Inorganic arsenic is found in soil, air or water. In recent years, the level of heavy metals, particularly arsenic, has increased in the environment due to natural sources, industrial processes and agriculture usage [43, 44]. Arsenic pollution is a serious problem in developing countries, such as in West Bengal (India), Bangladesh and the Red River delta of Vietnam, and is also a chronic problem in China and Thailand. Arsenic has been known to induce toxic effects on various systems or apparatuses. Furthermore, a previous study showed that arsenic-induced accumulation phenomena resulted in an increase in arsenic concentration in tissues [22]. Besides its implication in the heart or nervous system, there is a risk that arsenic intoxication may lead to damage of the respiratory system [2]. The lung is one of the major target organs of arsenic, either from contaminated air or from polluted water, and leads to the development of bronchiectasis, bronchitis, chronic obstructive pulmonary disease and malignancies [40]. Experimental studies have reported that consumption of drinking water contaminated with arsenic could cause lung cancer and nonmalignant illnesses in the pulmonary system [27]. Respiratory complications are found with lung cancer as a critical end point of arsenic toxicity. Numerous epidemiological studies have reported chronic cough, chronic obstructive pulmonary disease and interstitial lung disease as common respiratory complications among the affected population [31, 36]. There are studies documenting that arsenic affects the normal functioning of alveolar macrophages, resulting in pulmonary oxidative damage and production of proinflammatory cytokines [29].

Several reports have suggested that arsenic-mediated toxicity may be due to elevated production of intracellular reactive oxygen species (ROS) like superoxide anion, hydroxyl radical and hydrogen peroxide during the metabolic processing of arsenic [35, 46]. Disruption of the prooxidant/antioxidant balance can lead to tissue injury via the effects of oxidative stress on critical biomolecules, such as lipids, proteins and DNA [11]. The potential role of oxidative stress in the injuries associated with arsenic poisoning suggests that antioxidant strategies seem to be reasonable, which may enhance the efficacy of treatment protocols designed to mitigate arsenic-induced toxicity.

Resveratrol is a polyphenolic antioxidant mainly present in the skin of grapes, red wine, berries and peanuts, is partly responsible for the cardiovascular benefits associated with the “French Paradox” [7]. Over the years, this molecule has received considerable attention for its anti-inflammatory, anti-tumor [25] and antioxidant properties [9], as well as its ability to increase lifespan in lower organisms and improve general health in mammals [3]. Reports have demonstrated that resveratrol enhanced antioxidant activity, which increased resistance to oxidative injury in H9c2 cells [4]. A recent study showed that resveratrol had some antioxidative potential in the treatment of bleomycin-induced pulmonary fibrosis in rats [1]. In addition, oral administration of resveratrol efficiently reduced oxidative stress and maintained mitochondrial function [42].

The beneficial properties of resveratrol have been well
defined. However, to our knowledge, no study has ever been conducted in vivo with a single dose of trans-resveratrol in lung tissue exposed to arsenic trioxide (As$_2$O$_3$). Moreover, because of the extensive pulmonary dysfunctions induced by arsenic and the absence of effective treatments, nutritional supplementation approaches to arsenic-induced toxicity are an attentive possibility. Our findings maybe provide a better understanding of the action of resveratrol in modulating As$_2$O$_3$-induced oxidative damage and accumulation phenomena in the feline lung and provide a rationale for further clinical study of resveratrol used as a protective agent after As$_2$O$_3$ exposure.

MATERIALS AND METHODS

**Animals and treatment:** Since the pharmacokinetic profiles of drugs in cats and humans are similar, we selected cats for the present study [14]. The study was performed on twenty-four healthy Chinese Dragon Li cats of either sex (12 males, 12 females) maintained in an air-conditioned rooms at 21 ± 4˚C and kept in individual stainless steel cages (during experimental phases). Weights and ages ranged from 2.8 to 3.5 kg and 1.5 to 2 years, respectively. The housing and experimental facilities at the Northeast Agricultural University were approved by the Chinese Ministry of Agriculture, and animal care and conduct of the study were performed in accordance with the Ethics Committee for Animal Experiments (Northeast Agricultural University, Harbin, China).

Twenty-four cats were randomly divided into 4 groups with 6 animals each: the control, As$_2$O$_3$-treated, resveratrol + As$_2$O$_3$-treated and resveratrol-treated groups. All treatments were given via the foreleg vein on alternate days for 3 days (i.e., days 1, 3 and 5) with measurements made on the 6th day. Resveratrol (Sigma-Aldrich, St. Louis, MO, U.S.A.) (100 mg) was dissolved with a mixture of dehydrated alcohol injection (5 mL) and physiological (0.9%) saline solution (5 mL), and the final concentration was 10 mg/mL. The single dose of resveratrol and arsenic we selected were based on a previously published dosage regimen that was shown to be biologically and therapeutically active in experimental systems. In the control group, cats were injected with physiological (0.9%) saline (1 mL/kg); in the As$_2$O$_3$-treated group, cats were treated with As$_2$O$_3$ parenteral solution (1 mg/mL) (Yida Pharmaceutical Co., Ltd., Harbin, China); in the resveratrol + As$_2$O$_3$-treated group, cats were given resveratrol (3 mg/kg) 1 hr before As$_2$O$_3$ (1 mg/kg) administration. The resveratrol-treated group received resveratrol alone (3 mg/kg). Twenty-four hr after the last injection, all cats were euthanized by an overdose of pentobarbital sodium (100 mg/kg). Lung sections were collected and used for further experimentation.

**Biochemical determination:** Lung tissues were rapidly excised and homogenized in phosphate-buffered saline (pH 7.4) using an Ultra-Thurax T25 homogenizer. After centrifugation at 10,000 x g for 10 min at 4˚C, the supernatant was used for biochemical determination. Superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase activities and malondialdehyde (MDA) levels were measured with assay kits (Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturers’ instructions, respectively. The levels of total glutathione (GSH) and glutathione disulfide (GSSG) content in the supernatant of lung tissue were measured using total GSH and GSSG kits (Jiancheng Bioengineering Institute, Nanjing, China) as described in the manufacturer’s instructions, and the ratio was calculated as follows: ratio=GSH$_{\text{total}}$/2GSSG/GSSG.

**Determination of ROS levels in the lung:** The amount of ROS in the lung was measured using 2’,7’-dichlorofluorescin diacetate, which is converted into highly fluorescent dichlorofluorescin by cellular peroxides (including hydrogen peroxide). The assay was performed as described in the literature [37]. Fluorescence was determined excitation and emission wavelengths of at 488 nm and 525 nm, respectively, using a fluorescence plate reader (LS-55, Perkin-Elmer, Buckinghamshire, U.K.).

**Measurement of 8-hydroxy-2’-deoxyguanosine (8-OHdG) in lung tissues:** Lung tissues were homogenized in a medium consisting of mannitol (0.24 mol/l), sucrose (0.06 mol/l), EDTA (50 µmol/l) and Tris-HCl (5 mmol/l, pH 7.2). The homogenate was lysed by adding 1% sodium dodecyl sulfate (SDS) and proteinase K (2.5 mg/m) for incubation at 55˚C for 20 min. The level of 8-OHdG in the DNA was determined as described previously [39]. Briefly, DNA was heat denatured and then digested sequentially with nuclease P1 (Seikagaku Corporation, Tokyo, Japan) and alkaline phosphatase. The generated 8-OHdG was determined using an electrochemical detector (ECD, Coulorchem II; ESA, Chelmsford, MA, U.S.A.); the method was combined with previously described [12] high-performance liquid chromatography (IRICA, Kyoto, Japan).

**Lung tissue for histopathology:** Lung tissues from cats were fixed in 10% formaldehyde. Fixed tissues were trimmed, embedded in paraffin, sectioned into 2-µm sections and stained with hematoxylin and eosin (H&E). Morphological examination was conducted under a light microscope (BX-FM; Olympus Corp, Tokyo, Japan).

**Determination of total arsenic in the lung tissues:** Arsenic contents in the lung were analyzed following the method of Cui et al. [6]. Briefly, approximately 0.5 g lung tissue sample was digested with a mixture of HNO$_3$–HClO$_4$ solution (ratio 1.3 v/v) for 2 days at 130˚C. After HNO$_3$ was removed by evaporation, digested samples were diluted with deionized water. Arsenic concentrations were measured by atomic fluorescence spectrometry (AFS) (Beijing Jitian Instrument Co., Ltd., Beijing, China).

**Statistical analysis:** All values were presented as means ± SEM, and statistically significant differences (P<0.05) and extremely statistically significant differences (P<0.01) were determined among the various groups by ANOVA and Tukey’s test using the SPSS 12.0 statistical software.

RESULTS

**General health condition:** Cats in the As$_2$O$_3$-treated group showed symptoms of severe diarrhea, drowsiness, vomiting, skin pruritus and hyperpigmentation compared with the
control group. The cats treated with resveratrol along with As$_2$O$_3$ only showed slight diarrhea and vomiting compared with the group exposed to As$_2$O$_3$ alone.

Effect of resveratrol on body weight changes: The body weight changes of different groups are shown in Table 1. There were statistical differences between the growth rates of all the treatment-related groups and the control group. A significant ($P<0.01$) decrease in final body weight was observed in the As$_2$O$_3$-treated group as compared with the control. Treatment with resveratrol along with As$_2$O$_3$ significantly ($P<0.01$) protected against the decrease in body weight. Resveratrol-treated groups maintained normal body weights compared with the control, and this suggested that resveratrol had practically no adverse effect on the feline growth response.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight changes (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>110.2 ± 15.9</td>
</tr>
<tr>
<td>As$_2$O$_3$</td>
<td>-19 ± 16.7*</td>
</tr>
<tr>
<td>As$_2$O$_3$ + Rev</td>
<td>40.2 ± 16.8#</td>
</tr>
<tr>
<td>Rev</td>
<td>39.8 ± 6.3</td>
</tr>
</tbody>
</table>

Values are means ± SEM. * $P<0.01$ vs control group; # $P<0.01$ vs As$_2$O$_3$-treated group.

Effect of resveratrol on oxidative stress variables: The status of ROS, MDA and 8-OHdG in control and treated animals is presented in Fig. 1. As shown in Fig. 1a, As$_2$O$_3$ treatment at a dose of 1 mg/kg body weight caused a significant ($P<0.05$) increase in ROS levels compared with the control group in lung tissues. Administration of resveratrol along with As$_2$O$_3$ antagonized the toxic effects of As$_2$O$_3$ and significantly ($P<0.05$) decreased the ROS levels compared with the group exposed to As$_2$O$_3$ alone.

The production of MDA in the As$_2$O$_3$-treated group was significantly higher than that in the control group ($P<0.05$) (Fig. 1b). However, pretreatment with resveratrol caused a significant decrease ($P<0.05$) in the levels of MDA compared with those seen in the As$_2$O$_3$-treated group.

Additionally, the levels of 8-OHdG in the control and experimental groups are shown in Fig. 1c. A significant ($P<0.05$) increase in the 8-OHdG level of the As$_2$O$_3$-treated group was found compared with the controls. Supplementation of resveratrol resulted in significant suppression of 8-OHdG production.

Effect of resveratrol on pulmonary histology: Identification and alteration of histopathological changes during oxidative damage and the protective effect of resveratrol against As$_2$O$_3$-induced toxicity are depicted in Fig. 2. Histopathological examination in the control group showed a normal structure of the lung (Fig. 2a). Serious alveolar collapse with focal inflammation was observed in the As$_2$O$_3$-treated group.
The lung sections from the resveratrol + As2O3-treated group showed good protection against the damage, with a slightly thickened alveolar septa and mild alveolar collapse (Fig. 2c). Resveratrol-treated cats also had normal morphology (data not shown).

Effect of resveratrol on antioxidant enzymes: As shown in Fig. 3, the activities of SOD, GPx and catalase in the As2O3-treated group were reduced compared with those in the control group. However, pretreatment with resveratrol caused a significant increase ($P<0.05$) in their activities compared with those seen in the As2O3-treated group.

Effect of resveratrol on GSH level in the feline lung: The contents of GSH in the control and experimental groups are shown in Fig. 4a. Significant ($P<0.05$) arsenic-induced depletion of GSH was observed in the As2O3-treated group compared with the control. Simultaneous supplementation of resveratrol along with arsenic in the resveratrol + As2O3-treated group significantly ($P<0.05$) elevated the levels of GSH compared with the As2O3-treated group.

Effect of resveratrol on GSH/GSSG ratio: The glutathione redox ratios of lung tissues from different groups are shown in Fig. 4b. The glutathione redox ratio of the As2O3-treated group was significantly decreased ($P<0.05$) compared with the control group. However, pretreatment with resveratrol reversed the glutathione redox status of lung tissues ($P<0.05$).

Effect of resveratrol on arsenic accumulation in the feline lung: As shown in Fig. 5, exposure to arsenic resulted in a significant increase ($P<0.01$) in the arsenic concentration of the lung compared with the control group. However, pretreatment with resveratrol significantly attenuated ($P<0.01$) arsenic accumulation in the lung compared with the As2O3-treated group.

**DISCUSSION**

Arsenic exerts its toxic effects due to its direct binding with –SH groups or indirectly through generation of ROS. ROS had a role in the major stages of carcinogenesis by directly damaging DNA and other macromolecules [18]. Recently, some researchers clearly confirmed that the generation of ROS is specifically induced in mitochondria by evaluating the effects monomethylarsonious acid, an intermediate metabolite of arsenic [26]. Naranmandura et al. [26] also showed that arsenic metabolism induced ROS generation specifically through inhibition of the activities of complexes in mitochondria. While Zheng et al. [47] reported
that resveratrol had an important role in improving the activity of mitochondrial complexes, which was verified by an inhibitory effect of resveratrol on imbalance of the levels of ROS and antioxidants systems.

The antioxidant systems that act to detoxify ROS are enzymatic (SOD, GPx and catalase) and nonenzymatic (glutathione, thioredoxin, vitamins, etc.). In physiological conditions, there is a balance between free-radical sources and antioxidant systems. Resveratrol has been identified as a peroxisome proliferator-activated receptor activator that could increase the activity of antioxidant enzymes [30]. A fourteen-fold increase induced by resveratrol in the action of MnSOD (SOD2) was found by Robb et al. [33]. Zheng et al. [47] reported that resveratrol markedly ameliorated the As$_2$O$_3$-induced decrease in the activity of antioxidant enzymes in the heart.

Among the systems that limit oxidative stress, glutathione is a cofactor of several enzymes and plays an impor-
tangent role in the regulation of cell redox reactions as well as detoxification. In the present study, we found that after \( As_2O_3 \) treatment, cellular glutathione was depleted significantly compared with the control group, whereas resveratrol treatment attenuated this depletion. Moreover, glutathione depletion has been reported to increase sensitivity to \( As_2O_3 \)-induced oxidative stress in cells [15]. Resveratrol has been shown to preserve antioxidant defense by preventing depletion of GSH [10, 16]. In this study, resveratrol attenuated \( As_2O_3 \)-induced oxidative stress, and this might be mediated (at least in part) by preserving levels of antioxidant enzymes and glutathione in tissues.

GSH/GSSG is a most important and commonly measured redox couple used to obtain an estimate of cellular redox state because it is found at high levels in cells, and it is important in determining the redox status of cells [15]. The GSH/GSSG ratio is maintained by GSSG reductase, which uses the reducing power of NADPH to convert GSSG to GSH. The GSH/GSSG ratio is thus ultimately tied to NADPH levels, which are determined by the energy status of the cell. Resveratrol has been found to increase the expression and activity of the phase II enzyme NAD (P)H:quinone reductase in cultured cells [45]. In addition, resveratrol has also been shown to preserve antioxidant defense by preventing depletion of total glutathione and GSH [8, 16], which have caused the increase in ratio of GSH/GSSG in this study. Taken together, these data suggested that resveratrol may attenuate \( As_2O_3 \)-induced imbalance of antioxidant systems and ROS.

In the case of oxidative stress leading to imbalance, an excess of free radicals can oxidize lipids, DNA, and proteins and can subsequently cause cellular and organ injuries. The end product of lipid peroxidation (MDA), which is known as a biomarker, can be used to monitor the oxidative damage. Studies by Ramos et al. [32] demonstrated a tendency for a positive correlation between arsenic concentration and lipid peroxidation level in the liver, kidney and heart of rats following acute exposure to arsenic. The present results revealed an increase in the formation of MDA in the \( As_2O_3 \)-treated group, which is in agreement with the findings reported previously [17]. Arsenic-induced MDA production could be due to impairment of the natural protective system of cells and could be directly related to the GSH depletion. Franco et al. [13] reported that animals treated with resveratrol had lower lipid peroxidation and higher protein and mRNA expression of SIRT1. There is a study indicating that resveratrol inhibits its lipid peroxidation mainly by scavenging lipid peroxyl radicals within the membrane, like alpha-tocopherol [38]. Because oxidative stress is likely to be a potential stimulus for inducing cellular and molecular damage events, we investigated 8-OHdG generation, the predominant marker of oxidative damage in DNA. It is widely thought that continuous oxidative damage to DNA is a significant contributor to the major cancers, such as those of the lung, breast and prostate [19]. In the present study, the \( As_2O_3 \) treatment increased the oxidative stress and the 8-OHdG level. In the study of Kubota et al. [21], resveratrol pretreatment led to significant suppression of 8-OHdG generation at 3 hr after endotoxin-induced uveitis induction. Sener et al. [34] proposed that the changes in MDA and 8-OHdG levels could be the result of suppression of oxidative stress by resveratrol’s antioxidant nature (free radical scavenging properties).

The results of histopathology suggested that the arsenic damaged the normal architecture of the lung (Fig. 2). The lung histological and pathological alterations seem to be correlated with other biochemical results in the present study. The protective effect might result from the ability of resveratrol to inhibit the production of ROS and oxidative stress.

Though short-term arsenic administrations in the present study could not directly show tumor progression, the oxidative damage induced by arsenic plays a role (at least partly) in the carcinogenesis of arsenic [20], which is similar in short-term and long-term exposure. The cellular and biochemical advantages of resveratrol’s chemopreventive activity, including inhibition of cyclooxygenase 1 activity and/or reduction of cyclooxygenase 2 at the mRNA level [24] and regulation of the ornithine decarboxylase levels in normal tissue in vivo [19, 24], significantly reduce the risk of developing many cancers [28]. The above findings imply that resveratrol could reduce, at least partly, the risk of carcinogenesis in short-term or long-term arsenic exposure.

Another interesting finding in the present study was the ability of resveratrol to reduce arsenic burden in the target tissues, and this may play a key role in the protective effect of resveratrol against \( As_2O_3 \)-induced toxicity. In several instances, protection against arsenic in human cells has been shown to be associated with GSH-dependent arsenic efflux from the cell [23]. Depleting GSH has been reported to increase sensitivity to arsenic and its accumulation in mammalian cells [5]. To explain our findings, we hypothesize that GSH (which helps in the efflux of arsenic) may be over expressed in cells after pretreatment with resveratrol, as suggested by the concentration of GSH in Fig. 4a [16]. In addition, it has also been reported resveratrol possesses low-affinity chelating properties for metals, which might cause the reduction in lung arsenic levels.

In conclusion, the present study demonstrated that treatment with resveratrol at a dose of 3 mg/kg when arsenic is administered at the same time suppresses, at least in part,
the oxidative stress and lung injury induced by arsenic exposure in cats. Therefore, further studies are needed to investigate the effect of different doses of resveratrol and the effect of systemic administration. On the other hand, taking into consideration the safety profile of resveratrol in other studies [41], we plan to try treatment with resveratrol as a supplementary therapy for clinical cases of arsenic exposure in a future study.

ACKNOWLEDGMENT. This study was supported by the National Natural Science Foundation Committee of China (31101868), the Special Foundation of Postdoctoral Science Foundation of China (2012T50302), Heilongjiang Provincial Foundation for Young Scholars (QC2010057), Chinese Postdoctoral Science Foundation (20100481040), and Program for New Century Excellent Talents in Heilongjiang Provincial University (1253-NCET-007).

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