vitro* A549, alveolar epithelial cells were tested for cytokine production after exposure to culture medium containing ground JSC-1A-vf. The results showed that ground lunar simulant has been shown to promote the production of IL-6 and IL-8, pro-inflammatory cytokines, by alveolar epithelial cells[9].

Although these researches had made a preliminary exploration on lunar dust toxicity, especially on pulmonary toxicity, but due to the limitation of experimental conditions, the researches are very superficial, especially to understand the specific mechanism.

In the previous study, it was measured biochemical indicators, cell classifications and cell counts in Bronchoalveolar Lavage Fluid (BALF) of the rats lunar soil simulant (LSS) exposed and the MPO activity was also measured in lung tissue and the pathological changes were observed. The results showed that LSS can induce lung injury and inflammatory lesions. The changes of the biomarkers in the BALF were consistent with the results of the histopathological observations[10].

This experiment further discussed the lunar dust toxicity mechanism from two aspects, immune injury and oxidative stress. This study filled the gaps in the study of biological toxicity mechanism of lunar dust. At present, oxidative stress reaction in lunar dust pulmonary toxicity and the relationship between immune injury and oxidative stress has not been reported.

MATERIALS AND METHODS

Experimental groups: Forty eight healthy male SPF Wistar rats purchased from the animal house, Liaoning Changsheng Biotechnology Co., Ltd. (Animals License number: SCXK (Liao) 2010-0001), weighing (190-210 g) had been selected for the present study. They were kept in polycarbonate cages, artificial fed, with free access to water and food. The experimental rats were grouped into 8 groups of 6 rats each. The groups were designated as follows: 4 and 24 h control saline groups, 4 and 24 h $PM_{2.5}$ groups, low-dose 4 and 24 h LSS groups and high-dose 4 and 24 h LSS groups.

Intratracheal instillation of dust samples: Prior to instillation, each rat was anesthetized with ether. The anesthetized rats were secured on a framed platform. After the secretions in throat being removed by swabs and the rima glottidis exposed, each rat was instilled with a 1 mL aliquot of a freshly suspended and ultrasonicated dust in normal saline. Then the rats were rotated several times to evenly distribute the liquid. Lunar dust groups received 0.7 mg (low dose) or 7 mg (high dose) lunar dust; $PM_{2.5}$ group rats received 0.7 mg $PM_{2.5}$ air particles; controls received only saline. The animals were returned to their cages where they had free access to food and water. After 4 and 24 h, animals were killed for the next step of the experiment.

$PM_{2.5}$ sampling: During November, 2016-February, 2017, $PM_{2.5}$ samples were collected on cross-site of Guangrong Street a typical urban setting of Shenyang, using TE-6070 high volume air sampler (Tisch Environmental Inc., Miami, FL, USA) at a flow rate of 6-8 L min^{-1}. The $PM_{2.5}$ air particles composition analyses were described by Ma *et al.*[11]. After sampling, the sampled filter paper was cut into a 1×1 cm size, put into distilled water and sonicated for 20 min with a sonicator (Model JL-180H, China). The $PM_{2.5}$ air particles suspension was treated through multilayer gauze for filtration. And then the centrifuged (1000 rpm at 4°C) was dried by vacuum-freeze machine (Labconco, USA) and stored at -80°C.

CAS-1 lunar soil simulant: Chinese Academy of Sciences-1(CAS-1) lunar soil (Chinese standard LSS) was obtained from National Astronomical Observatories. The mineral, chemical compositions and physical properties were very similar to the lunar sample from Apollo 14[12]. As described by Sun *et al.*[10], the CAS-1 simulated lunar dust with a diameter size under 5 μm was obtained by screening with 5 μm aperture sieving.

Serum preparations and determination of cytokines and immunoglobulin: At 4 and 24 h post-exposure, the rats were anesthetized with ether and fixed on the platform. The rats were sacrificed by drawing blood from the descending abdominal aortic bifurcation to a sterile test tube with sterile disposable syringe. The blood was static cooled down for 1 h and centrifuged (1000 rpm at 4°C) for 10 min, then serum was put in -80°C until analyses. Tumor Necrosis Factor alpha (TNF-α), Interleukin 6 (IL-6), Immunoglobulin A (IgA) and Immunoglobulin G (IgG) in the serum were measured using the ELISA Kits Biosource International, Camarillo, USA. The specific steps in strict accordance with the kit manual.

BALF collections and determination of cytokines and immunoglobulin: After taking blood, neck tracheas of rats were exposed and the lung lavage needle was inserted into the tracheas, ligated and fixed. Then the left main bronchus was closed by hemostat. The lungs were lavaged 3 times with 3 mL Phosphate-Buffered Saline (PBS, pH 7.2) to get BALF. BALF was collected in 5 mL-sized sterile centrifuge tubes and centrifuged (1500 rpm at 4°C) for 10 min to separate the cells from the supernatant. The supernatant was collected and stored at -80°C until assay of TNF-α, IL-6, IgA and IgG. Determination method is the same as above.

Homogenate and biochemical analysis of lung tissue: Non lavaged right lung was quickly placed in liquid nitrogen and then stored at -80°C. On the ice, the homogenate (the concentration was 10%) was prepared with an electric homogenate and centrifuged (3000 rpm at 4°C) for 10 min. The supernatant was collected and stored at -80°C until analyses. Malondialdehyde (MDA), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) in the supernatant were measured using the ELISA Kits Nanjing Jiancheng Bioengineering Institute, Nanjing, China. The specific steps in strict accordance with the kit manual.

Statistical analysis: The experimental data were expressed as the mean±standard deviation (Mean±SEM). The analysis among multiple groups was performed using statistical software (SPSS16.0, Chicago, USA) with a one-way analysis of variance (ANOVA). Correlation between cytokines and biochemical indexes was performed using Pearson test. The level of $p<0.05$ was defined as statistical significance.

RESULTS

Determination of the cytokines and immunoglobulin in the serum: The changes of TNF-α, IL-6, IgA and IgG in serum are provided in Table 1. The changes in TNF-α, IL-6, IgA and IgG of all the groups treated with dust showed no significant differences compared with those in the saline group after 4 h ($p>0.05$). However, the changes in TNF- α, IL-6 and IgA of the high-dose LSS group showed significant differences compared with those in the saline group after 24 h ($p<0.05$). The changes in IgG had an increasing trend, but did not reach a significant level.

Determination of the cytokines and immunoglobulin in the BALF: The changes of TNF-α, IL-6, IgA and IgG in BALF are provided in Table 2. The changes in TNF- α, IL-6, IgA and IgG of all the groups treated with dust showed no significant differences compared with those in the saline group after 4 h ($p>0.05$). The changes in TNF- α, IL-6 and IgA of the high-dose LSS group increased significantly after 24 h ($p<0.05$). The changes in IgG had an increasing trend, but did not reach a significant level.

Table 1: Changes of cytokines and immunoglobulin in the serum after infection (n = 6)

Group	Amount of dust (mg)	Time (h)	IgA (μg mL^{-1})	IgG (μg mL^{-1})	TNF-α (ng L^{-1})	IL-6 (ng L^{-1})
NS	0.0	4	18.20±5.99	264.71±21.91	244.55±39.48	19.08±6.49
$PM_{2.5}$	0.7	4	24.68±7.09	252.70±12.97	273.52±58.57	26.48±7.57
LSS (low-dose)	0.7	4	20.48±2.92	261.14±18.35	275.70±22.83	21.89±2.04
LSS (high-dose)	7.0	4	25.58±4.97	273.13±74.52	284.80±18.65	27.07±5.35
NS	0.0	24	24.28±2.61	310.90±56.98	266.73±45.66	25.08±2.72
$PM_{2.5}$	0.7	24	27.67±1.01	301.60±16.91	286.41±49.80	28.72±1.30
LSS (low-dose)	0.7	24	27.29±3.46	319.90±32.74	287.40±34.20	28.33±3.82
LSS (high-dose)	7.0	24	29.70±3.50*	323.10±16.90	338.90±39.53*	31.19±3.51*

Data are presented as Mean±SEM, compared with the saline control group, *$p<0.05$, NS: Normal saline, IgA: Immunoglobulin A, IgG: Immunoglobulin G, TNF-α: Tumor Necrosis Factor alpha and IL-6: Interleukin 6

Table 2: Changes of cytokines and immunoglobulin in the BALF after infection (n = 6)

Group	Amount of dust (mg)	Time (h)	IgA (μg mL^{-1})	IgG (μg mL^{-1})	TNF-α (ng L^{-1})	IL-6 (ng L^{-1})
NS	0	4	38.74±10.56	397.50±58.19	536.120±110.57	40.33±17.53
$PM_{2.5}$	0.7	4	55.88±14.83	500.63±109.92	749.370±137.27	58.57±15.14
LSS (low-dose)	0.7	4	52.66±15.73	424.75±158.24	673.000±172.39	55.05±16.99
LSS (high-dose)	7	4	53.86±16.92	644.62±265.45	729.500±222.59	55.47±11.49
NS	0	24	44.23±8.28	481.62±192.16	535.120±155.26	45.94±8.41
$PM_{2.5}$	0.7	24	67.86±18.01	658.12±244.40	805.870±204.31	64.44±13.74
LSS (low-dose)	0.7	24	60.85±4.30	565.25±174.33	677.125±202.91	60.68±4.95
LSS (high-dose)	7	24	79.28±16.32**	757.12±197.97	1277.500±252.59*	76.25±16.32**

Data are presented as Mean±SEM, compared with the saline control group, *p<0.05 **p<0.01, NS: Normal saline, IgA: Immunoglobulin A, IgG: Immunoglobulin G, TNF-α: Tumor Necrosis Factor alpha and IL-6: Interleukin 6

Table 3: Changes of SOD, GSH-Px and MDA in lung tissue (n = 6)

Group	Amount of dust (mg)	Time (h)	SOD (IU mg^{-1})	GSH-Px (IU mg^{-1})	MDA (nmol mg^{-1})
NS	0.0	4	28.49±7.44	561.60±94.91	0.85±0.31
$PM_{2.5}$	0.7	4	19.82±7.96	513.39±64.33	0.80±0.41
LSS (low-dose)	0.7	4	20.61±4.32	490.21±104.77	0.96±0.45
LSS (high-dose)	7.0	4	18.06±4.40	403.19±110.21	1.14±0.44
NS	0.0	24	25.33±7.93	459.90±82.21	0.98±0.40
$PM_{2.5}$	0.7	24	16.37±4.94	317.59±76.41	1.25±0.52
LSS (low-dose)	0.7	24	13.36±5.90*	325.80±82.10	1.35±0.69
LSS (high-dose)	7.0	24	8.45±3.33**	246.99±94.08**	2.18±0.83*

Data are presented as Mean±SEM, compared with the saline control group, *p<0.05 **p<0.01, NS: Normal saline, SOD: Superoxide dismutase, GSH-Px: Glutathione peroxidase and MDA: Malondialdehyde

Determination of the oxidative stress index in lung tissue: The SOD, GSH-Px activity and MDA content were measured in lung tissue. SOD and GSH-Px are two important antioxidant enzymes. MDA is the most important product of membrane lipid peroxidation, which reflects the degree of oxidative damage of membrane system.

The changes in SOD, GSH-Px and MDA are provided in Table 3. The changes in SOD, GSH-Px and MDA of all the groups treated with dust showed no significant differences compared with those in the saline group after 4 h ($p>0.05$). After 24 h, it was found that the SOD of the LSS groups (low-dose and high-dose) decreased significantly and the GSH-Px of the high-dose LSS group decreased significantly. It was also found that compared with the saline control group, the MDA of the high-dose LSS group increased significantly.

Correlations between TNF-α, IL-6 in BALF and SOD, GSH-PX, MDA in lung tissue: There were significant negative correlations between TNF-α and SOD ($r = -0.50$), TNF-α and GSH-Px ($r = -0.53$), IL-6 and SOD ($r = -0.58$), IL-6 and GSH-Px ($r = -0.64$) and there were significant positive correlations between TNF-α and MDA ($r = 0.67$), IL-6 and MDA ($r = 0.50$). It was indicated that in the lung tissue, there was a significant correlation between oxidative stress and inflammatory reaction caused by particulate matter exposure.

DISCUSSION

The purpose of this study was to investigate the mechanism of acute lung injury exposed to lunar dust. So far, to be sure, lunar dust can cause lung injury indeed, but the mechanism reports are still few. Although intratracheal instillation of collected and extracted samples has limitations, this method is very useful when the material to be studied is in very limited supply and extremely precious, as is the case with lunar soil simulant. The doses for intratracheal instillation in this study were chosen on the basis of information from Henderson *et al.*[13]. It was reported that in rats exposed by inhalation to 10 mg m^{-3} of quartz or TiO_2 for 1 week, the lung contained 0.76 mg quartz or 0.44 mg TiO_2. If it is assumed that dust deposition in the lung is roughly proportional to the minute respiratory volume (MRV) of the exposed animals, then the dust burden of the rat lung used would have also been about 0.76 mg quartz or 0.44 mg TiO_2. In the present study, a single dose of 0.7 mg/rat was chosen as a low dose and 7 mg as a high dose. This study explored the mechanism of immunity and oxidative stress for respiratory damage caused by lunar dust simulant through intratracheal instillation in rats and it was a useful complement to lunar dust biological toxicological studies.

The inflammatory response is the defense and repair of the body's stimulation and damage caused by various endogenous and exogenous substances, but it can also cause damage to normal tissues. IL-6 and TNF-α are pro-inflammatory cytokines[14]. Some studies reported that the synthesis increase and combined effects of some pro-inflammatory regulatory factors could cause systemic inflammation[15,16].

TNF-α is an endothelial activation factor, which can promote the neutrophile granulocyte and eosinophils

granulocyte adhere to endothelial cells, in order to facilitate their migration to inflammatory sites and enhance their toxic effects. After being activated by the particles, macrophages, epithelium and lymphocytes can produce IL-6 through the induction of TNF-α. Some studies showed that TNF-α could be enhanced by $PM_{2.5}$ inhalation in pulmonary artery. It can also lead to fibroblast proliferation and collagen synthesis[17,18]. Therefore, it was believed that the increased TNF-α in BALF might be an sensitive early marker for diagnosis of pulmonary fibrosis[19,20]. IL-6 is one of the main signs of acute inflammation, which has the function of immune regulation, participating in inflammation and anti-infection. Inappropriate expression of IL-6 is one of sensitive indicators of early inflammatory response in the body[21]. The results showed that after 24 h in the high dose LSS group, the level of TNF- α and IL-6 in the serum and BALF increased significantly compared with the normal saline group. It is suggested that LSS can produce inflammatory stimulation effect on lung tissue, which is consistent with the previous research results.

IgG is the main antibody in serum. It can easily spread into the organization, playing a passive immune function, which participates in immune defense[22]. IgA is one of the major antibody that plays an important immunological effect in serum and extracellular fluid, mainly produced by mucosa-associated lymphoid tissue. IgA in the secretion is the most important factor in the local mucosal immunity. It can inhibit the pathogenic microorganisms from adhering to mucosal target cells and neutralizing toxins so, IgA is the main antibody that participates in the local mucosal immunity and plays an important role in the local anti-infection[23,24]. In this study, the main immunoglobulins in the serum and lung lavage fluid were detected and the results showed that the IgA and IgG increased after LSS exposed. But due to the short exposure time, IgG content had an increasing trend, without reaching the significant level. These results reflect the effect of LSS on the production of immunoglobulins, suggesting that after particulate entering the body, the body's humoral immunity and local mucosal immune response are stimulated.

SOD and GSH are key antioxidants to regulate oxidative stress in cells. SOD can catalyze the transformation of superoxide free ions into oxygen and hydrogen peroxide. It plays a crucial role in the balance between oxidation and antioxidation. This enzyme can clear the superoxide anion radical and protect the cell from damage. Its activity is inversely proportional to the antioxidant capacity of the body. Therefore, the level of SOD activity is closely related to aging, inflammation, tumor, autoimmune disease, kidney disease and so on.

GSH is a small three peptide molecule, which is widely existed in the living body. Its antioxidant effect is mainly to reduce peroxidation induced by free radicals based on redox reaction, to keep the thiol containing enzymes (ATP enzyme) from heavy metals and oxidant. It can antagonize oxidizing poison, maintain intracellular calcium homeostasis and regulate enzyme activity[25]. Some studies have indicated that the imbalance of oxidized and antioxidant in rats lung could be induced by endotracheal instillation of $PM_{2.5}$ and accompanied with the decrease of GSH[26]. SOD and GSH are oxide decomposing enzyme, can remove peroxide metabolites, block lipid peroxidation chain reaction, protect the cell membrane and the activity reflects the ability for scavenging oxygen free radicals. The free radicals induced by $PM_{2.5}$ can directly act on antioxidant enzyme system(e.g. SOD, GSH, CAT) and reduce their activity *in vivo* and *in vitro*[27,28]. MDA is a product of lipid peroxidation between oxygen free radical and biological membrane polyunsaturated fatty acids. The amount of MDA was positively correlated with the amount of oxygen free radicals and the degree of lipid peroxidation. In addition, MDA can trigger a chain reaction of oxygen free radicals and enlarge the role of reactive oxygen species. Therefore, the detection of MDA can reflect the level of oxygen free radicals and the degree of oxygen free radical damage in the body[29]. In this study, it was found that the activity of SOD and GSH in LSS groups decreased, while the content of MAD increased, especially in the high dose LSS group. These results suggested that LSS particles can break the balance between oxidative and antioxidant and reduce antioxidant capacity. The lipid peroxidation was enhanced and the cells were subjected to a certain degree of oxidative stress or oxidative damage, which changed the permeability of the membrane. In order to explore the intrinsic relationship between LSS exposure and lung toxicity, the correlation between inflammation and oxidative stress response in lung tissue was analyzed. The results showed that oxidative stress and inflammatory reaction caused by LSS exposure were closely linked. There may be two reasons: First, the LSS used in this experiment contains Cr, Zn, Cu and other trace elements. These elements can stimulate tissue to produce a large amount of oxygen free radicals Reactive Oxygen Species (ROS). It can activate some regulatory proteins and up regulate the expression of many cytokines, causing immune disorders[30,31] and second: The inflammatory response promotes oxidative damage. Some elements of $PM_{2.5}$ can play roles of antigen and hapten and induce the body's immune response, resulting in the deposition of particulate matters in the lungs.

The particles can be in contact with epithelial cells, alveolar macrophages and lung cells after depositing in the lungs. Such contact can stimulate or enhance the activity of ROS formation, which can lead to the occurrence of pulmonary interstitial and/or alveolar inflammation (This was consistent with previous findings in lung pathology)[32]. The redox balance was broken and a large number of free radicals were generated in the cells in a short time. The interaction between oxidative stress and immune response promoted the formation of a positive cycle, which enhanced each other. However, further investigations, it will conduct subacute toxicity and long-term toxicity studies and conduct a mechanism study on the toxicity of lunar dust components and further examinations to explore the pathways of immune and oxidative stress are indispensable. It is expected that the research results will be useful for the accurate assessment of lung toxicity induced by lunar dust and provide a reference for the future lunar base construction and lunar manned landing mission.

CONCLUSION AND FUTURE RECOMMENDATIONS

In this study, the mechanism of immunity and oxidative stress in rats' acute lung injury induced by LSS was studied. The results showed that significant immune and oxidative stress response in respiratory system were caused by LSS acute exposure and the immune and oxidative stress response promoted each other, resulting in toxic effect on pulmonary parenchymal cells and membrane of rats.

SIGNIFICANCE STATEMENT

This study discovers the inflammation induced by lunar dust related to immune responses and redox reactions that can be beneficial for exploration on the mechanism of toxic dust. This study will help the researchers to uncover the critical mechanism of the lunar dust toxicity that many researchers were not able to explore. Thus, a new theory on the diagnosis and treatment of lunar dust poisoning may be arrived at.

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REFERENCES

1. Carrier, W.D., 1973. Lunar soil grain size distribution. The Moon, 6: 250-263.
2. Jiang, X. and Y. Sun, 2016. A review of biological toxicology of lunar dust. J. Shenyang Med. Coll., 18: 214-216.
3. Holland, J.M. and R.C. Simmonds, 1973. The mammalian response to lunar particulates. Space Life Sci., 4: 97-109.
4. Kustov, V.V., O.F. Ostapvenko and V.G. Petrukhin, 1974. Research on the Biological Effect of a Fine Fraction of Lunar Soil Sent to Earth by the Unmanned Station Luna-16. In: Lunar Soil from the Sea of Fertility, Vinogradov, A.P. (Ed.)., M. Nauka, Moscow, pp: 592.
5. Kustov, V.V., V.I. Belkin and G.G. Kruglikov, 1989. Biological effects of lunar soil. Probl. Kosm. Biol., 61: 1-103, (In Russian).
6. Lam, C.W., R.R. Scully, Y. Zhang, R.A. Renne and R.L. Hunter *et al*., 2013. Toxicity of lunar dust assessed in inhalation-exposed rats. Inhalation Toxicol. Int. Forum Respirat. Res., 25: 661-678.
7. Lam, C.W., J.T. James, R. McCluskey, S. Cowper, J. Balis and C. Muro-Cacho, 2002. Pulmonary toxicity of simulated lunar and Martian dusts in mice: I. Histopathology 7 and 90 days after intratracheal instillation. Inhalation Toxicol. Int. Forum Respirat. Res., 14: 901-916.
8. Lam, C.W., J.T. James, J.N. Latch, R.F. Hamilton Jr. and A. Holian, 2002. Pulmonary toxicity of simulated lunar and Martian dusts in mice: II. Biomarkers of acute responses after intratracheal instillation. Inhalation Toxicol. Int. Forum Respirat. Res., 14: 917-928.
9. Wallace, W. and A.S. Jeevarajan, 2002. Lunar dust and lunar simulant activation, monitoring, solution and cellular toxicity properties. NASA Technical Reports Server (NTRS). https://ntrs.nasa.gov/search.jsp?R=20090007462
10. Sun, Y., J.G. Liu, Y.C. Zheng, C.L. Xiao and B. Wan *et al*., 2018. Research on rat's pulmonary acute injury induced by lunar soil simulant. J. Chin. Med. Assoc. 81: 133-140.
11. Ma, M., S. Li, H. Jin, Y. Zhang and J. Xu *et al*., 2015. Characteristics and oxidative stress on rats and traffic policemen of ambient fine particulate matter from Shenyang. Sci. Total Environ., 526: 110-115.
12. Zheng, Y.C., S.J. Wang, Z.Y. Ouyang, Y.L. Zou and J.Z. Liu *et al*., 2009. CAS-1 lunar soil simulant. Adv. Space Res., 43: 448-454.
13. Henderson, R.F., K.E. Driscoll, J.R. Harkema, R.C. Lindenschmidt, L. Chang and E.B. Barr, 1995. A comparison of the inflammatory response of the lung to inhaled versus instilled particles in F344 rats. Toxicol. Sci., 24: 183-197.

14. Zhao, H., W. Li, Y. Gao, J. Li and H. Wang, 2014. Exposure to particular matter increases susceptibility to respiratory *Staphylococcus aureus* infection in rats via reducing pulmonary natural killer cells. Toxicology, 325: 180-188.
15. Zhang, W., T. Lei, Z.Q. Lin, H.S. Zhang and D.F. Yang *et al*., 2011. Pulmonary toxicity study in rats with PM_{10} and $PM_{2.5}$: Differential responses related to scale and composition. Atmos. Environ., 45: 1034-1041.
16. Myatt, T.A., M.S. Vincent, L. Kobzik, L.P. Naeher, D.L. MacIntosh and H. Suh, 2011. Markers of inflammation in alveolar cells exposed to fine particulate matter from prescribed fires and urban air. J. Occupat. Environ. Med., 53: 1110-1114.
17. Ishii, H., S. Hayashi, J.C. Hogg, T. Fujii and Y. Goto *et al*., 2005. Alveolar macrophage-epithelial cell interaction following exposure to atmospheric particles induces the release of mediators involved in monocyte mobilization and recruitment. Respirat. Res., Vol. 6, No. 1. 10.1186/1465-9921-6-87.
18. Davel, A.P., M. Lemos, L.M. Pastro, S.C. Pedro and P.A. de Andre *et al*., 2012. Endothelial dysfunction in the pulmonary artery induced by concentrated fine particulate matter exposure is associated with local but not systemic inflammation. Toxicology, 295: 39-46.
19. Kandhare, A.D., S.L. Bodhankar, V. Mohan and P.A. Thakurdesai, 2015. Effect of glycosides based standardized fenugreek seed extract in bleomycin-induced pulmonary fibrosis in rats: Decisive role of Bax, Nrf2, NF-κB, Muc5ac, TNF-α and IL-1β. Chemico-Biol. Interact., 237: 151-165.
20. McGrath, E.E., A. Lawrie, H. Marriott, P.F. Mercer and S.S. Cross *et al*., 2011. S111 The role of TNF-related apoptosis inducing ligand (TRAIL) in pulmonary fibrosis. Thorax, 66: A51-A52.
21. Rodriguez-Cotto, R.I., M.G. Ortiz-Martinez, E. Rivera-Ramirez, V.L. Mateus, B.S. Amaral, B.D. Jimenez-Velez and A. Gioda, 2014. Particle pollution in Rio de Janeiro, Brazil: Increase and decrease of pro-inflammatory cytokines IL-6 and IL-8 in human lung cells. Environ. Pollut., 194: 112-120.
22. He, M., T. Ichinose, M. Kobayashi, K. Arashidani and S. Yoshida *et al*., 2016. Differences in allergic inflammatory responses between urban $PM_{2.5}$ and fine particle derived from desert-dust in murine lungs. Toxicol. Applied Pharmacol., 297: 41-55.
23. Zhao, J., Z. Gao, Z. Tian, Y. Xie and F. Xin *et al*., 2013. The biological effects of individual-level $PM_{2.5}$ exposure on systemic immunity and inflammatory response in traffic policemen. Occup. Environ. Med. 10.1136/oemed-2012-100864.
24. Lai, H.T.L., M.G.B. Nieuwland, A.J.A. Aarnink, B. Kemp and H.K. Parmentier, 2012. Effects of 2 size classes of intratracheally administered airborne dust particles on primary and secondary specific antibody responses and body weight gain of broilers: A pilot study on the effects of naturally occurring dust. Poult. Sci., 91: 604-615.
25. Liu, Y., L. Wang, F. Wang and C. Li, 2016. Effect of fine particulate matter ($PM_{2.5}$) on rat placenta pathology and perinatal outcomes. Med. Sci. Monit., 22: 3274-3280.
26. Szigeti, T., C. Dunster, A. Cattaneo, D. Cavallo and A. Spinazze *et al*., 2016. Oxidative potential and chemical composition of $PM_{2.5}$ in office buildings across Europe-The OFFICAIR study. Environ. Int., 92: 324-333.
27. Pamplona, R. and D. Costantini, 2011. Molecular and structural antioxidant defenses against oxidative stress in animals. Am. J. Physiol. Regul. Integr. Comp. Physiol., 301: R843-R863.
28. Luo, B., H. Shi, L. Wang, Y. Shi and C. Wang *et al*., 2014. Rat lung response to $PM_{2.5}$ exposure under different cold stresses. Int. J. Environ. Res. Public Health, 11: 12915-12926.
29. Dellinger, B., W.A. Pryor, R. Cueto, G.L. Squadrito, V. Hegde and W.A. Deutsch, 2001. Role of free radicals in the toxicity of airborne fine particulate matter. Chem. Res. Toxicol., 14: 1371-1377.
30. He, J., Y.M. Wang, Z.M. Zhao, W. Zhou and X.Y. Yuan *et al*., 2016. Oxidative damage related to $PM_{2.5}$ exposure in human embryonic stem cell-derived fibroblasts. Chin. J. Preventive Med., 50: 705-709.
31. Lu, S., W. Zhang, R. Zhang, P. Liu and Q. Wang *et al*., 2015. Comparison of cellular toxicity caused by ambient ultrafine particles and engineered metal oxide nanoparticles. Particle Fibre Toxicol., Vol. 12, No. 1. 10.1186/s12989-015-0082-8.
32. Lakey, P.S., T. Berkemeier, H. Tong, A.M. Arangio, K. Lucas, U. Poschl and M. Shiraiwa, 2016. Chemical exposure-response relationship between air pollutants and reactive oxygen species in the human respiratory tract. Scient. Rep., Vol. 6. 10.1038/srep32916.