Chronic exposure to high fat diet exacerbates arsenic-induced lung damages in male mice: Possible role for oxidative stress

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Abstract

Arsenic is a common environmental and occupational contaminant worldwide which can influence the development of respiratory diseases. In recent years, alteration in the lifestyle as well as food habits have led to increased consumption of food containing high levels of fat. The present study was designed to evaluate the effects of chronic exposure to a high-fat diet (HFD) on arsenic-induced damages and oxidative stress in the lung tissue of mice. This is the first study to reveal the effect of diet-induced obesity on arsenic-induced lung damages. Seventy-two male Naval Medical Research Institute (NMRI) mice were divided into six groups and fed an HFD or standard diet (SD) while being exposed to 25 or 50 ppm of arsenic through drinking water for 20 weeks. At the end of the experiment, the lung weight to body weight ratio; oxidative stress markers, nitrite level, and hydroxyproline content in the lung tissue; and lung histology were evaluated. The results demonstrated that arsenic exposure leads to a significant decrease in the glutathione level and catalase enzyme activity, and significantly increased reactive oxygen species, malondialdehyde, and nitrite level, but it did not affect the superoxide dismutase activity and hydroxyproline content in the lung tissue. Consequently, all the parameters studied aggravated when HFD was consumed along with arsenic. These findings were confirmed by histological examination. Our study showed that HFD increased arsenic-induced lung damages through oxidative stress in mice. These findings could be important for clinical research to protect against arsenic-induced respiratory toxicity in humans.

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Introduction

Arsenic (As) is a heavy metalloid found in water, soil, and air. Although contact to arsenic occurs through both environmental and industrial sources, the common source of exposure worldwide is through inhalation and drinking water [1]. Many studies have linked arsenic exposure with an increased risk of cancers [2] and non-malignant diseases [3]. Consumption of arsenic in drinking water has also been associated with respiratory diseases [4]. Epidemiological data have associated arsenic exposure through the use of drinking water to chronic cough, chronic bronchitis, bronchiectasis, and obstructive lung diseases [5].

Recent studies have shown that arsenic induces toxicity by producing reactive oxygen species (ROS), and the intracellular peroxide level is associated with arsenic-induced cellular apoptosis [6]. Human lung tissue may be affected by arsenic toxicity because of the high partial pressure of oxygen and dimethylarsine, a gas that is excreted *via* the lungs [7]. Although lung is one of the main targets that is affected by arsenic, little evidence has been presented for the production of ROS in arsenic-exposed lung cells.

Industrialization and financial progress of modern society have led to modifications in diets of the people. A number of studies have discovered that diet is an important factor in arsenic-induced toxicity. HFD improved arsenic-induced hepatofibrogenesis [8] and heightened the gathering of arsenic in the liver tissue [9]. Many studies have indicated the presence of an imbalance between the formation of ROS and antioxidant defense system in the presence of respiratory damage or obesity [10]. For example, in patients with asthma or obesity, elevation of oxidative stress and changes in antioxidant defenses have been reported [11]. Albuali established that the serum level of malondialdehyde (MDA), a biomarker of oxidative stress, is increased whereas the serum levels of glutathione (GSH), an antioxidant, is decreased in obese children compared with that in the normal control group [12].

Because there is no evidence to show the effects of chronic exposure to HFD on arsenic toxicity in the respiratory system, the present study was designed to determine the relationship between consumption of HFD and arsenic-induced lung damages in mice.

Materials and Methods

Animals and treatment

Seventy-two adult male Naval Medical Research Institute (NMRI) mice (25-30 g) were purchased from the animal facility of the Ahvaz Jundishapur University of Medical Sciences. The animal experiments

pime EDITRICE

were conducted in accordance with animal care guidelines with an ethics committee approval No. IR.AJUMS.REC.1395.405. The mice consumed standard diet (SD) or HFD and arsenic in water for 20 weeks. The levels of arsenic in HFD (58% fat) and SD (16% fat) were 5 and 7 ppb which contain very low concentration of arsenic compared to the ordered concentration (25 and 50 ppm) in this study. All mice were divided into six groups (n=12) as follows: SD (control group), SD +As25ppm, SD +As50ppm, HFD (control group), HFD+As25ppm, and HFD+ As50ppm [13].

Sample preparation and analytical procedures

At the end of the 20-week experimental period, the animals were sacrificed and the lung were removed, weighed, and stored frozen at -70°C until analysis. For the estimation of arsenic, a mineral extract of these samples was prepared by wet ingestion in a mixture of nitric acid, sulfuric acid, and perchloric acid. For complete conversion of arsenic V into arsenic III, potassium iodide and hydrochloric acid were added to the mineral extracts and incubated for 1h. Arsenic in the mineral extracts was determined by a hydride generation atomic absorption spectrophotometer [14].

Preparation of lung homogenates

The lung tissue washed with ice-cold saline and a 10%homogenate (0.03 M sodium phosphate buffer, pH-7.4) of it was prepared by using an Ultra-Turrax T25 homogenizer at a speed of 9500 rpm [15]. The homogenized tissue preparation was used for biochemical analysis.

Determination of ROS, GSH and MDA levels in lung tissue

The level of ROS in lung tissue was measured by using 2, 7-dichlorofluorescindiacetate (DCFDA) that converted into highly fluorescent DCF by cellular peroxides. Fluorescence was calculated using a fluorimeter, at 488nm excitation and 525 nm emission wavelength [16]. GSH contents were measured according to Thomas and Skrinska method. The GSH reacts with DTNB and forms a yellow-colored complex with it. The absorbance was read at 412 nm. The result was expressed as µmoles of GSH/mg protein [17]. The extent of lipid peroxidation in terms of malondialdehyde formation was measured. Briefly homogenate lung sample containing 1 ml was mixed with 1 ml TCA (20%), 2 ml TBA (0.67%) and heated for 1 h in boiling water bath. After cooling, mixture centrifuged and absorbance of the supernatant measured at 532 nm against suitable blank. The amount of TBARS was calculated by using a molar extinction coefficient of $\varepsilon=1.56\times10^5$/M/cm and expressed as mol/mg protein [18].

Determination of catalase enzyme and superoxide dismutase (SOD) in lung tissue

Catalase activity was assayed according to the method used by L. Goth. 500 µl of 0.05 mmol Tris-HCl, 1 ml H_2O_2 and 50 µl of sample were mixed and incubated for 10 min, and then Reaction was stopped by adding 500 µl Ammonium molybdate solution 4%. The absorbance was read at 410 nm. The result was expressed as U/mg protein [19]. The activity of SOD was determined using a xanthine/xanthine oxidase system for production of superoxide radical and subsequent measurement of Cytochrome-*C* as a scavenger of the radicals. Optical density was determined using a spectrometer (UV- 1601, Shimadzu) at 550 nm. One unit of enzyme activity was defined as the quantity of SOD required to inhibit the rate of reduction of Cytochrome-*C* by 50%. SOD activity is presented as units per milligram of protein (U /mg protein) [19].

Determination of nitrite and hydroxyproline level in lung tissue

Nitrite was estimated using Greiss reagent which served as an indicator of nitric oxide production. An amount of 100 µL Greiss reagent (1:1 solution of 1% sulphanilamide in 5% phosphoric acid and 0.1% napthaylamine diamine dihydrochloric acid in water) was added to 100 µL of supernatant and absorbance was measured at 542 nm [20]. Lung hydroxyproline can be used as an indicator of collagen deposition. Lung hydroxyproline was determined spectrophotometrically and the results were expressed as micrograms of hydroxyproline per lung [21].

Histopathological studies

The right lungs tissues of mice were excised, fixed in buffered 10%formalin solution for 24 h and embedded in paraffin wax, then sectioned and stained with Hematoxylin and Eosin stain for histological examination under light microscope.

Results

Effect of diet and arsenic exposure on the body weight, average of daily water and arsenic intake

As expected, the HFD control mice weighed more than the SD control group ($p<0.001$), but coadministration of HFD and both concentrations of arsenic resulted in a significant weight loss in comparison with that in the HFD control group ($p<0.01$). The results of daily water intake showed that HFD control mice drank less water compared with the SD control group ($p<0.01$). Furthermore, administration of both concentrations of arsenic in the SD and HFD groups decreased the water intake compared with that in their control groups ($p<0.01$). The results of daily arsenic exposure indicated a significant and dose-dependent increase in the arsenic intake in the SD and HFD groups ($p<0.05$). Moreover, there was a significant difference between the SD groups and their counterparts in the HFD groups ($p<0.05$) (Table 1).

Effects of diet and arsenic exposure on oxidative and nitrosative stress in lung tissue

The results of the lipid peroxidation analysis revealed that the lung tissue MDA level was significantly different in the two control groups. Also, arsenic exposure at a concentration of 50ppm significantly increased the MDA level in the HFD- and SD-fed mice when compared with that in their respective controls ($p<0.05$ and $p<0.01$).

The glutathione assessment results showed that both arsenic doses significantly decreased the GSH level in the SD and HFD groups ($p<0.01$) and ($p<0.001$), and further, there was a significant difference between the HFD groups and their counterparts in the SD groups ($p<0.01$). Also, 50 ppm arsenic exposure in the HFD and SD groups induced a significant increase of ROS in the lung tissue ($p<0.001$), but ROS formation was not statistically significantly different between the HFD and SD control groups. Also, the results showed that HFD induced a significant rise in the nitrite levels in the lung tissue ($p<0.001$). Exposure to 50 ppm arsenic increased this variable in the SD- and HFD-fed mice ($p<0.05$) (Table 2).

Effects of HFD and arsenic on catalase and SOD activity in lung tissue

The results showed that catalase enzyme activity significantly decreased in the HFD control group compared with that in the SD control group ($p<0.001$). Furthermore, arsenic exposure at both doses signifi-

cantly decreased this antioxidant enzyme in the SD- and HFD-fed mice ($p<0.001$). Also, the SOD activity was not statistically significantly different between the HFD and SD control groups. Arsenic exposure at 50ppm significantly increased the SOD activity in the HFD- ($p<0.05$) but not SD-fed mice when compared with their respective controls (Table 2).

Effect of diet and arsenic exposure on lung weight to body weight ratio and lung distribution of arsenic

The average total lung to body weight ratio after 20 weeks training was significantly lower in the HFD control mice compared with that in the SD controls ($p<0.05$). Further, arsenic exposure increased the lung to body weight ratio in both SD and HFD mice at 25 and 50 ppm ($p<0.01$). This ratio increased in the HFD+As50ppm compared with that in the SD+As50ppm group ($p<0.05$) (Figure 1A). Exposure to arsenic at 25 and 50ppm resulted in the accumulation of arsenic in the lungs of SD- and HFD-fed mice ($p<0.001$). Also, there was a significant difference between the HFD groups and their counterparts in the SD groups ($p<0.05$) (Figure 1B).

Effects of arsenic and diet on hydroxyproline content in lung tissue

As shown in figure1.C, exposure to a combination of HFD and 50 ppm arsenic significantly increased the content of hydroxyproline compared with that in the control group. Also, the content of hydroxyproline increased in the HFD+As 50ppm compared with that in the SD+As 50ppm group ($p<0.05$).

Histopathological analysis

Figure 2 A-C shows the histopathological assessments of the lung tissue of the experimental animals. A normal architecture of the pulmonary tissue was observed in the HFD and SD control groups, but in the SD+As 50ppm group, pulmonary edema was observed. In the HFD+As 50ppm group, inflammation and alveolar wall degeneration were observed.

Discussion

The present study has shown that HFD noticeably aggravates arsenic-induced lung injury by the induction of oxidative stress and reduction of antioxidant enzymes. Lung is one of the main target tissues in arsenic toxicity because of the high partial pressure of oxygen and dimethylarsine, which are excreted *via* the lungs [7]. Epidemiological data have associated arsenic exposure through the use of drinking water to chronic cough, chronic bronchitis, bronchiectasis, and obstructive lung diseases [5]. In one study, arsenic-induced skin lesions were linked to increased risk of respiratory diseases [22]. Arsenic is the only lung carcinogen that is active by both inhalation and ingestion and may be unique in its ability to increase the risk of numerous lung diseases via ingestion rather than inhalation [23]. Arsenic decreases the body weight by interfering with several metabolic pathways [24]. In the present study, we found that the consumption of HFD for 5 months led to increased body weight, but when HFD was administered along with arsenic, body weight decreased. Also, when HFD was administered with arsenic, the lung weight to body weight ratio increased. Based on these results, we proposed that arsenic exposure led to hypertrophy of the lung tissue. In addition, arsenic decreased water intake, and the same effect was revealed in the HFD control group when compared with the SD control group. Even though the animals treated with 50 ppm arsenic and HFD drank less water compared with the SD control group, the results showed that they received a significant amount of arsenic. It was shown that arsenic collected in many tissues and organs. Our results indicated that chronic exposure to arsenic increases its level in the lungs of mice, and HFD suppressed this effect. A number of studies

Table 1. Effect of diet and arsenic exposure on the average of daily water drink, arsenic and calorie intake, and body weight.

Groups / Variables	Standard diet			High fat diet		
	Control	As 25ppm	As 50ppm	Control	As 25ppm	As 50ppm
Body weight (g)	38.58±3.1	36.40±5.1	35.75±3.3	46.33±8.6^{a*}	35.16±3.5^{b*}	29.08±2.4^{b*}
Water drink (ml/day)	11.46±1.3	7.52±0.7^{a*}	4.56±1.5^{a**}	8.67±0.9^{a*}	6.20±1.6^{b}	4.29±1.4^{b*}
Arsenic intake (µg/day)	0	188.01±16.5	228.31±24.9^{c}	0	154.83±31.9^{c}	214.51±38.4^{ed}

Each value was presented as means ± SEM (n=12). [a]Significantly different from control SD group ($p<0.05$); [b]significantly different from control HFD group ($p<0.05$); [c]significantly different from SD + arsenic 25 ppm group; [d]significantly different from SD + As50 ppm group ($p<0.05$); [e]significantly different from HFD + arsenic 25 ppm group ($p<0.05$); $^{a*, b*, e*}p<0.01$; $^{a**, b**}p<0.001$. p-values from one-way ANOVA, followed by Tukey's test for multiple comparisons.

Table 2. Effect of high fat diet and arsenic on antioxidant marker in lung tissue.

Groups / Variables	Standard diet			High fat diet		
	Control	As 25ppm	As 50ppm	Control	As 25ppm	As 50ppm
MDA (nmol/mg protein)	6.3±0.4	8.2±0.25	11.9±1.05^{a*}	10.3±10.4^{a}	11.5±0.43	14.56±0.7^{b}
GSH (µg/mg protein)	109±4.5	87±5.1^{a*}	75.2±4.4^{a**}	62±5.4^{a**}	52±5.5^{c*}	43±5.5^{b*d*}
Catalase (U/mg protein)	152.7±1.9	115.7±10.1^{a**}	55.6±5.2^{c**a**}	105±5.6^{a**}	46.5±3.6^{b**c**}	33.3±3.97^{b**}
ROS (% control)	160.2±28	202.5±13.67	221.3±17.1^{a**}	192.5±10.2	215.6±12.8	96.8±26.22^{d**b**}
SOD (U/mg of protein)	9.43±1.43	9.31±2.75	11.76±1.98	11.04±3.54	12.08±2.43^{a}	14.86±1.98^{a*b}
Nitrite levels (% of control)	100±8.5	116.667±18.4	138±11.9^{a*}	160±11.3^{a**}	139.667±12.5^{a*c}	156±12^{a**d}

Data are meanSD; n=12. MDA, malondialdehyde; ROS, reactive oxygen species; GSH, glutathione. [a]Significantly different from control SD ($p<0.05$); [b]significant difference from control HFD ($p<0.05$); [c]significant difference from SD + As 25 ppm ($p<0.05$); [d]significant difference from HFD + As 25 ppm ($p<0.05$); [e]significant difference from SD + As 50 ppm ($p<0.05$); $^{a* b*}p<0.01$, $^{a**, b**}p<0.001$. p-values from one-way ANOVA, followed by Tukey's test for multiple comparisons.

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have reported that consumption of HFD can produce cell membrane dysfunction by the induction of permeability and increase in lung inflammation [8]. So, according to the present results, it could be suggested that HFD can increase permeability and dysfunction of the cell membrane and cause more arsenic accumulation in the lung tissue.

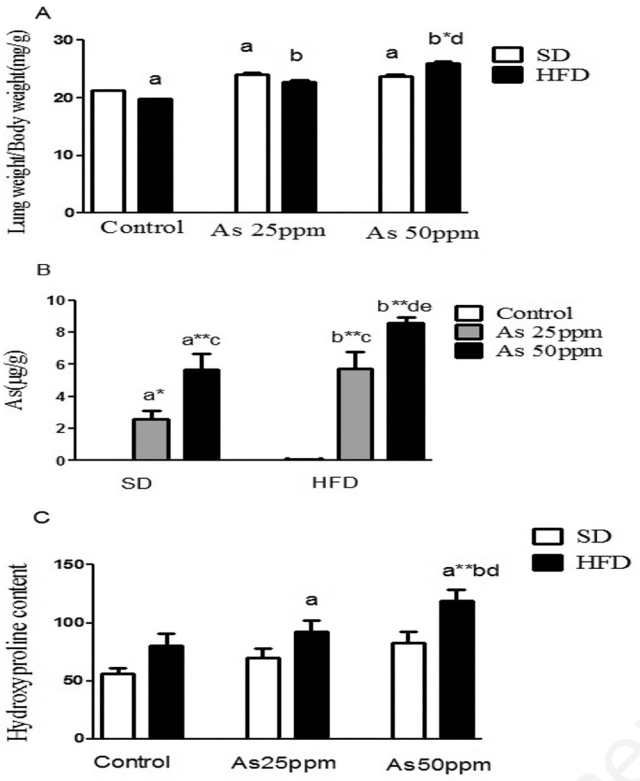

Figure 1. Effects of arsenic and diet on A) lung weight to body weight ratio; B) lung distribution of arsenic; C) lung hydroxyproline content (mean SE; n=12). a, significant difference from control SD ($p<0.05$); b, significant difference from control HFD ($p<0.05$); c, significant difference from SD + As 25 ppm ($p<0.05$); d, significant difference from HFD + As 25 ppm ($p<0.05$); e, significant difference from SD + As 50 ppm ($p<0.05$); a*, b*, $p<0.01$; a**, b**, $p<0.001$. p-values rom one-way ANOVA, followed by Tukey's test for multiple comparisons.

In this study, oxidative stress factors in the lung tissue have been evaluated. Previous studies have shown that oxidative stress plays a basic role in various types of respiratory diseases [25]. Also, many studies have shown that arsenic causes oxidative stress, oxidative damage, and decrease in the levels of antioxidants. Oxidative stress induced by arsenic may cause a change in the expression of antioxidant genes which contributes to the generation of free radicals such as ROS, activates the process of lipid peroxidation, elevates the level of tissue MDA, and decreases the GSH content [26]. Reduced glutathione can be the result of decreased synthesis or increased degradation/utilization of GSH by increased oxidative stress [27].

HFD increased the production of ROS as well as reduced the antioxidant defense mechanisms. Some studies have shown that on feeding an HFD, there are greater levels of oxidative stress biomarkers that are also associated with reduced antioxidant enzyme activities [28]. Evidence shows that an imbalance between the formation of ROS and antioxidant defense system is present in obesity [10].

The serum levels of MDA are significantly increased in obese children while the serum levels of glutathione are decreased [11]. Therefore, it seems that there is a relationship between obesity, respiratory diseases, and the elevation of oxidative stress. Hence, in agreement with the previous study, the findings from the present study indicate that arsenic in combination with HFD leads to an increase in the production of ROS and depletion of glutathione levels in the lung tissue.

The membrane polyunsaturated fatty acids are exceedingly susceptible to free radical-induced oxidative damage. The interaction between the free radicals and polyunsaturated fatty acids induces self-disseminating lipid peroxidation reactions which result in impaired membrane function and generation of MDA [29]. Our study also showed that arsenic exposure alone and in combination with HFD increases the lipid peroxidation in the lung tissue. Hence, a combination of both factors leads to excessive oxidative stress as a result of ROS production and lipid peroxidation of the membranes, thereby causing degradation of phospholipids, and finally, cellular deterioration in the lung tissue. The excessive oxidative stress could be due to the accumulation of free radicals as a result of increased lipid peroxidation due to HFD consumption and also free arsenic ions in the lung tissue.

Arsenic produces not only ROS but also hydrogen peroxide, hydroxyl radical species, dimethyl arsenic peroxyl radical, and dimethyl arsenic radical, which are known to be generated during arsenic toxicity [30]. In the present study, we showed that arsenic decreased the activity of the antiperoxidative enzyme, catalase, in the mice lung. This enzyme causes scavenging of hydrogen peroxides that are produced by arsenic

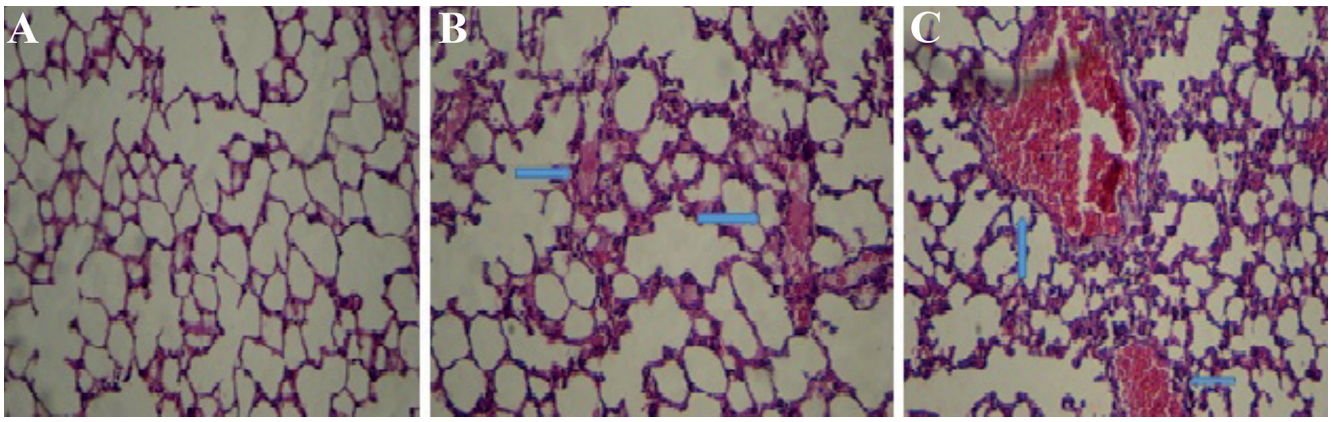

Figure 2. Effects of arsenic and diet on A) histopathological maps of lung, control groups; B) histopathological maps of lung, SD + arsenic 50 ppm; C) histopathological maps of lung, HFD + arsenic 50 ppm. H&E (200x magnification).

[31,32]. Thus, we suggest that arsenic could impair the ability to detoxify H_2O_2 *via* catalase resulting in the accumulation of H_2O_2 in the lung tissues.

Our results also indicated an increase in the nitrite level in the arsenic- and HFD-fed mice. The induction in nitrite level by arsenic and HFD may be due to its effect on the inducible nitric oxide synthase (iNOS) expression and upregulation of its expression in the lung tissue. Moreover, another study proposed that the source of airway oxidative stress in obesity is related to the generation of anion superoxide from airway iNOS [33].

Furthermore, in the current study, collagen deposition was determined by evaluating the total hydroxyproline content of the lung tissues. Our data found that arsenic and HFD increased the content of hydroxyproline in the lung tissue, and this rise was not significant, but exposure to a combination of HFD and 50 ppm arsenic increased this variable significantly when compared with that in the control group. These results concur with that of a previous study that showed that treatment with arsenic elevated lung hydroxyproline content. These results indicated that the consumption of HFD along with arsenic-induced pulmonary inflammation and, probably, fibrosis in mice, but more studies are needed to prove it.

Lung injury that is induced by arsenic and HFD may affect the airways, lung parenchyma, mediastinum, pleura, pulmonary vasculature, and/or the neuromuscular system (34). Recognition of lung diseases is difficult because the clinical, radiological, and histological findings are nonspecific. The pulmonary tissues in the SD+As 50ppm group showed pulmonary edema. The alveolar wall capillaries seemed dilated, with slight inflammatory reaction, but showed little indication of degenerative modification. The HFD+As 50ppm group showed congestion, inflammation, and alveolar wall degeneration.

Conclusions

In conclusion, our study results reveal that chronic arsenic exposure through the drinking water produced lung injury, and HFD significantly enhanced it in mice. This effect may be explained by the reduced antioxidant activities and/or increased oxidative stress in the mice lung tissue. These findings could be important for a clinical intervention to protect against or prevent arsenic-induced respiratory damages in humans.

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