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Original Article

Protective Effects of Grape Seed Extract (GSE) against the Respiratory System following Formalin Exposure on Balb/c Mice

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Abstract

Background and Aim: As it has been reported in previous studies, the prevalence of respiratory distress is related to a variety of environmental factors. Among them, formaldehyde exposure also irritates the respiratory tract and augments the risk of airway diseases. The aims of this study was to determine the protective impacts of grape seed extract against (GSE) following formaldehyde exposure on Balb/c mice.

Materials and Methods: Twenty-four adult Balb/c mice were divided into the following groups: 1-control (Ctrl) group, 2-formaldehyde exposure (FOR) group, 3-formaldehyde exposure and grape seed extract treatment (FOR+GSE) group and 4- grape seed extract treatment (GSE) group. The mice in groups 3 and 4 received 200 mg/kg GSE intraperitoneally and the mice in groups 2 and 3 were exposed to 3 mg/m3 formalin from 8 a.m. to 3 p.m., 7 h/day, 6 days/week, for 4 weeks. At the end of experimental periods, the tissue samples were removed from the trachea and the lungs and immersed in 10% formalin to be prepared for histological examination. Finally, the level of malondialdehyde (MDA) was evaluated in the plasma of the blood samples.

Results: Data analysis showed that formaldehyde exposure could significantly reduce the weight of the mice *($p<0.05$)*. Moreover, formaldehyde caused epithelial metaplasia and edema of sub-epithelial space in the trachea. The wall of blood vessels was thickened. However, GSE consumption moderated some of formaldehyde effects. Treatment with GSE significantly reduced the MDA level $p<0.05$.

Conclusion: GSE could reduce the marker of oxidative stress and stimulate antioxidant defenses against formaldehyde inhalation.

Keywords: Formaldehyde, Grape seed extract, Malondialdehyde, Mice

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Introduction

Prevalence of respiratory diseases is related mainly to environmental factors. Among them formaldehyde causes airway inflammation and bronchial irritation (1). Formalin is a mummifying fluid which is used in anatomy laboratories for cadaver fixation. United States Environmental Protection Agency confirmed that employees in pathology, anatomy, and histology laboratories are at remarkably greater risk of cancer than others (2). Researches showed that formaldehyde is the smallest and most reactive aldehyde that could induce wide effects in malignancy progression,

particularly in the respiratory airway (3). Although the formaldehyde lesions may occur directly through eyes or skin, inhalation is the principal source of formaldehyde exposure. Formaldehyde was absorbed in the upper respiratory tract and rapidly broken to small particles (4). Formaldehyde is one of the primary substances widely used in various products, and nearly all tissues in the human body catalyze it to fewer toxic particles (5). Formaldehyde irritated the upper respiratory airways and augmented the risk of squamous-cell carcinomas of the nasal cavities and nasopharyngeal cancers (6). Researcher have indicated that formaldehyde cause destructive effects on ventilation, alimentary, and genital as well as nervous systems (7). Formaldehyde affects people from multiple sources such as cigarette smoking, cloth or wood production, and construction materials (5), and occasionally causes contact dermatitis. Some researchers have shown that formaldehyde exposure causes general toxicity and carcinogenicity in rats. Furthermore, formaldehyde inhalation induces squamous cell carcinomas in the nasal epithelium of rats and mice (8).

Herbal medicines have traditionally been used in the treatment of various diseases. Thus, with the development of extraction methods, components and extracts of plants have been isolated and antioxidant effects of some of them have been proven (9). For instance, neuroprotective impacts of polyphenol-rich pomegranate juice intake in pregnancies on infants with intrauterine growth have been proven (10).

Evidence from previous studies on mice suggests that grape seed extract could ameliorate endothelial dysfunction and reduce blood pressure in pregnant mice also it recover cardiovascular and kidney remodeling indexes against oxidative stress (11). It has been indicated that oxidative stress modulates acute injury in the respiratory system and progresses chronic scarring in bronchopulmonary structure (12). Moreover, antineoplastic effects of grape seed extract inhibited the proliferation of cell lines in adenocarcinoma and small cell lung cancer (13). Hence, grape seed extract has been shown to cause protective effects on some organs that have been exposed to formalin. Hence, the present study was designed to investigate the protective impacts of grape seed extract on respiratory system toxicity following formaldehyde inhalation in Balb/c mice.

Materials and Methods

This study was designed to assess the effects of formaldehyde exposure on plasma oxidative stress index (Malondialdehyde) and histopathological changes of the respiratory tract as well as to examine the protective effects of GSE against formaldehyde.

Grape seed extract (Vitis Vinifera) capsule was purchased from Sigma-Aldrich (Darmstadt, Germany), and formaldehyde was purchased from Kimia Tehran Acid (Tehran, Islamic Republic of Iran). Malondialdehyde kits were purchased from Kushan Zist (Tehran, Islamic Republic of Iran) and the Balb/c mice of this study were purchased from the animal room of North Khorasan University of Medical Sciences.

To perform this study, 24 adult Balb/c mice were used. The mice were approved by the Animal Ethics Committee of North Khorasan University of Medical Sciences with number IR.NKUMS.REC.1398. 124. These animals were divided into 4 groups with six mice in each group: 1: control (Ctrl) group, 2: formaldehyde exposure (FOR) group, 3: formaldehyde plus grape seed extract (FOR+GSE) group, 4: grape seed extract (GSE) group. The mice in groups 3 and 4 received 200 mg/kg GSE (14) and mice in the animals in groups 2 and 3were exposed to 3 mg/m^3 formalin vapor from 8 a.m. to 3 p.m., 7 h/day, 6 days/week, for 4 weeks in separate glass chambers. All the mice were allowed to have free access to food and water, and were housed under a constant 12h light/dark cycle at 21–23°C.

At the end of the experimental periods, the mice in all the groups were sacrificed by cervical dislocation and the sample tissue from the trachea and lungs were immersed in 10% neutral buffered formalin for 48 hours. Then, the specimen's sections from each group were routinely stained with H & E.

The Malondialdehyde (MDA) level was measured in blood samples to evaluated the oxidative stress in plasma. For this purpose, the blood samples were collected into micro tubes and quickly centrifuged at 5,000 rpm for 5 min. Plasma was stored at −80°C and the assay was carried out according to the manufacturer's instructions, and results were expressed in µM.

Statistical analysis of the data was conducted by version

20 of SPSS software. One-way ANOVAs with post-hoc Tukey tests were utilized in order to make a comparison between the groups. P values P<0.05 was considered significant. The data were expressed as Means ± SD.

Results and Discussion

The results of this study showed that formaldehyde exposure could significantly decrease the weight of female mice $P < 0.01$. However, data analysis did not reveal any difference in body weight of the mice that received GSE compared with the control group. Interestingly, with regard to the male mice that received formalin + GSE, no significant alterations were observed in the weight of the mice compared with the control group (Fig 1).

Histological findings of trachea showed that formaldehyde could cause epithelial metaplasia. Furthermore, vascular congestion and rare oedema were observed in the subepithelial space. However, hyperchromatic nuclei were observed in tracheal epithelium (Fig 2).

Analysis of slides after hematoxylin & eosin staining showed that formaldehyde inhalation could cause histological changes in the upper part of the respiratory tract in both male and female mice. Moreover, paraffin sections revealed that formaldehyde could cause epithelium of nasal cavity loses their cilia, and cell metaplasia was observed in female section.

Moreover, there were some extent congestion and moderate subepithelial edema in nasal cavity and pharynx of the mice in the FOR group but hyperchromatic nuclei were observed in laryngeal epithelium. However, formaldehyde inhalation reduced the mucosal folds of nasal cavity and thickened the blood vessel walls. Depending on the location, epithelium proliferation was lower in the FOR group than other groups. Cell proliferation of the GSE+FOR group increased significantly compared with the FOR group. Also, induction of cellular metaplasia was not observed in the upper part of the respiratory tract in the FOR+ GSE group.

According to the results of this study, male mice were more resistant to formaldehyde because female mice had severe weight loss compared with male mice. Following formaldehyde exposure, evidence of bulla spaces was observed in the lung tissue due to rupture of inter alveolar septum (Fig 3). Walls of blood vessels were thickened, and numerous cellularity were observed in the alveolar wall, that leads to wall thickening (Fig 3). Our findings showed that histopathological changes were more severe in the upper part of the respiratory tract than the lower part (Fig 2).

The present study showed that GSE consumption could moderate formaldehyde effects. Our findings also indicated that there were some differences in the

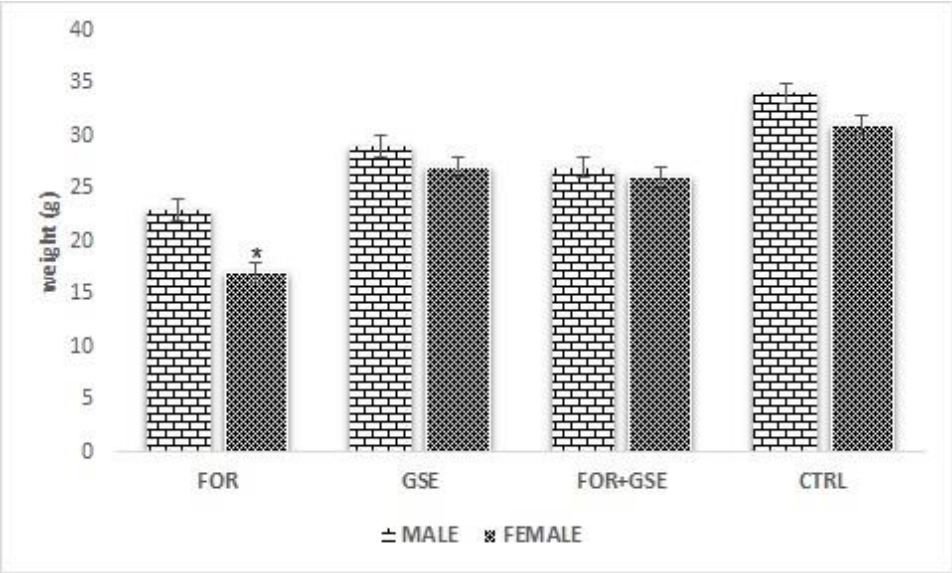

Figure 1. Impacts of formaldehyde exposure on weight change (g) of the male and female mice. The data have been shown as mean ± SE, *p<0.05, compared with the control group (CTRL), formaldehyde group (FOR), GSE group, and FOR+GSE group.

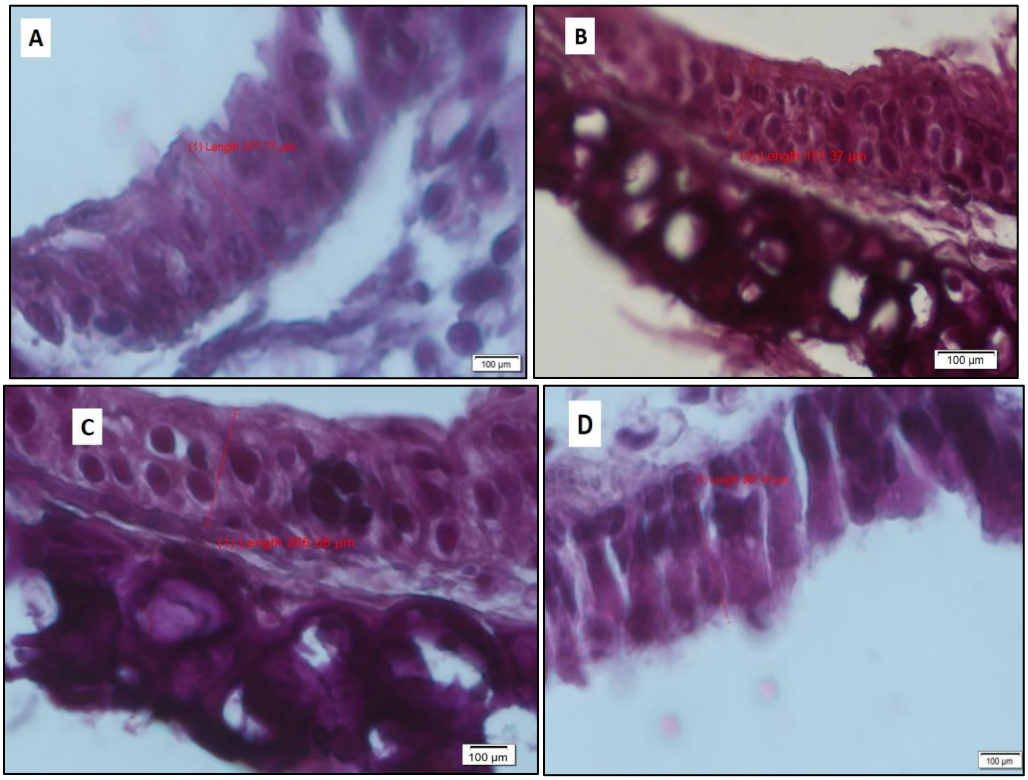

Figure 2: H&E staining of the trachea tissue sections of the BALB/c mice in the experimental groups (Image magnification: ×200; scale bar = 100 µm). A) the control group, B) formaldehyde group, C) FOR+GSE group and D) GSE group. Image analysis showed that epithelium high decreased in the formaldehyde group. However, no significant histologic differences were observed between A and D. Image C showed that GSE was able to protect the epithelium

microscopic observation of male and female respiratory systems such as lumen diameter and muscle bulk of airways (Fig 2,3).

MDA concentrations in plasma were significantly increased in the formaldehyde group compared with the control $P < 0.05$ (Fig 4). However, treatment with GSE significantly affected the marker of lipid peroxidation, and reduced the MDA level ($P < 0.01$). Interestingly, this value did not change in the male mice. The results of this study revealed that antioxidant effects of GSE were improved and the level of MDA significantly decreased in plasma of the GSE and GSE+FOR groups compared with the FOR group $P < 0.01$ (Fig 4) . Our data demonstrated that GSE could decrease oxidative stress and stimulate antioxidant defenses against formaldehyde exposure.

The results of this study confirmed previous findings concerning local cytotoxic effects in the upper part of the respiratory tract after formaldehyde inhalation. In the present study, the antioxidant effects of GSE on formaldehyde inhalation in the respiratory system were reported. We demonstrated that GSE was able to inhibit some deleterious effects of formaldehyde on the respiratory tract. Our results showed that formaldehyde inhalation decreased the weight of female mice. However, plasma levels of MDA increased in the mice that inhaled formaldehyde, and its concentrations substantially were reduced by GSE.

Although many biological studies have reported potential effects of GSE to improve human health, there is little information about health benefits of GSE on DNA, protein damage, and enzyme inhibitory effects (15). However, the scavenging activity of GSE was attributed to the presence of the phenolic group. The phenolic group was advantageous in the protection and treatment of free radical related diseases (16). Evidence from previous studies showed that GSE could have natural antioxidant effects with a great potential for

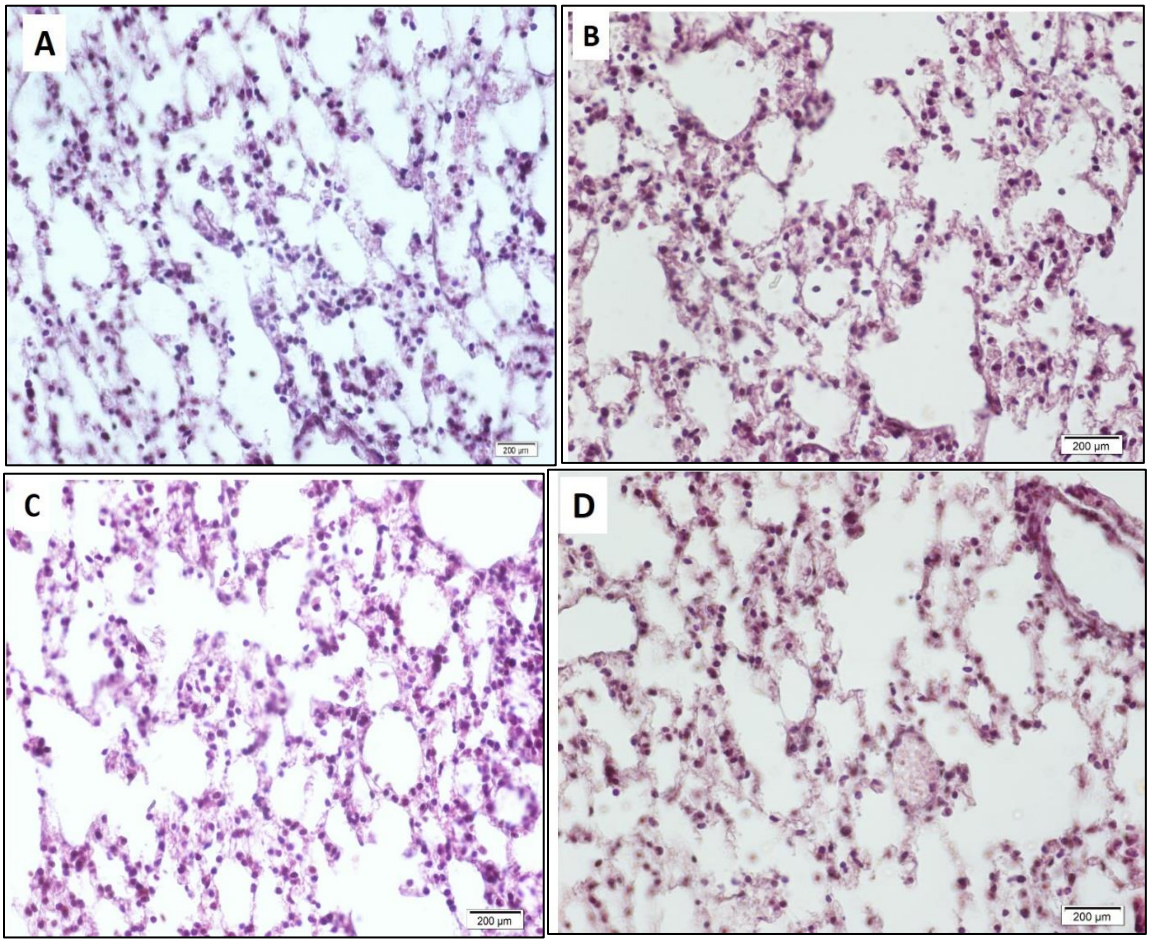

Figure 3: H&E staining of the lung tissue sections of the BALB/c mice in the experimental groups (Image magnification: ×200; scale bar = 100 µm). A) The control, B) FOR+GSE group, C) FOR group and D) GSE group. Image analysis showed that inter alveolar septum decreased in B and C. However, no significant histologic differences were observed between A and D

protection against oxidative stress which could protect the endothelial function and decrease blood pressure (17). Moreover, some researchers have confirmed that GSE might have therapeutic effects on bronchoconstriction disease via signaling pathways (18). It has been demonstrated that GSE intoxication on lung injury arises from the stimulation of the AMPK/Nrf2/p62 signaling pathway. Furthermore, GSE could reduce the release of inflammatory factors in addition to the inhibition of apoptosis induction in the lungs (19). According to the results of this study, formaldehyde could increase the MDA level in plasma, which is in agreement with results of previous studies that reported significantly raised levels of MDA in mice caused by formaldehyde (20). It might be due to free radical damage or lacking endogenous antioxidant status that free radicals can attack cell membranes and allow cell contents release in blood and generate MDA as an intermediate product that depends on the severity of membrane damage. *In vitro and in vivo* studies have indicated that scavenging activity of GSE provides greater protection against lipid peroxidation and DNA fragmentation compared with vitamins in hepatic and brain tissues of mouse models (21).

A key process in the pathogenesis of pulmonary fibrosis is alveolar apoptosis mediated by oxidative stress, and grape seed extract inhibits the pulmonary fibrosis

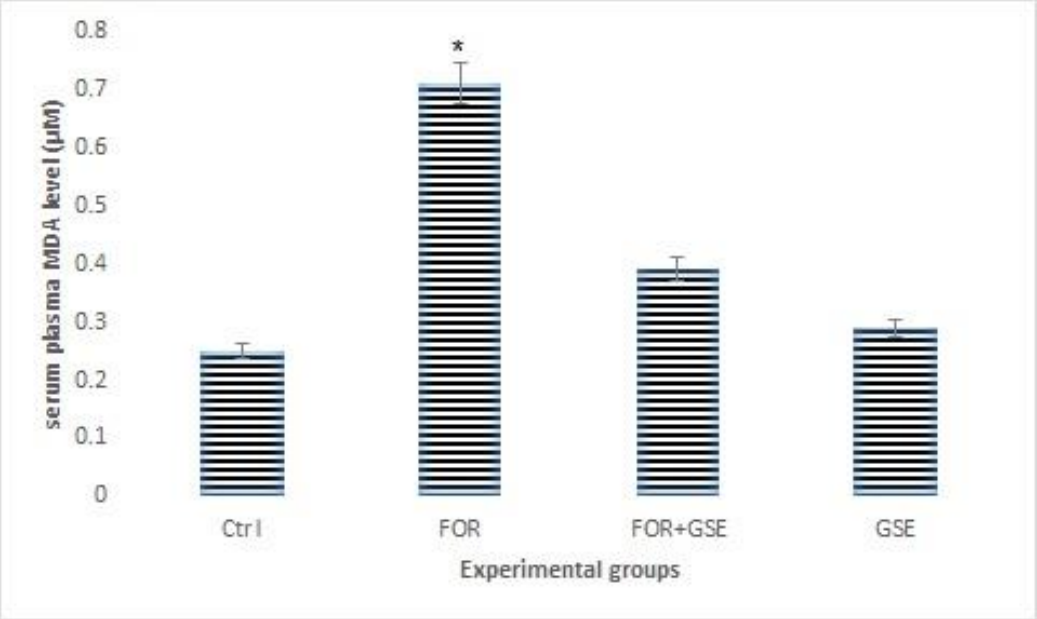

Figure 4. Impacts of formaldehyde exposure on weight change (g) of the male and female mice. The data have been shown as mean ± SE, *p<0.05, compared with the control group (CTRL), formaldehyde group (FOR), GSE group, and FOR+GSE group.

through the inhibition of oxidative stress and collagen deposition in lungs. GSE also reduces the expression of IL-6. Furthermore, GSE attenuates mitochondrial apoptosis through decreasing the Bax/Bcl-2 ratio (22). An association has been made between the excessive production of free radicals with the pathophysiology of a variety of complications. In agreement with recently conducted investigations in mouse models (11), it was found that circulating concentrations of MDA were increased in the plasma of mice that inhaled formaldehyde compared with the control groups. However, our results showed that supplementation with GSE noticeably decreased this marker of lipid peroxidation. Previous studies have confirmed that reactive oxygen species have a significant role in the progress of lung injury. MDA is the main metabolite product of lipid peroxide and is one of the biomarkers of oxidative stress. Moreover, GSE is able to protect the lungs against oxidative stress injury (23).

Conclusion

It can be stated that histopathological alterations were more noticeable in the mice exposed to formaldehyde than the mice exposed to the composition of formaldehyde and GSE with a greater impact on the upper part of the respiratory system than the lower one. In any case, formaldehyde may increase the risk of upper respiratory system cancer, particularly in female mice.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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