Original Article

Oxidative stress indices in rats' lung tissues following simultaneous exposure to noise and styrene

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Abstract

Received: April 2017, **Accepted**: May 2017

Background: Simultaneous exposure to noise and organic solvents such as styrene is an indispensable part of today's industries. Numerous studies were done related to ototoxic effects of co-exposure to noise and styrene while some evidences showed the adverse effects of such exposure on other organs. In this study, we aimed to assess the subacute effects of combined exposure to noise and styrene on rats' lung tissue.

Materials and Methods: Twenty-four Wistar male rats were divided in four groups including: A) control, B) rats exposed to 100 dB octave band of noise, C) rats exposed to 750 ppm styrene alone, and D) rats exposed to combination of 100 dB noise and 750 ppm styrene. Following the last day of exposure, the rats were euthanized and their lung tissues were excised, homogenized and assayed for biological analysis of malondialdehyde (MDA), glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD). The results were analyzed using SPSS software.

Results: MDA increased significantly ($P < 0.001$) at the end of experiment in the group exposed to styrene and noise-styrene. GSH concentrations decreased significantly in styrene and noise-styrene group ($P < 0.001$). SOD ($P < 0.001$) and CAT ($P < 0.05$) activities were determined to be significantly lower for the styrene and noise-styrene groups compared to the control group.

Conclusions: In conclusion, our results indicated that exposure to combination of noise and styrene caused oxidative stress, increased lipid peroxidation, and antioxidant depletion. These results appear to support the fact that co-exposure to noise and styrene might cause oxidative stress-induced damage to the lung tissue. Since simultaneous exposure to noise and styrene has an additive effect in this regard, further studies are necessary to be carried out on the effects of noise and organic solvents co-exposure.

Keywords: Noise, Styrene, Oxidative Stress, Rat

Introduction

Styrene is an important volatile industrial chemical, which can also be found in urban atmosphere and indoor air. This substance was classified by the International Agency for Research on Cancer (IARC) as a possible human carcinogen (group 2B) (1). Styrene is widely utilized for a number of purposes with significant human exposure, especially reinforced plastics industry, plastic packaging, rubber, and resins (2, 3). Styrene exposure seems to be associated with hypersensitivity pneumonitis, occupational asthma, and diffuse cell damage involving the tracheal, bronchiolar, and alveolar epithelium (4). In rodents, styrene causes both liver and lung damage (5, 6). According to the literatures, reactive oxygen species (ROS) formation is the most known cause of styrene toxicity (7, 8). Free radicals, which contain one or more unpaired electrons, include ROS and reactive nitrogen species (9). They are essential for cellular life processes; however, in excess they

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damage cellular lipids, proteins, and DNA (10).

Exposure to noise brings about many health problems such as hearing loss, sleep disturbance as well as hypertension and blood pressure (11). Its damaging effects, particularly the productions of free radicals are not limited to the auditory organ (12). Noise exposure exceeding more than 90 dB has been deemed to be source of oxidative stress (10). Noise exposure firstly increases levels of ROS such as superoxide radicals, hydroxyl radicals and hydrogen peroxide (13). Long-term exposure to noise can produce excessive free radicals and reduce antioxidant status such as superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) (14). Malondialdehyde (MDA) which is an indicator of lipid peroxidation processes, increases during oxidative stress. It has been shown that workers are exposed to styrene vapor in workplace where noise pollution is also common (15).

The overall aim of this study was to assess the effects of styrene and noise, each alone and in combination, on biomarkers of oxidative stress in rats' lung tissue.

Material and Methods

Twenty-four Wistar male rats weighing 180-220 g (upon arrival) were obtained from Pasteur institution in Tehran, Iran. Rats were divided randomly into four groups of 6 rats and housed in standard polyurethane cages (40 cm × 25 cm × 15 cm) in a temperature-regulated (20 ± 2 °C) room and relative humidity of 40%-50% with routine 12-hour (12-h) light/dark (8 am to 8 pm) conditions. They had free access to water and food at all times. Average sound level in a typical vivarium room was 65 dB SPL (16). As for adaptation, the experiment began 10 days after housing. All animals received pre-exposure auditory function test (DPOAE). The exposure procedure started for all groups in 2016 from September 10 for one month.

Experimental groups: Group A (control): rats were placed in exposure chamber without being exposed to either styrene or noise for period of 6 hours/day 6 days/week in 4 consecutive weeks.

Group B: rats were exposed to 100 dB (A) noise for 6 hours/day 6 days/week during 4 consecutive weeks in exposure chamber.

Group C: rats were exposed to 750 ppm styrene for 6 hours/day 6 days/week during 4 consecutive weeks.

Group D: rats were simultaneously exposed to 750 ppm styrene and 100 dB (A) noise for 6 6 hours/day 6 days/week for 4 consecutive weeks.

Exposure system: Exposures process was conducted in a custom made reverberant chamber (Figure 1) with stainless steel structure and walls made of glass. The chamber was made in a dynamic flow basis with a continuous air flow through them. Rats were individually placed in stainless steel wire cages (20 cm × 20 cm × 18 cm) with wire-mesh floors. The cages were positioned symmetrically on shelf within the styrene exposure chambers.

For generation and maintaining of styrene concentration at 750 ppm (17) in exposure chamber, liquid styrene (above 99% purity CAS No.100-42-5 from Daejung Chemicals and Metals Co., LTD) was vaporized in an impinger with room air bubbled through it. The highly dense generated vapor was diluted with room air in a mixing vessel to achieve the desired concentration. Styrene concentration was continuously monitored with Miran 1A PhoCheck (Wilks Scientist Corp., USA). The temperature was maintained at 21 ± 1 °C and relative humidity at 50 ± 10% and was monitored by a digital thermometer and humidity meter.

Noise generation system: The Noise was generated on a computer by a Filtered Noise Generator software (Timo Esser's Audio software, version 1.2), recorded and played by the Cool Edit Pro software (Syntrillium Software Corporation, version 2.1), amplified by an audio amplifier (Pejvak Ava

Corporation, Model AP12), and delivered by loudspeakers (JBL GT6-6) located approximately 12 cm above the wire cages (Figure 1). The noise levels within the chambers were measured at the level of rats' ear (8 cm) in each cage using a sound level meter (Casella CEL 480). Overall, the noise levels varied less than ± 1 dB (100 ± 1 dB) (18) between the measuring points and the frequency distribution of the noise matched with high-pass white noise spectrum. Such spectrum (HPWN/8kHz) was selected to cause hearing losses where auditory sensitivity is the highest for the rats (19). The cages in the chambers were daily rotated to maintain the most equal exposure for all rats.

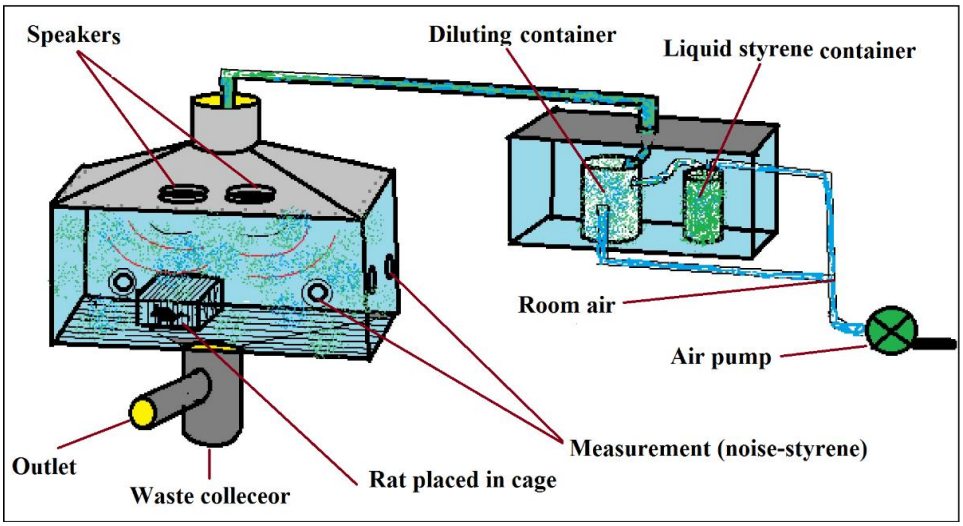

Figure 1: Exposure and mixing chambers

Biological analysis: Following last exposure on the 4th week, all the animals were euthanized after intraperitoneal (IP) injection of ketamine (40 mg/kg body weight) and xylazine (10 mg/kg body weight) (20). The lung tissues were homogenized for biological analysis. MDA as a secondary product of lipid peroxidation (LPO) , was estimated in the lung tissue utilizing the colorimetric reaction of thiobarbituric acid, the method described by Draper and Hadley (21). The amount of GSH as an essential antioxidant and free radical scavenger, was assessed according to the method of Sedlak and Lindsay (22). The activity of SOD enzyme was assayed using the method of Beauchamp and Fridovich (23). SOD is responsible for turning the superoxide anion (very reactive) into the less reactive hydrogen peroxide (H_2O_2) molecule. Furthermore, the hydrogen peroxide later is changed to H_2O by CAT enzyme (24). CAT activity was also measured according to the method of Aebi (25). All chemicals used in this study were purchased from Merck Company (Germany).

Statistical analysis: All values were expressed as mean ± standard deviation. Data were analyzed using one-way ANOVA by SPSS software (version 21.0, IBM Corporation, Armonk, NY, USA) followed by Tukey's HSD multiple range test and the differences below $P < 0.05$ were considered significant.

Results

The concentration of MDA (Figure 2) in lung tissue was significantly high in styrene plus noise-styrene ($P < 0.001$) compared to control. In spite of slight increase in the level of MDA in noise group, it was not statistically significant. Furthermore, the MDA level in noise-styrene group increased significantly compared to styrene group ($P < 0.050$ and $P < 0.001$, respectively).

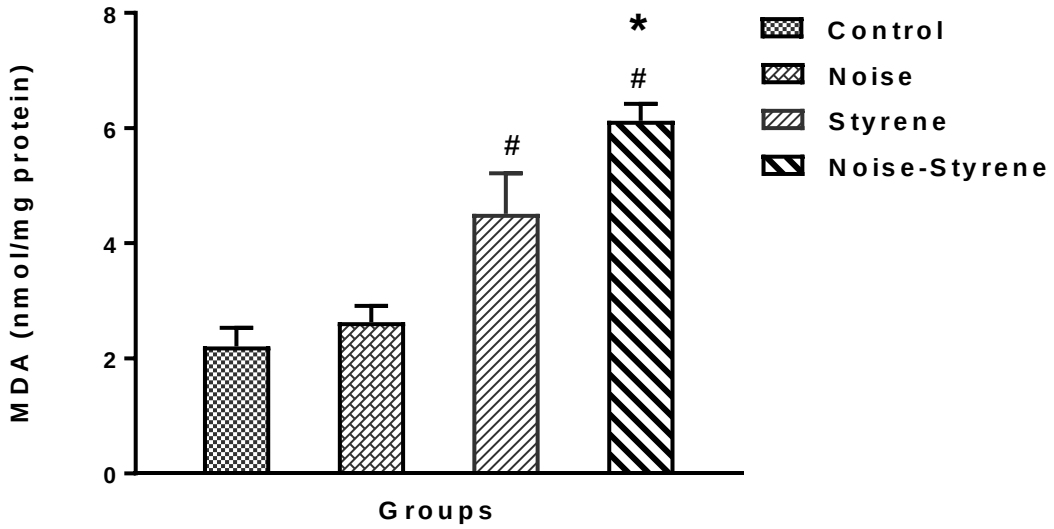

Figure 2: Lung tissue malondialdehyde (MDA) levels, each value represents the mean ± SD (n = 6), styrene and also noise-styrene groups were significantly higher than the control group (# P < 0.001), noise-styrene group was significantly higher than styrene (*P < 0.050)

GSH level (Figure 3) in styrene and noise-styrene groups significantly decreased (P < 0.001). The concentration also moderately decreased in noise group which was not significant.

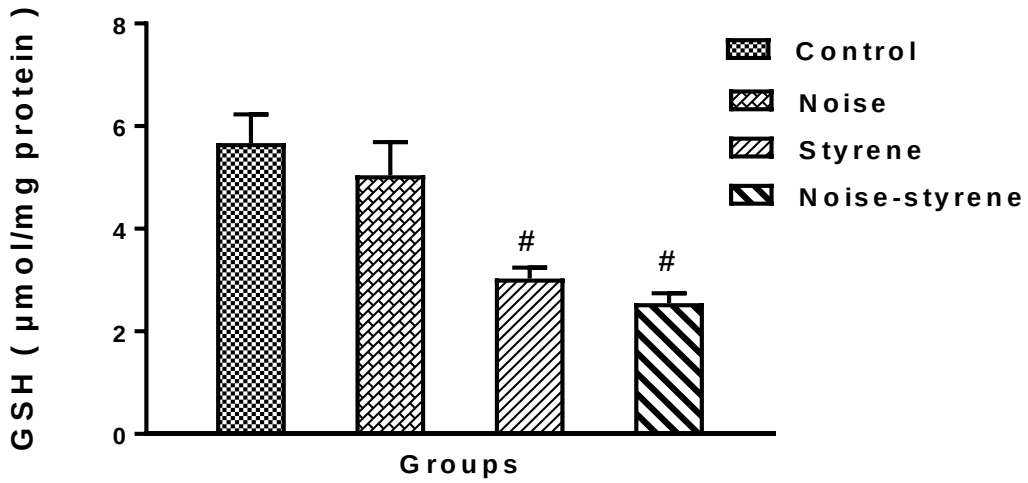

Figure 3: Glutathione (GSH) level in the lung tissues of rats following subacute exposure, each value represents the mean ± SD (n = 6), noise-styrene and styrene group were significantly lower compared to the control group (# P < 0.001)

A significant decrease in SOD activity (Figure 4) was observed at the end of the 4th week in both styrene and noise-styrene groups (P < 0.001) whereas increase in SOD activity of noise-styrene group was not found to be significant compared to styrene group (P > 0.050).

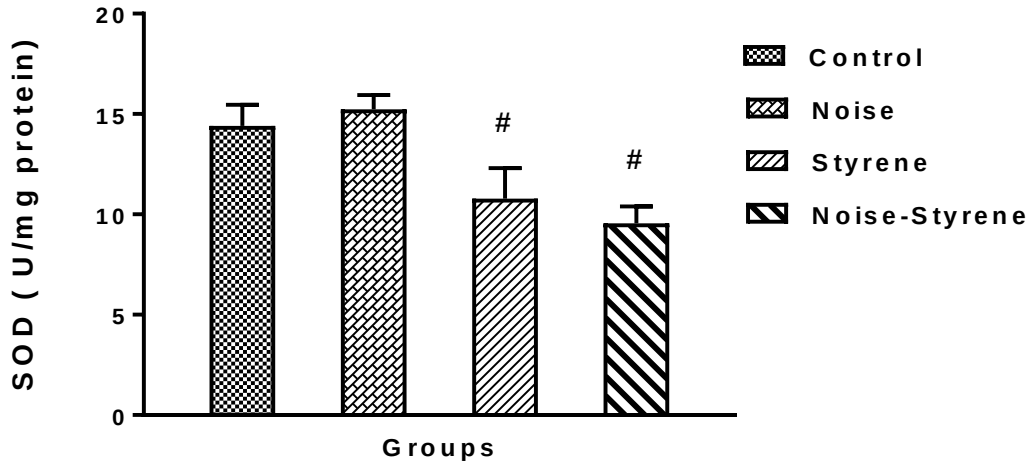

Figure 4: Superoxide dismutase (SOD) activity in the lung tissues of rats following subacute exposure, each value represents the mean ± SD (n = 6), noise-styrene and styrene groups are significantly lower (# P < 0.001) compared to the control group

Compared to the control group, reduction in the CAT activity (Figure 5) of styrene and noise-styrene groups were statistically significant (P < 0.050). No significant relationship was observed in noise and noise-styrene groups compared to control and styrene groups, respectively.

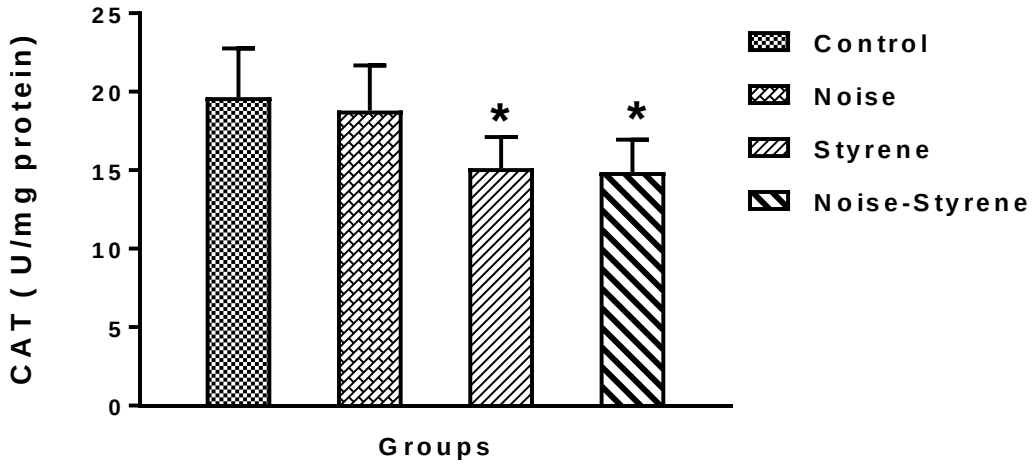

Figure 5: Catalase (CAT) activity in the lung tissues of rats following subacute exposure, each value represents the mean ± SD (n = 6), noise-styrene and styrene group were significantly lower compared to the control group (*P < 0.050)

Discussion

The studies which have been carried out on the potential hazards of noise and chemical interactions are mostly limited to the auditory organ. Data from such studies suggest that at relatively high ototoxic levels of styrene and noise, an interaction is seen which can be at least additive. These effects are mainly resulted from formation of ROS following exposure to noise and styrene solely (26, 27). Several effects have been observed in experimental animals following exposure to

styrene including pneumotoxicity, hepatotoxicity, neurotoxicity and ototoxicity (28). The present study was conducted to assess the oxidative status of rats' lung exposed to styrene and noise alone and simultaneously.

Styrene exposure may be a factor enhancing individual susceptibility to other pneumotoxicants of environmental importance, in particular those causing oxidative stress and free-radical mediated injury (6).

Phillip and farmer suggested that the adverse effects of styrene are mediated through styrene oxide (SO) (29). The lung has been identified as a location for biotransformation of styrene to styrene oxide. Clara cells and type II cells are responsible for a significant portion of the oxidative metabolizing capacity of the lung (30). Reduction in activity of SOD which is essentially a protective enzyme that scavenges the superoxide ions produced as cellular byproducts during oxidative stress, can lead to adverse effects because superoxide anions are extremely toxic and may accumulate in the cells. Noise exposure firstly increases levels of ROS such as superoxide radicals, hydroxyl radicals and hydrogen peroxide. Secondly, activity of antioxidants and related enzymes increases in order to eliminate the overproduced ROS due to noise (13). Hakan Mollaogo et al. have shown that MDA level, an indicator of lipid peroxidation, significantly increased in noise group (18).

In our study, MDA concentration in the lung tissues showed significant increase in styrene as well as noise-styrene group compared to control group which means that high MDA concentration is an indication of high lipid peroxidation concentration and as a result, oxidative damage will be greater. GSH is the most abundant non-protein thiol source in the cells and serves many vital physiological functions including protection of cells from ROS, detoxification of exogenous compounds, and amino acid transport. We assessed significant decrease in GSH concentrations in lung tissues of experimental groups and this reduction was significant in styrene and noise-styrene group. A close relationship between decreased GSH level and increased MDA concentration has been found. These findings are partly in line with the literature (31, 32). Ravenzwaay et al. did a study on the adverse effects of styrene on lung tissue in rat and mice. They concluded that exposure to styrene caused glutathione depletion, increased lipid peroxidation and also DNA damages in mouse and rats (in higher concentration) (33).

The activity of SOD in styrene and noise-styrene groups decreased in a significant manner. SOD is essentially a protective enzyme which scavenges the superoxide ions produced as cellular byproducts during oxidative stress. These products can lead to adverse effects because superoxide anions are extremely toxic and may accumulate in the cells. In Carlson et al.'s study on mice, the Clara cells were exposed to 3-hour styrene and styrene oxide in an in vitro study. The results indicated that the occurred oxidative stress led to increase in ROS and SOD (34).

Similar to SOD, CAT serves as a first line defense mechanism against oxidative stress and protects the cellular constituents against oxidative damage. In current study, CAT followed the same reduction pattern as SOD. When ROS generation increases, antioxidant defense systems fail, and damage occurs. This may explain why the CAT and SOD activities decreased in the lung tissue (9, 35, 36).

Conclusion

After this preliminary investigation, we conclude that the effect of noise on styrene group assessed as noise-styrene group was synergistic in MDA level while it was fairly additive in GSH content as well as activity of CAT and SOD.

Low levels of tissue antioxidant enzymes are likely to result in high levels of tissue damage that are reflected as lipid peroxides, protein carbonyls, etc. Conversely, elevated levels of antioxidant enzymes would reduce this oxidative damage to tissues. Noise, as a

physical stressor, has been specified to increase oxidative species in different parts of body, whereas based on the findings, the styrene role in overwhelming the endogenous defense system is undeniable. These results appear to support the fact that co-exposure to noise and organic solvents (styrene) might have more adverse effects and could produce higher amount of reactive oxygen and nitrogen species than each item alone. Thus, other studies need to be done in the area of simultaneous exposure of noise and substance (organic solvents) in order to revise or redefine the current standard exposure limits in occupational environments and workplaces.

Acknowledgments

The present study is a result of Mr. Mojtaba Highlight's MS thesis approved by research deputy of Tarbiyat Modares University. The author would like to thank Dr. Mohammad Faridan and Dr. Ali Seyedkhani for their contributions, valuable suggestions, support and guidance throughout this work.

Conflict of interest: None declared.

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