Toxicology Reports 13 (2024) 101849

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Investigating the effects of co-exposure to noise and benzene on serum oxidative stress in rat

Amirreza Shalili [a], Hassan Assilian Mahabadi [b,*], Ali Safari Variani [c]

[a] Department of Occupational Health and Safety Engineering, Faculty of Medical Science, University of Tarbiat Modarres, Tehran, Iran
[b] Department of Occupational Health and Safety, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[c] Department of Occupational Health and Ergonomic, Qazvin Medical University, Qazvin, Iran

ARTICLE INFO

Handling Editor: Prof. L.H. Lash

Keywords:
Benzene
Noise
Rat
Occupational exposure
Co-exposure
Oxidative stress

ABSTRACT

Occupational exposures are generally complex, workers are exposed with more than one hazardous agent in work environment. Combined exposure to noise and benzene is common in occupational environments. Subacute exposure to benzene vapors can induce oxidative stress in serum. Additionally, noise exposure leads to non-auditory effects, such as oxidative stress. However, In the authors' research scope, a study aimed at investigating the effect of co-exposure to noise and benzene on the oxidative stress of rat serum has not been found. The purpose of this study is to investigate the effect of co-exposure to noise and benzene on oxidative stress. In this study, 24 Wistar albino male rats were divided into four groups: the control group (1), the group exposed to white noise with an intensity of 100 dB and a frequency of 250–8KHz (2), the group exposed to benzene vapors with a concentration of 300 PPM (3), and the group co-exposed to white noise with an intensity of 100 dB and benzene vapors with a concentration of 300 PPM. Oxidative stress induced was investigated by serum oxidative stress indices, including lipid peroxidation (MDA), total oxidative capacity (TOS), and antioxidant activity indices (SOD) and (GSH), as well as total antioxidant capacity (TAC).The results showed that exposure to noise and benzene, both separately and combined, can lead to oxidative stress in rat serum, increasing serum oxidant indices MDA and TOS while decreasing serum antioxidant indices TAC, SOD, and GSH was detected in groups exposed to noise and benzene. The findings indicate that the serum oxidative stress caused by the co- exposure to noise and benzene is significantly higher than separate exposure to noise or benzene, also Co-exposure to noise and benzene can have an almost additive effect on increasing serum oxidative stress in rats. This study highlights the importance of studying co-exposure to physical and chemical hazardous agents in the work environment.

1. Introduction

Today, due to the complexity of the industrial production process, the risk of exposure to hazardous factors has increased [1]. Extensive studies investigate the effects of exposure to hazardous factors in the work environment on various aspects of the health of industrial workers [2–7]. The results of studies show that there are occupational exposures to volatile organic compounds (VOCs) in environments where co-exposure to noise is likely [8]. There are also concerning epidemiological evidence regarding co- exposure to noise and benzene, one of the most important volatile organic compounds (VOCs) [9]. Volatile organic compounds (VOCs) are widely used in the structure of many household, industrial, and commercial products and are practically an integral part of human daily life, These compounds are present in the atmosphere of urban areas at low concentrations due to seasonal, weather, and geographical conditions [10]. Benzene is considered one of the most toxic compounds in the volatile organic compound (VOC) category, and the International Agency for Research on Cancer (IARC) classifies benzene as a definite blood carcinogen (A1) for humans based on conclusive evidence of its carcinogenicity [11]. Exposure to benzene and its compounds among employees of various industries, including those related to spray paint and resin [12], industrial solvents [13], petrochemicals [14], automobile manufacturing [15], and shoes industries [16], has been reported. Most exposures to benzene in industrial environments occur chronically and through inhalation [17]. The effects of exposure to benzene on the body depend on the dose of benzene that enters the

* Correspondence to: Department of Occupational Health Engineering, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.
E-mail address: asilia_h@modares.ac.ir (H.A. Mahabadi).

https://doi.org/10.1016/j.toxrep.2024.101849
Received 18 September 2024; Received in revised form 21 November 2024; Accepted 3 December 2024
Available online 4 December 2024

body through oral or inhalation routes. The first complication of exposure to benzene is usually related to the central nervous system (CNS) and Acute exposure to benzene can lead to anesthesia, delirium, convulsions, and even suffocation due to respiratory muscle paralysis and sudden death [18]. study shows that chronic exposure to benzene generally leads to various disorders in the entire hematopoietic system [19]. Despite control measures, cases of aplastic anemia caused by benzene and leukemia are still observed in industries [20]. On the other hand, there are also reports of chronic benzene toxicity caused by non-occupational exposures, including leukemia among people who did not have occupational exposure to benzene, but whose leukemia is attributed to benzene exposure [21]. One of the fundamental effects of exposure to benzene is development of reactive oxygen species in different tissues, known as oxidative stress [22]. Oxidative stress refers to the imbalance between free radical species and antioxidants (Fig. 1) in cells or tissues [23]. In a balanced state between oxidants and antioxidants, all oxidants can be neutralized by the donation of electrons from antioxidants [24]. Under conditions of oxidative stress, the concentration of free radicals (Oxidant) increases, leading to lipid peroxidation, which is associated with an increase in MDA and TOS level [25]. A significant source of free radicals and oxidative stress is exposure to environmental and occupational hazardous agents [26,27]. The results of studies show that the amount of reactive oxygen species (ROS) increases in various tissues during infections, physical activity, and exposure to physical and chemical pollutants in the work environment [28]. The increase oxidant species without enough antioxidants for neutralization can cause oxidative stress and persistence of oxidative stress is associated with a wide range of disturbances and diseases [29].

Increasing the concentration of free radical species will increase the oxidation of fats and decrease the proper and useful function of the cell. On the other hand, with the increase in the concentration of free radicals, there is an increase in the peroxidation of fats, which itself leads to an increase in serum TOS (Total Oxidative Stress) and MDA (Malondialdehyde) [32]. The activity of antioxidant defense aims to create a balance between oxidant and antioxidant groups. The increase of oxidants inside the cell leads to a reduction of antioxidant species in the cell, and the overall activity of antioxidants eventually decreases. As a result, the concentration of SOD (Superoxide Dismutase), TAC (Total Antioxidant Capacity), and GSH (reduced glutathione) serum, which is considered an important antioxidant index, decreases [33]. Exposure to noise as a physical hazardous agent is considered one of the most common hazardous exposures in work environments [34]. According to their intensity and frequency, noise waves can cause direct effects such as hearing loss, tinnitus, and imbalance, as well as indirect effects such as increased blood pressure, heart rate, nervous disorders, and digestive disorders [34,35]. One of the most important side effects of uncontrolled exposure to noise is the occurrence of stress, which can be psychological or cellular [36].

Previous studies have shown that exposure to noise (a source of oxidative stress) with an intensity greater than 90 dB causes serum oxidative stress and is associated with an increase in oxidative indices and a decrease in antioxidant indices [37]. Studies regarding exposure to benzene indicate a decrease in antioxidant factors and an increase in oxidant indices in various tissues, including blood [38].studies show that exposure to noise and other hazardous factors in the work environment are hazardous factors that chemically intensify each other's effects [39]. The results of comparing the oxidative stress of different tissues with blood show that development of oxidative stress in blood can reflect the development of oxidative stress in other tissues [28]. The aim of this study is to investigate the effects of exposure to noise and benzene vapors, both individually and in combination, on oxidative stress biomarkers in rat serum.

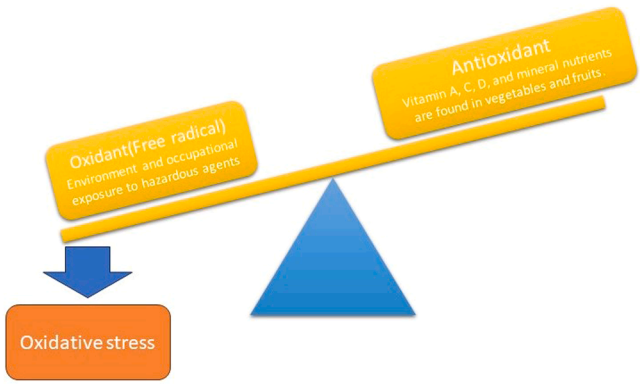

Fig. 1. oxidative stress and imbalance of antioxidant[30] and oxidant[31].

2. Materials and methods

In this study, 24 Wistar albino male rats weighing 206 ± 17 (gr) were used. The rats were purchased from the Biophysics-Biochemistry Research Center of Tehran University, Iran, and were kept for one week before the start of the study. The rats were acclimatized to the laboratory conditions to become accustomed to the environment and the researcher. The conditions of laboratory housing included a temperature range of 20 ± 2 degrees Celsius, humidity of 40–50 %, and a 12-hour light and 12-hour dark cycle (from 8 AM to 8 PM). Throughout their stay in the laboratory, the animals had free access to water and food. The rats were randomly divided into four groups, with 6 rats placed in each group. All rats were weighed before the exposure. The exposures started on December 23, 2023, and lasted for 15 days.

2.1. Tested groups

24 Wistar albino male rats were divided into four groups (Fig. 2): the control group: Group A (Control Group): This group include 6 male Wistar albino rats, which were placed in an exposure chamber for 10 days, 8 hours each day, but there was no exposure to benzene; the concentration in the exposure chamber of the control group was zero, and the noise intensity was measured at 27 decibels. Group B: This group include of 6 Wistar albino male rats that were exposed to white noise (100 dB) with range of frequency 250–8 KHz for 8 hours every day and 10 days، but there was no exposure to benzene; the concentration in the exposure chamber of the group B was zero. Group C: This group include 6 male Wistar albino rats that were exposed to benzene vapor (300 PPM) for 8 hours every day and 10 days. the noise intensity was measured at 35 decibels in their exposure chamber. Group D: This group included 6 Wistar albino male rats that were exposed to combined noise (100 dB) and benzene (300 PPM) vapors for 8 hours every day for 10 days.

All the groups had free access to food and water during the resting time, unlike the exposure time. They were exposed to 27 dB noise while resting, and they were not exposed to benzene.

Exposure chamber specifications: In this research, a reverberant chamber with dimensions of 30x40x80 cm and a thickness of 3 mm was made of plexiglass. Plexiglass is a transparent thermoplastic that is resistant to volatile organic compound (VOC) [37] and has not significant noise absorption properties [40]. To perform the exposure, the rats were placed in small cages with dimensions of 24x16x16 cm, and then the cages containing the rats were placed in the exposure chamber. The dimensions of the chamber were designed to accommodate 6 cages containing rats inside.

Production of benzene vapors. In this study, the saturated vapor method was used to produce the required concentrations of benzene vapors at 300 ppm. This method involves an impinger to vaporized liquid benzene (above 99 % purity, CAS No. 100–42–5 from Merk Chemicals and Metals Co., LTD). Air from outside the chamber is bubbled into the impinger by a manual low flow pump, and at its output, saturated vapor containing benzene at high concentration is produced. The concentration created in the impinger, after proper mixing with

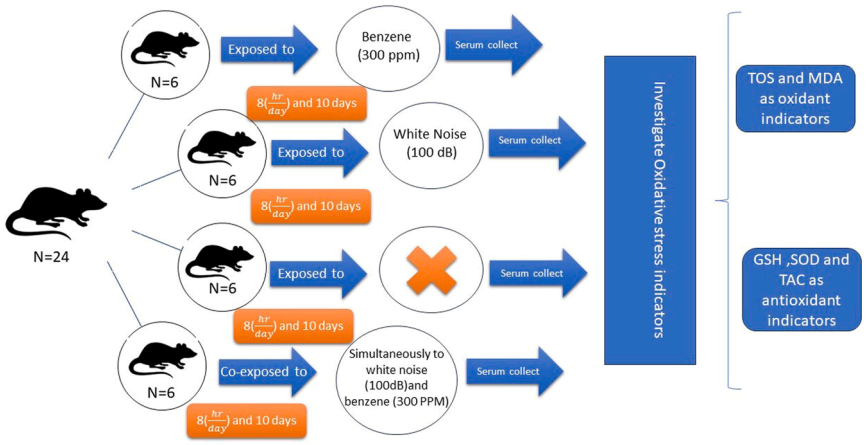

Fig. 2. Exposed groups to noise (B), Benzene (C), Noise-Benzene (D) and control group.

fresh air, adjusted temperature of (22 ± 1) °C and humidity of (50 ± 8 %) enters to the exposure chamber. Benzene concentration was continuously monitored using TIGER PhoCheck (Ion Science Ltd, Cambridge, England).

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 above the wire cages. The noise levels within the chambers were measured at the level of the rats' ears (8 cm) in each cage using a noise level meter (Casella CEL 480). Overall, the noise levels varied less than ± 2 dB (100 ± 2 dB), between the measuring points, and the frequency distribution of the noise has white noise spectrum. Such spectrum (250–8 kHz) was selected to cause hearing losses where auditory sensitivity is the highest for the human [41]. The cages in the chambers were rotated daily to maintain the most equal exposure for all rats.

Biological analysis: After two days of final exposure, all animals were weighed, and to test the serum oxidative stress indicators, blood was taken from the animals' hearts. A solution containing ketamine (90 mg/kg) and xylazine (9 mg/kg) was prepared, and all the animals were anesthetized by intraperitoneal (IP) injection [42]. Blood was then collected from their hearts. To prevent hemolysis of the red blood cells after blood collection, the blood was slowly drained into a tube without anticoagulant and centrifuged for 15 minutes at a speed of 2000 revolutions per minute. The separated blood serum was extracted from the laboratory tubes with a micropipette and transferred to small laboratory tubes with lids. The final concentration of serum oxidative stress indicators was determined after sample preparation using special assay kits, performed by spectrometry at a specific wavelength with an ELISA reader device (a specific spectrophotometer for wavelengths 400–700 nm, Biotech-reflex 800 model made in America). TOS (Total Oxidative Stress) and MDA (Malondialdehyde) concentrations were prepared and calculated as serum oxidative stress indicators using special ZB-TOS and ZB-MDA kits. Additionally, the concentrations of GSH and TAC, as well as the activity of the SOD enzyme, were measured as serum antioxidant defense indicators using ZB-GSH, ZB-TAC, and ZB-SOD special kits.

Statistical analysis: All values were expressed as mean ± standard deviation. Data were analyzed using one-way ANOVA with SPSS software (version 26.0, IBM Corporation, followed by Tukey's HSD multiple range test. Differences with $P < 0.05$ were considered significant.

3. Results

The results showed that the mean concentration of MDA in blood

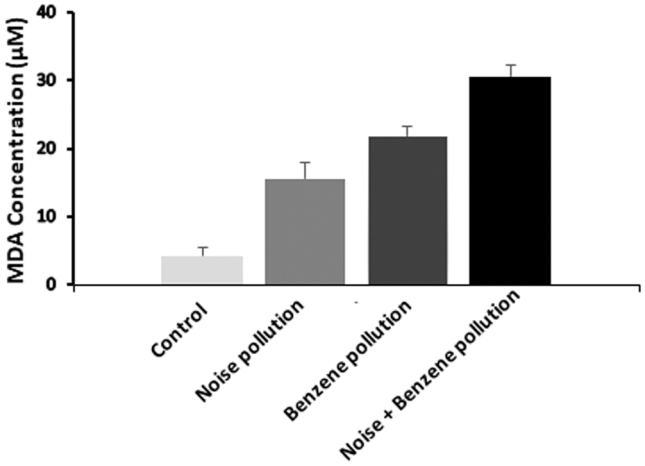

Fig. 3. Serum MDA concentration in different exposure group (each value represents the mean ± SD (n = 6), benzene, noise and also noise+benzene groups were significantly higher than the control group ($P < 0.001$), noise+benzene group was significantly higher than benzene or noise exposure ($P < 0.05$)).

(Fig. 3) in the exposure group to benzene or noise, as well as the co-exposure group (noise and benzene), had a significant increase ($P < 0.01$) compared to the control group. Additionally, the co-exposure group exhibited a significant increase ($P < 0.01$) in MDA concentration compared to the groups exposed to benzene or noise alone. Furthermore, the blood MDA concentration in the benzene exposure group was significantly higher than that in the noise exposure group.

The average activity of blood glutathione (GSH) (Fig. 4) in group exposed to benzene, noise, and co- exposure group showed a significant decrease ($P < 0.001$) compared to control group. Additionally, results of the statistical test indicated a significant decrease ($P < 0.001$) in blood glutathione (GSH) activity in co-exposure group compared to exposure group with benzene or noise. The blood glutathione (GSH) regeneration activity among group exposed to benzene was significantly ($P < 0.05$) lower than that of the group exposed to noise.

The results of the statistical test showed that the mean antioxidant activity (SOD) of blood (Fig. 5) in the group exposed to benzene, noise, and the co-exposure group had a significant decrease ($P < 0.05$) compared to the control group. Additionally, the results of the tests indicated a significant decrease ($P < 0.001$) in antioxidant activity (SOD) of blood in the co-exposure group compared to the group exposed to benzene. The group exposed to noise exhibited a significant difference ($P < 0.001$) in antioxidant activity (SOD) of blood compared to the co-

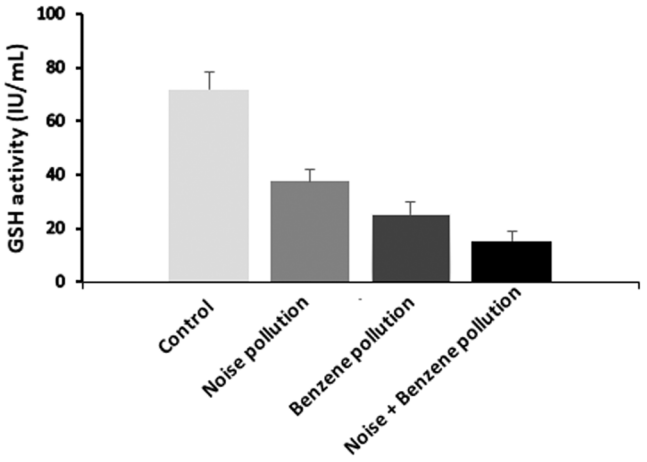

Fig. 4. Serum GSH activity in different exposure groups (each value represents the mean ± SD (n = 6), benzene, noise and also noise+benzene groups were significantly lower than the control group ($P < 0.001$), noise+benzene group was significantly lower than benzene or noise exposure ($P < 0.05$)).

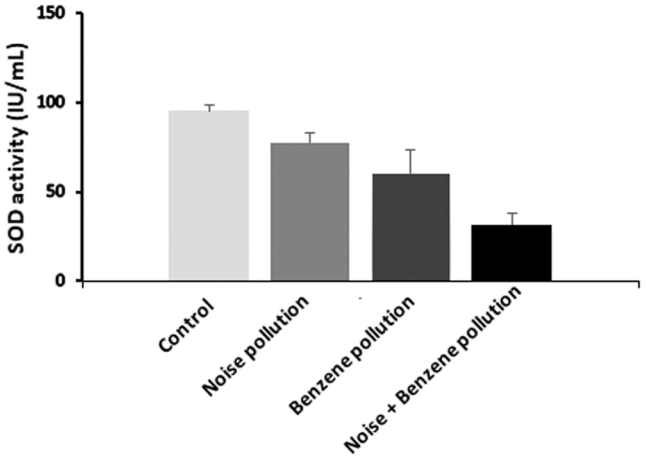

Fig. 5. Serum SOD activity in different exposure groups. (Each value represents the mean ± SD (n = 6). The benzene, noise, and noise+benzene groups were significantly lower than the control group ($P < 0.001$). The noise+benzene group was significantly lower than the benzene and noise exposure groups ($P < 0.05$)).

exposure group. Furthermore, the activity of the antioxidant enzyme (SOD) in blood serum in the group exposed to benzene showed a significant decrease ($P < 0.05$) compared to the group exposed to noise.

Statistical analysis showed that the average blood concentration (TOS) in the group exposed to benzene, noise, and co- exposure group increased significantly ($P < 0.001$) compared to the control group. Additionally, the results of the tests indicated a significant increase ($P < 0.05$) in blood concentration (TOS) in the co- exposure group compared to the group exposed to benzene. The group exposed to noise shows a significant difference ($P < 0.001$) in blood antioxidant concentration (TOS) compared to the group co-exposed to benzene and noise. Furthermore, blood serum total oxidant concentration (TOS) in the group exposed to benzene showed a significant increase ($P < 0.001$) compared to the group exposed to noise (Fig. 6).

The statistical analysis showed that the average serum concentration (TAC) in the group exposed to benzene, noise, and co- exposure group had a significant decrease ($P < 0.001$) compared to the control group. The results of the tests also indicated a significant decrease ($P < 0.001$) in serum concentration (TAC) in the co- exposure group compared to the group exposed to benzene and the group exposed to noise. Additionally,

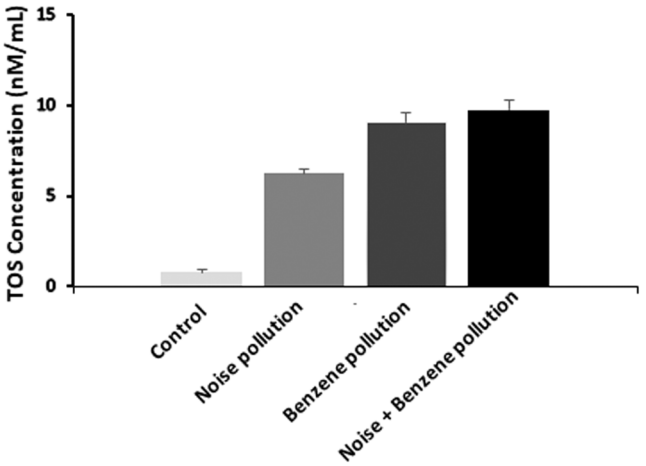

Fig. 6. Serum TOS concentration in different exposure groups (each value represents the mean ± SD (n = 6), benzene, noise and also noise+benzene groups were significantly higher than the control group ($P < 0.001$), noise+benzene group was significantly higher than benzene or noise exposure ($P < 0.05$)).

the antioxidant concentration (TAC) of blood serum in the group exposed to benzene showed a significant difference ($P < 0.05$) compared to the group exposed to noise (Fig. 7).

4. Discussion

In the workplace, workers are co-exposed to multiple hazardous agents, including physical, chemical, biological, and psychological [43]. Exposure to multiple occupational and environmental hazardous agents can be associated with health disturbance [44]. In many industries, exposure to noise and chemical solvents occurs simultaneously [45], Benzene is used as one of the most important solvents in industries; on the other hand, it is considered a definite carcinogen(A_1) for humans [46]. Studies conducted to evaluate the effects of co- exposure to hazardous agents in the work environment can show the actual conditions of exposure more clearly [37,47].This group of studies usually focuses on a specific tissue, examining the adverse effects of co- exposure to hazardous factors in a specific tissue. Saraei *et al.* studied the effect of co-exposure to solvent and noise on the human auditory system [45].

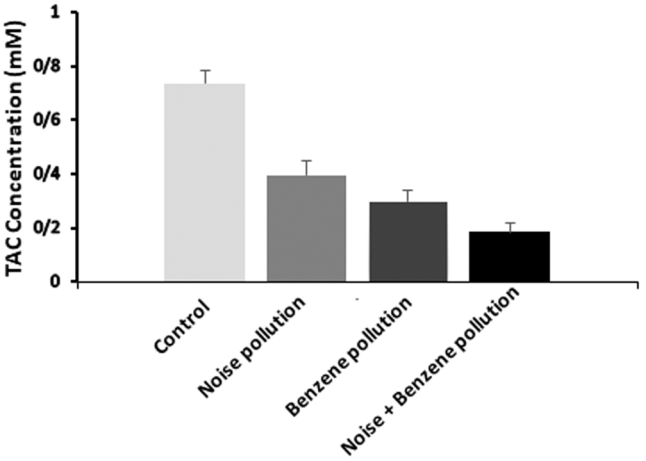

Fig. 7. Serum TAC concentration in different exposure group (each value represents the mean ± SD (n = 6), benzene, noise and also noise+benzene groups were significantly lower than the control group ($P < 0.001$), noise+benzene group was significantly lower than benzene or noise exposure ($P < 0.05$)).

Tehrani *et al.* investigated the effect of co- exposure to noise exceeding the occupational limit of 8 hours and styrene on the oxidative stress of rat liver tissue [37]. haghighat *et al.* studied the oxidative stress caused by co- exposure to noise and styrene on rat lung tissue[48]. The study conducted to compare oxidative stress in blood and different tissues shows a significant correlation between blood oxidative stress indices and tissue oxidative stress, Development of serum oxidative stress is an indicator of the occurrence of oxidative stress in the tissue. Additionally, due to the interaction of blood and tissue, the serum oxidative stress index can be an accurate cumulative measure of oxidative stress caused by different exposure pathways to agents [28]. Studies show the correlation between serum oxidative stress and inflammation in rat and human animal models [49]. The present study was conducted to investigate serum oxidative stress caused by single and co- exposure to noise and benzene vapors on male rats. In fact, this study aims to validate the hypothesis that co-exposure to noise and benzene can lead to exacerbation of serum oxidative stress compared to single exposure. Different oxidative stress indicators are used to assess the balance of oxidants and antioxidants. Among these indicators are the concentration of malondialdehyde (MDA) and total oxidant capacity (TOS) as indicators of the occurrence of oxidative stress, along with decreasing activity indicators such as superoxide dismutase (SOD) enzyme, reduced glutathione concentration (GSH), and total antioxidant capacity (TAC), which have been proposed as indicators of the decreasing of antioxidant defense due to increased oxidative stress in related studies [50]. Previous studies aimed at investigating serum oxidative stress in rats have also used similar oxidative stress indices [37,51]. Studies on benzene pollution in the environment show that even very small concentrations of benzene in urban air can cause serum oxidative stress in people exposed to benzene [52]. Benzene in work environments has a much higher concentration than in the general environment; exposure to benzene in these environment, as a carcinogenic agent, can lead to oxidative stress in exposed personnel and thereby cause genetic damage [53]

Findings of the present study show that MDA concentration and TOS capacity in the serum of rats exposed to benzene have increased significantly compared to the control group, while SOD and GSH activity, as well as TAC antioxidant capacity in the serum, have also decreased significantly compared to the control group. According to the necessary balance between oxidants and antioxidant activities, results of the present study indicate development of oxidative stress in rats exposed to a concentration of 300 ppm benzene. A study on workers exposed to benzene also shows development of oxidative stress due to exposure to benzene in the serum, supporting the results of the present study [54]. Similar results were observed in a study on fuel station personnel, where the incidence of serum oxidative stress was noted even at concentrations lower than the permissible limits of occupational exposure to benzene and BTEX compounds [55]. A similar study also showed the incidence of oxidative stress in male albino Wistar rats due to exposure to BTEX vapors, which also contain benzene vapors [56]. Study of kidney and liver function in rats exposed to benzene vapors demonstrated development of serum and tissue oxidative stress, confirming the results of the present study [57]. Yonggang Ma *et al.* showed in a review study that exposure to cadmium is associated with the development of cardiovascular diseases, kidney disorders, and neurological issues caused by the development of oxidative stress [58]. The link between oxidative stress and the development of cardiovascular diseases was established years ago in the study by Steinberg and his colleagues [59]. Hwang et al., study human population and identified a connection between a broader range of diseases and oxidative stress. According to their results, in addition to diseases previously attributed to oxidative stress, the development of Parkinson's disease, as a nervous system disorder, was also considered related to oxidative stress [60]. The studies of the last decade have created a deeper understanding of the effects of oxidative stress; today, the aging process and the reduction of telomere length are clearly attributed to oxidative stress [61,62]. Recent studies have identified oxidative stress as the main cause of Alzheimer's disease [63].

Study of the complications caused by noise exposure shows that these complications are not limited to auditory system and include a wide range of non-auditory complications as well [64]. One of the most significant effects of exposure to noise is development of oxidative stress; this oxidative stress caused by noise can be the main mechanism affecting hearing los s[65]. A study conducted on the serum of rats exposed to noise at an intensity of 100 decibels showed that concentration of the MDA index in the exposed group increased significantly compared to the control group, and exposure to noise caused a significant decrease in the indicators of antioxidant activities, specifically SOD and GSH, compared to the control group [36]. results of the present study indicate that concentration of MDA and the capacity of TOS in the serum of rats exposed to noise have increased significantly compared to the control group, while activity of SOD, GSH, and antioxidant capacity of serum TAC have also decreased significantly compared to control group. According to the necessary balance between oxidants and antioxidant activities, results of the present study indicate development of oxidative stress in rats exposed to noise at an intensity of 100 dB. findings of tissue studies also shows that oxidative stress caused by exposure to noise cause tissue disturbance, including brain tissue secretions [66]. Jafari *et al.* study on the effect of noise and formaldehyde on the oxidative stress indices of rats showed that noise at an intensity of 100 dB can lead to significant changes after 28 days of exposure, including a decrease in GSH and an increase in MDA compared to the control group [67]. As a result, this study confirms the findings of the present study regarding the incidence of oxidative stress in rats exposed to noise. Koc *et al.* investigate the effect of rosuvastatin on the process of creating oxidative stress caused by exposure to noise, they found that exposure to noise in rats leads to an increase in the oxidative stress index of MDA and a decrease in the antioxidant activity of GSH and SOD, which is completely consistent with the results of our studies regarding the effect of exposure to noise on oxidative stress, Also They found that rosuvastatin can lead to a reduction in oxidative stress indicators caused by exposure to noise [68].

Examining the effect of several different types of exposure is very difficult and confusing [69]. combined effects of different exposures to hazardous factors can be synergistic, additive, or potentiating [70,71]. Studies on interactions between noise and organic solvents are often limited to auditory effects [72–74]and are usually attributed to development of oxidative stress [36,65]. Some studies have investigated effects of interactions between organic solvents and noise on specific tissues such as the lungs [47,48], liver tissue [75], and skin [76]. Since the blood is in contact with and interacts with all tissues, the oxidative stress indices of the blood can be affected by the oxidative stress of different tissues and serve as an indicator of the oxidative stress of all tissues [28]. It can be the best target for evaluating the effects of co-exposure to hazardous factors that affect body through different exposure routes, such as noise, which affects auditory tissue [77], and thus can influence serum oxidative stress indicators, and benzene, which is absorbed through the olfactory system [78] and can lead to changes in serum oxidative stress indices. Finally, examining serum oxidative stress indicators can serve as an indicator of the impact of various exposures [23]. In the researcher's scope of the present study, no study has been found that investigates the effect of co-exposure to noise and benzene on serum oxidative stress.

Results of the present study show that rats co- exposed to benzene vapors at a concentration of 300 ppm and noise at an intensity of 100 decibels have significantly higher oxidative stress indices (MDA and TOS) than those exposed to noise or benzene alone. Additionally, the antioxidant defense indices of SOD and GSH activity and TAC capacity were lower than those observed with single exposure to noise or benzene. The changes in serum oxidative stress factors clearly indicate the intensification of oxidative stress in rats that were exposed to both noise and benzene. This finding supports the main hypothesis of the study and

demonstrates that co-exposure to benzene and noise can be far more hazardous than exposure to noise or benzene alone.

A study conducted on the auditory system of workers exposed to noise and a combination of toluene, xylene, and ethylbenzene showed that co- exposure to noise and chemical solvents can have an intensified effect on the hearing loss of employees [79]. A study on the oxidative stress of rat lung tissue caused by co-exposure to noise and styrene has also shown that the effects of co- exposure are intensified compared to the individual ones [48]. Also, Tehrani *et al.* study on liver tissue stress shows the intensification of effect of co- exposure to noise and styrene on liver enzyme changes [37]. These studies support the result of the present study because they can prove the occurrence of the intensified effect of co- exposure to noise and organic solvents.

5. Conclusion

Results of the study show that Co-exposure to noise and benzene have an almost additive effect on increasing serum oxidative indices (MDA and TOS) and decreasing serum antioxidant activity indices (SOD, GSH, and TAC). The lower levels of antioxidant indices, along with increasing oxidative indices, lead to a disturbance of oxidative balance and development of oxidative stress. Serum oxidative stress represents the occurrence of tissue oxidative stress and serves as a cumulative index to investigate oxidative stress caused by various exposures to chemical and physical agents. The results of the present study reinforce the hypothesis that co- exposure to noise and organic solvents such as benzene can have more hazardous effects than exposure to noise or benzene alone. In conclusion, more studies are needed to provide a clear view of the co-exposure's effects in occupational environments on employees. Additionally, extensive research based on human and animal models is necessary to demonstrate the need of reducing threshold limits value of benzene and noise in occupational Environments that are likely to be co-exposed. Furthermore, the analysis of the present study shows that the serum oxidative stress indices of the oxidative type (MDA and TOS) and antioxidant activity (TAC, GSH, and SOD) are suitable for measuring exposure in different ways, such as noise and organic solvents (benzene). Further studies regarding exposures aimed at measuring serum oxidative stress indicators can yield very useful information about real effects of co- exposure. Co-exposure to noise and benzene in the environment and industries should be limited, and more extensive studies are needed on the effects of co-exposure to physical and chemical agents on various tissues.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Amirreza Shalili: Writing – review & editing, Writing – original draft, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Hassan Assilian Mahabadi:** Visualization, Validation, Supervision, Resources, Conceptualization. **Ali Safari Variani:** Supervision.

Declaration of Competing Interest

All authors declare that they have no conflicts of interest. It is important to note that this study was conducted independently without any funding or financial support.

Acknowledgements

The present study is a result of Mr. Amirreza Shalili's MS thesis, which was approved by the research deputy of Tarbiat Modares University, Tehran, Iran

Data Availability

Data will be made available on request.

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