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Research Paper

Co-exposure to Toluene and Noise Made Synergistic and Antagonistic Effects on Some Neurotoxic Parameters in New Zealand White Rabbits

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ABSTRACT

Introduction: Numerous physical and chemical agents can induce destructive effects on the brain tissue. Noise and toluene, which are some of these harmful agents, have significant adverse effects on the brain tissue. This work aimed to investigate the neurotoxic changes induced by co-exposure to toluene and noise.

Methods: A total of 24 male white New Zealand rabbits were randomly segregated into four groups, including toluene exposure, noise exposure, co-exposure to noise and toluene, and control. This in vivo study tested the neurotoxic effects of exposure to 1000 ppm toluene and 100 dB noise during two weeks (8 h/day). The serum levels of brain-derived neurotrophic factor-α (BDNF-α), malondialdehyde (MDA), glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase and total antioxidant capacity (TAC) values in the brain tissue were measured. Moreover, hematoxylin and eosin (H&E) staining was utilized for brain pathological analysis.

Results: Exposure to noise increased TAC values in the cerebral cortex. Co-exposure to toluene and noise increased the serum levels of BDNF-α. Nevertheless, exposure to noise decreased the levels of BDNF-α in serum. On the other hand, histopathological examinations using H&E staining exhibited that different signs of inflammation, such as lymphocyte infiltration, pyknosis, vacuolization, and chromatolysis were induced by exposure to noise and toluene in the cerebellum, hippocampus, and frontal section in the brain tissue. In addition, simultaneous exposure to toluene and noise induced antagonistic and synergistic changes in some neurotoxic parameters.

Conclusion: Exposure to noise and toluene, which caused inflammation in the brain tissue cells, could be a noticeable risk factor for the neurological system.

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Highlights

- Exposure to noise increased total antioxidant capacity.
- Exposure to toluene decreased brain-derived neurotrophic factor-α.
- Exposure to noise decreased brain-derived neurotrophic factor-α.
- Co-exposure to noise and toluene increased brain-derived neurotrophic factor-α.
- Noise and toluene induced some histopathological effects on the brain tissue.

Plain Language Summary

The brain tissue can be adversely affected by various agents, including noise and toluene. This study aimed to examine the effects of simultaneous exposure to noise and toluene on the nervous system. Twenty-four healthy male white New Zealand rabbits were randomly divided into four groups: control, noise, toluene, and simultaneous exposure to noise and toluene. The study involved a two-week in-vivo experiment, subjecting the rabbits to 100 dB noise and 1000 ppm toluene for eight hours per day. This study showed that exposure to noise and toluene changed different parameters relating to the neurological system. Furthermore, noise and toluene induced some adverse effects on the brain tissue. This study suggested that exposure to noise and toluene can lead to harmful effects on the brain tissue, posing a significant risk to the neurological system.

1. Introduction

Noise is a fundamental property of different activities produced by changes in air pressure (Snyder, 2000). Excessive noise hurts various systems in the body, including the cardiovascular system, auditory system, psychological system, and neural system (Babisch, 2003; Brunoni et al., 2008). It has been proven that exposure to noise causes some disorders in the brain tissue, such as congestion, edema, blood vessel expansion, neuroglial hyperplasia, and mild atypia (Xue et al., 2014). Regrettably, the main cause or intensifying factors of many disasters and accidents, which could be related to the neurological and psychological disorders provoked by exposure to noise, such as enhancing the occurrence of violence and increasing workplace accidents (Toppila et al., 2009) may not be well detected or resolved.

Toluene is one of the most suitable solvents with various applications in the plastic, rubber, leather, adhesives, and pharmaceutical industries (Acton, 2013). Previous studies have demonstrated that exposure to toluene induces different diseases in various organs of the body, including the liver and kidneys (Schardein & Macina, 2006). The psychological and neurotoxic effects of toluene are complicated (Lowinson et al., 2005) and include the loss of balance, headache, weakness, fatigue, and joy feelings. In addition, toluene has a devastating effect on the nervous tissue of the brain (Dobbs, 2009).

There are many different physical and chemical stressors in the industry. Noise and toluene are some of the prevalent physical and chemical stressors in different workplaces. Previous studies have indicated that different stressors could deteriorate some brain disorders (Demır et al., 2017; Xiu et al., 2009).

The debate about the health effects of cocoa exposure to chemical and physical pollutants has been highlighted by many discussions about the interaction between different chemical and physical harmful agents (Sexton & Linder, 2011; Streffer et al., 2013). As stated by numerous reports and studies, many people in various occupations are not only exposed to noise (such as constructions, factories, nightclubs, and airports) (Tripathy, 2008) or only toluene (such as petrochemical, plastics factories, adhesive factories, and pharmaceutical factories) (Acton, 2013; Miller, 2002) but also simultaneously exposed to noise and toluene (Dobie, 2015; Rea & Patel, 2014). This indicates a need to understand various perceptions of neurotoxicity induced by simultaneous exposure to noise and toluene. Therefore, this experimental study assessed how co-exposure to toluene and noise changes biochemical factors and brain tissue in rabbits.

2. Materials and Methods

Animals and groups

Twenty-four healthy adult male white New Zealand rabbits (Pasteur Institute, Iran) weighing 2.83±0.41 kg were used in this experiment. Animals were kept on a 12:12 hour light/dark circulation period at controlled ambient temperature (21±2°C) and with a humidity of 50 to 70% while having access to food (standard rabbit pellet, Behparvar Feed Manufacturing Company, Tehran) and treated water. Animals were housed in the laboratory for 14 days before the experiments to adapt to the environmental conditions of the laboratory. They were randomly segregated into four groups (n=6 per group) including, noise exposure group (group 1), toluene exposure group (group 2), co-exposure to toluene and noise group (group 3), and control group (group 4). Exposure duration was 14 consecutive days (8 h/day from 9:00 AM to 5:00 PM) for all groups. The control group was placed in the exposure chamber with clean air flowing (toluene=0 ppm) and <50 dBA noise (background noise) with an exposure duration of 14 consecutive days. Then, group 1 was placed in the exposure chamber to receive 100±5 decibel (dBA) noise. After that, group 2 was placed in the same exposure to receive toluene (1000±50 ppm) for the exposure duration. Then, group 3 was placed in the exposure chamber and exposed to noise and toluene with the same specifications as groups 1 and 2 for the exposure duration. The temperature was 23±3°C and the relative humidity was 65%-80% inside the exposure chamber for all the experimental groups.

Exposure chamber

Animals in all groups (n=6) were exposed in the same exposure chamber with the dimensions of 50×60×90 cm^3 made of clear and transparent polycarbonate sheets, which were coated with transparent polyethylene terephthalate (PET) adhesive tape. Specifications of the exposure chamber were chosen according to the animals' welfare requirements (Gad, 2006) and based on the reverberant chamber features (Cobo et al., 2009; Moreno et al., 2000) to generate the same noise specifications inside the exposure chamber and to make noise intensity independent from distance in the chamber.

Noise exposure set-up

White noise at 100±5 dBA (70-20000 Hz) was produced by the White Noise Generator Software, version 1.3.12 and monitored by Cool Edit Pro Software, version 2.1 and Syntrillium Software version 2.0. The selected noise was amplified by a power amplifier (3030 W, multi tone) and transported to a loudspeaker. The loudspeaker was placed directly above the chamber on the chamber roof. The noise level was continuously measured using a sound level analyzer (TES 1358 sound analyzer real-time 1/1 & 1/3 octave band analyzer) in a 1/3 octave band during the exposure (groups 1 and 3). The sound level meter microphone was placed at the same level as the ears of the animals by checking the designed outlets (made of rubber tape and coated with PET adhesive tape) every 30 min in order to monitor noise specifications.

Toluene exposure set-up

Exposure to 1000±50 ppm toluene happened through its surface evaporation. In summary, constant generation of toluene vapors was done using an impinger (capacity of 250 mL) with the content of 100 mL toluene liquid (Merck KGaA, extra pure, Darmstadt, Germany) and via injecting 20 mL toluene into the impinger every 90 min on the periodical basis. The toluene concentration was analyzed during pilot studies using a calibrated humidity-resistant real-time instrument (Phocheck, Ion Science Ltd, Cambs UK 07-01782) and also charcoal absorbents with gas chromatography (model agilent 7890A GC system) established on the 1501 NIOSH method. Accordingly, changing the toluene concentration was negligible (5%) during the exposure time in the pilot and real experiments. According to the pilot experiments, the pure air flow rate was 30 L/min and the toluene vapor-contained air flow rate was 3 L/min. Moreover, before starting real exposures, the purity of the toluene vapors was confirmed by air sampling from the exposure chamber (active charcoal samples) and using a gas chromatography-mass spectrometry instrument (Agilent 6890/5973 GCMS). To obtain the target concentration of toluene constantly and continuously, a mixer chamber with the same materials and specifications as the exposure chamber with the dimensions of 50×50×20 cm^3 was used before entering the air into the exposure chamber. To monitor and check toluene concentration, the same real-time instrument (Phocheck), which had been already calibrated and utilized in pilot experiments was used. Air samples were prepared from inside the exposure chamber through checking outlets designed on four sides in the surrounding walls of the exposure chamber (checking outlets).

Blood sampling

Blood samples were collected from the marginal vein of the ears of each animal in five stages, as mentioned in Figure 1.

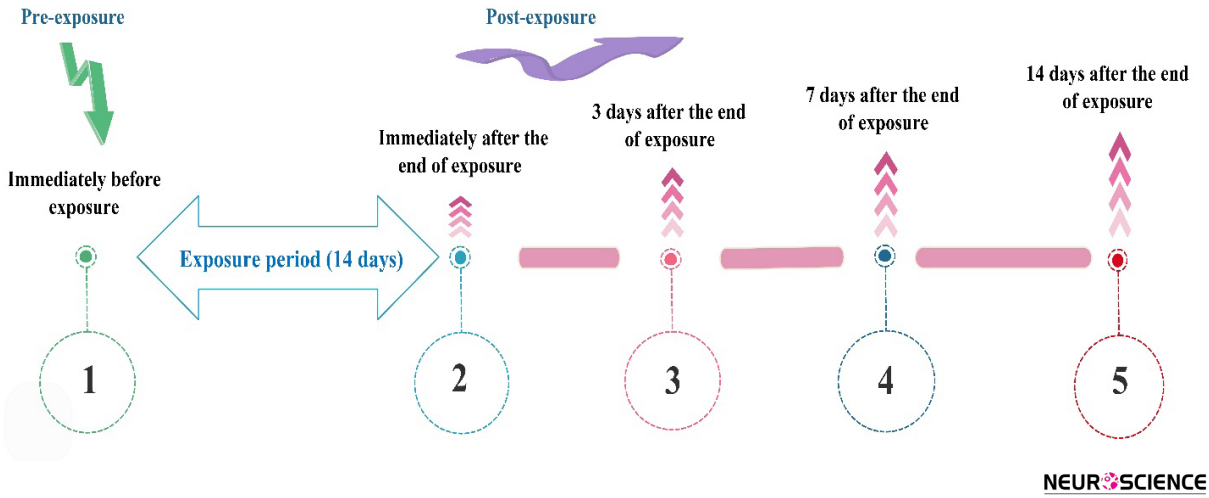

Figure 1. Stages of blood sampling

The blood samples were collected immediately before exposure and at different moments after the end of exposure in each group.

Tissue sampling and processing

Rabbits were sacrificed after anesthesia with 35 mg/kg ketamine and 5 mg/kg xylazine (IM injection). The animals were sacrificed 14 days after the end of the exposure in each group. The whole brain consisting of the cerebral cortex, hippocampus, and cerebellum was dissected immediately after the animals were sacrificed and fixed in 10% formalin solution (formaldehyde solution, Merck KGaA, Darmstadt, Germany) at pH 7.2. After the fixation, paraffin blocks were prepared. Moreover, for biochemical analyses, the cerebral cortex of the brain tissues was immediately collected and kept at -80˚C until the time of biochemical experiments. Dehydration and paraffin impregnation phases of the tissue samples were made by an autotechnicon device. Then, paraffin molding was carried out and the slides were prepared using a microtome with a typical diameter of 5 µ. Eventually, the slides were stained with hematoxylin and eosin (H&E) and histopathologic changes were assessed by light microscopy (Nikon Eclipse E100, Japan).

Homogeneity of brain tissue

First, 100 g of the cerebral cortex of the brain samples was weighed out of all the groups to measure the oxidant/antioxidant parameters of the brain tissue. Afterward, they were dissolved in 1 cc phosphate-buffered saline and homogenized by a homogenizer device (ultrasonic homogenizer Hielscher UIP-1500HD). Then, the samples were centrifuged by a centrifuge device at 1000 g at 4˚C for 20 min. Required samples were taken from the clear supernatant liquid of the centrifuged samples using a sampler and placed in microtubes. The supernatant protein liquid of the cerebral cortex was measured using a commercial kit (Pars Azmoon Company, Karaj, Iran). Finally, the parameters in the supernatant liquid were normalized by measuring the amount of supernatant protein liquid of the brain tissue and valued per mg of tissue protein.

Determining oxidant and anti-oxidant factors in the cerebral cortex using the spectrophotometric method

Total antioxidant capacity (TAC) was measured by a Naxifer™ diagnosis kit (TAC assay kit, Navand Lab Kit, Urmia, Iran). The levels of glutathione peroxidase (GPx) were measured by a Biorexfars diagnosis kit (production code: BXC0551, Shiraz, Iran). Catalase (CAT) levels were measured by a Cayman chemical company kit (CAT assay kit, Item No. 707002), and superoxide dismutase (SOD) levels were measured by a non-enzymatic method Nasdox™ diagnosis kit (Navand Lab Kit, Urmia, Iran). All the measurements were done according to the instructions provided by the kit manufacturers; in addition, malondialdehyde (MDA) levels were measured using the thiobarbituric acid (TBA) method (Janero, 1990; Stewart & Bewley, 1980).

Determining the serum levels of brain-derived neurotrophic factor-α (BDNF-α) using the ELISA method

BDNF-α levels were measured by Rabbit ELISA kits (Hangzhou Eastbiopharm CO., LTD, Hangzhou, China) and based on the manufacturer's instructions (Cat. No: CK-E91855). In this experiment, the State Fax 2100 ELISA plate reader (Awareness Technology, Inc., USA)

and the State Fax 2600 ELISA plate washer (Awareness Technology, Inc., USA) were employed.

Statistical analysis

The generalized estimating equation (GEE) statistical method was used to assess BDNF-α levels so that differences between different repeats in different groups were compared by SPSS software, version 25. Moreover, analysis of variance (ANOVA) followed by Tukey's post hoc test was used to compare the oxidant and anti-oxidant parameters and the mean brain tissue weight/body weight in different groups in Minitab software, Version 18. The statistical significance level was considered at 0.05. The logarithm of abnormal parameters was used for GEE statistical analysis.

3. Results

Biochemical changes

According to Figure 2E, TAC values in group 1 were significantly higher than those in groups 2-4. Figure 2 A, B, C & D shows CAT, GPx, SOD, and MDA levels in group 1.

Exposure to noise significantly decreased BDNF-α levels compared to the control and toluene exposure groups on the 14th day after exposure. Moreover, co-exposure to noise and toluene significantly increased BDNF-α levels compared to the noise and toluene exposure groups on the 3rd day after exposure. Furthermore, co-exposure to noise and toluene significantly decreased BDNF-α lev-

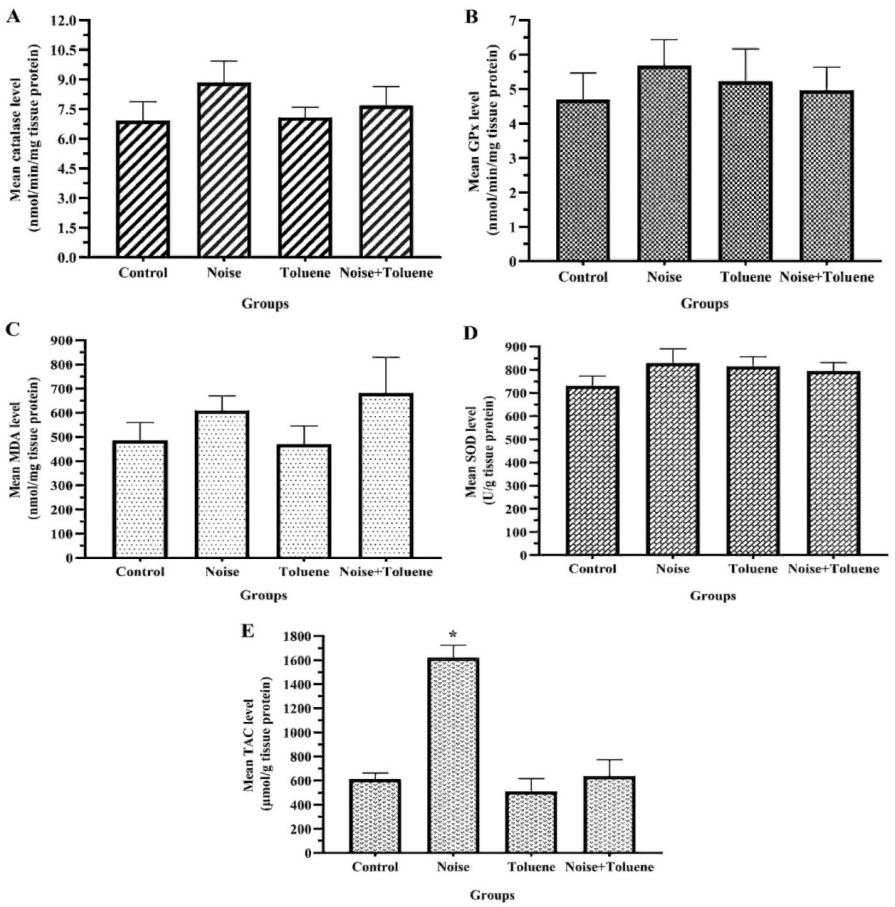

Figure 2. Different oxidant and anti-oxidant parameters in the brain tissue

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Abbreviation: GPx: Glutathione peroxidase; MDA: Malondialdehyde; SOD: Superoxide dismutase; TAC: Total antioxidant capacity.

*Significant differences between the noise and other groups (P<0.001), Routes of exposure: Inhalation exposure to toluene and hearing noise.

Note: Factors were measured 14 days after the end of exposure to 100±5 dB noise (noise group, n=6), 1000±50 ppm toluene (toluene group, n=6), co-exposure to 100±5 dB noise and 1000±50 ppm toluene (noise+toluene group, n=6), and no treatment (control group, n=6). Bars represent the arithmetic Mean±SE.

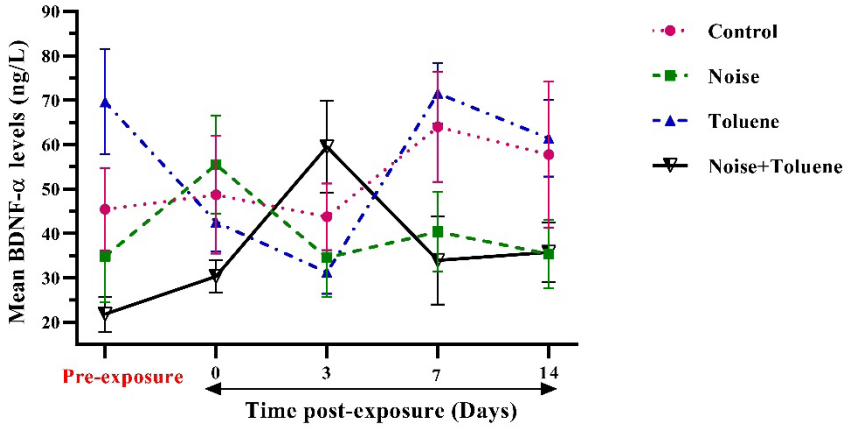

Figure 3. Serum levels of BDNF-α in exposure groups in different stages

Note: Points represent the arithmetic Mean±SE. Groups include the control group (group 4, n=6), exposure to noise at 100±5 dB (group 1, n=6), exposure to toluene at 1000±50 ppm (group 2, n=6), and co-exposure to 100±5 noise and 1000±50 ppm toluene (group 3, n=6). Stages include: Stage 1) Pre-exposure; Stage 2) Immediately after the end of exposure; Stage 3) 3 days after the end of exposure; Stage 4) 7 days after the end of exposure; Stage 5) 14 days after the end of exposure.

els compared to the toluene exposure group on the 7th and 14th days after exposure (Figure 3).

Brain tissue weight changes

According to Figure 4, the average brain weight/body weight in the noise exposure group was significantly lower than that in the toluene exposure group.

Histopathological changes

The cerebellum was in normal conditions in the control group. However, the population of Purkinje cells decreased in the cerebellum in group 1; in addition, pyknosis appeared in Purkinje cells in this group. The relative reduction in the number of cells was visible in the granular layer in groups 1 and 2. Granular cells not only reduced relatively but also appeared massively in group 2. Pathological changes were more specific in the cer-

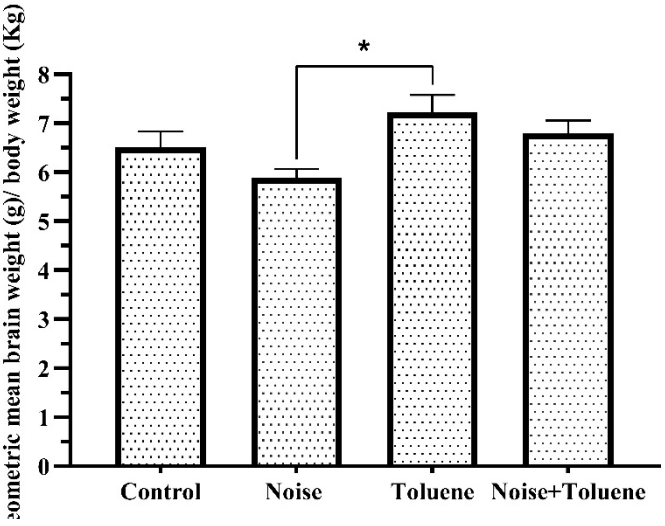

Figure 4. Geometric mean of brain weight/body weight in different groups

*Significant differences between the noise and toluene exposure groups (P<0.05).

Note: This parameter was measured 14 after exposure to 100±5 dB noise (noise group, n=6), 1000±50 ppm toluene (toluene group, n=6), co-exposure to 100±5 dB noise and 1000±50 ppm toluene (noise+toluene group, n=6), and no treatment (control group; n=6). Bars represent the geometric Mean±SE. Exposure routes: Inhalation exposure to toluene and hearing noise.

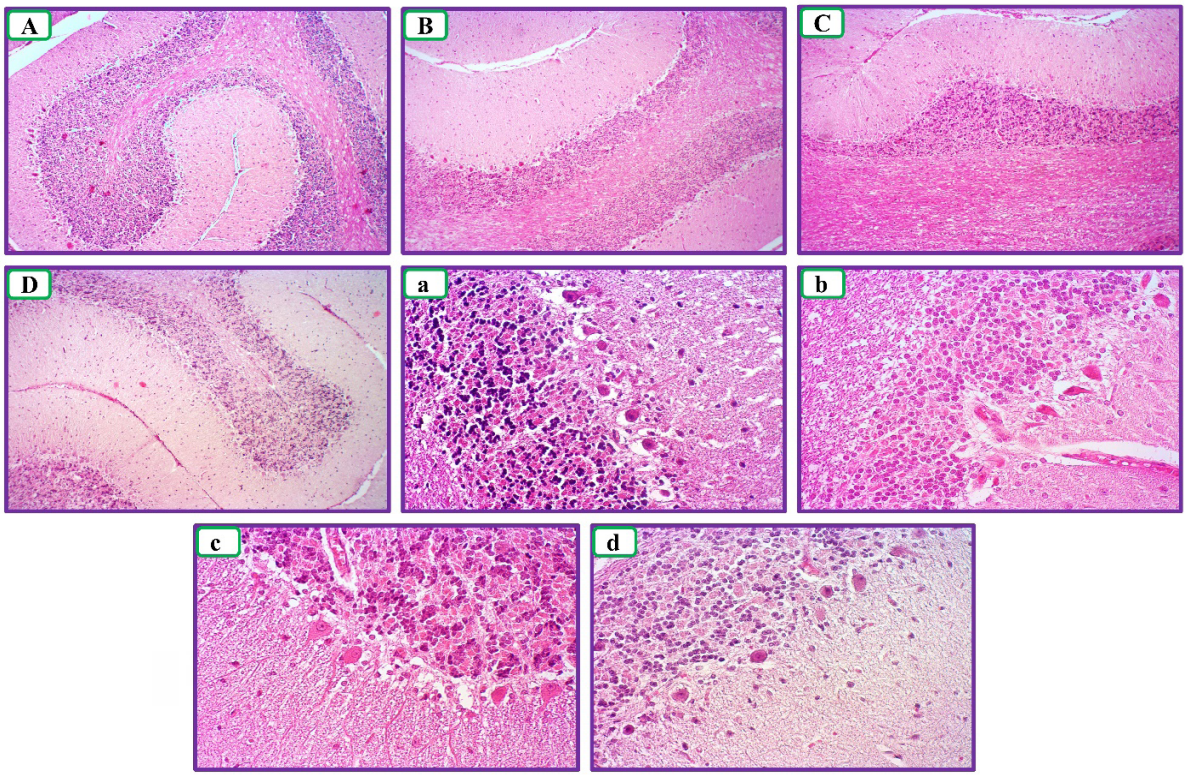

Figure 5. Light micrograph of cerebellum lesions stained with H&E at 10x (A, B, C & D) and 40x (a, b, c & d)

Note: Lesions were removed 14 days after the exposure to 100±5 dB noise (B & b), 1000±50 ppm toluene (C & c), co-exposure to 100±5 dB noise and 1000±50 ppm toluene (D & d), and without treatment (A & a).

Exposure routes: Inhalation exposure to toluene and hearing noise.

ebellum area in group 3. A decrease in the population of Purkinje cells, a decrease in the thickness of the granular layer, and a decrease in granule cells were some of the significant changes observed in the cerebellum in group 3 (Figure 5).

The cortex area in the frontal part of the brain was normal in the control group. Nevertheless, gliosis was detected in the prefrontal cortex area in groups 1-3 compared to the control group. The number of pyknotic cells in groups 1 and 3 appeared higher than group 2 in the prefrontal cortex area. Minor lymphocyte infiltration, which was possibly induced by the retrograde effect of toluene and transmitted to the frontal part of the brain cortex through olfactory nerves, was visible in the prefrontal cortex area in group 2. Some pathological changes were noticed in the prefrontal cortex area in group 3, such as disorganization in the cortex layer, relative reduction of pyramidal cells, vacuolization, and chromatolysis (Figure 6).

The light micrographs of the hippocampus area were normal in the control group. Dark cells were reduced in population and also were pyknotic in the hippocampus area in group 1, even more severely than in group 2. However, it was not remarkable compared to group 3. Moreover, the pyramidal cells in group 1 noticeably decreased in population compared to group 2 in the hippocampus area. But it was not remarkable compared to group 3. All the changes reported for group 1 also appeared in group 2 in the hippocampus area but were milder than in group 3 so the pathological changes appearing in group 3 were more severe than those in groups 1 and 2 (Figure 7).

4. Discussion

The results of this study showed that the noise caused an increase in TAC in the cerebral cortex. In a study on the effects of industrial noise stress on workers, the serum levels of CAT and TAC were significantly higher in workers exposed to noise stress (Bagheri Hosseinabadi et al., 2019). Increased SOD levels were reported

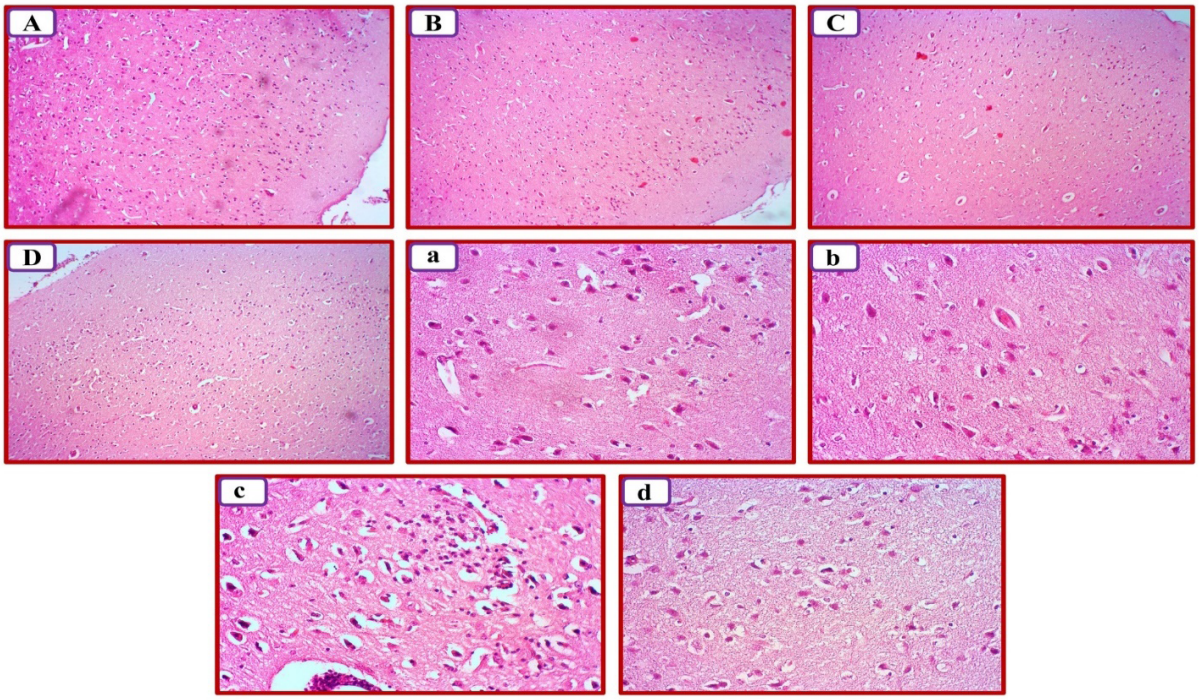

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Figure 6. Light micrograph of cortex lesions of frontal section stained with H&E at 10x (A, B, C, & D) and 40x (a, b, c, & d)

A & a) Without treatment; B & b) Lesions were removed 14 days after exposure to 100±5 dB noise; C & c) 1000±50 ppm toluene; D and d) Co-exposure to 100±5 dB noise and 1000±50 ppm toluene

Exposure routes: Inhalation exposure to toluene and hearing noise.

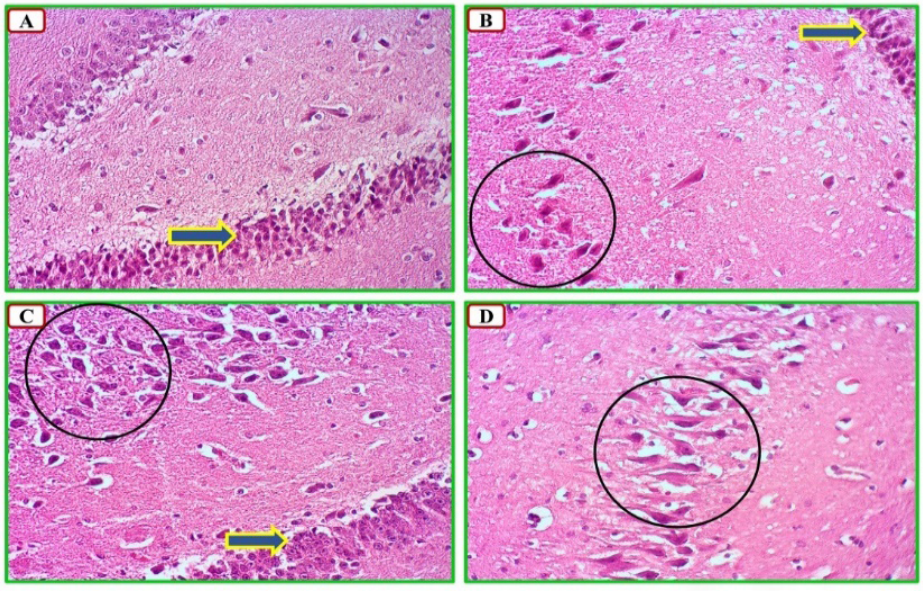

Figure 7. Light micrograph of hippocampus lesions stained with H&E (40x)

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A) Without treatment; B) Lesions were removed 14 days after exposure to 100±5 dB noise; C) 1000±50 ppm toluene; D) Co-exposure to 100±5 dB noise and 1000±50 ppm toluene.

Note: The control group (A), found without noticeable pathological changes. Some histopathological changes were observed in the noise exposure group (B), in the toluene exposure group (C), in the simultaneous exposure group (D) as marked in the figure. The signs marked in the photos presented pyramidal cells (dentate gyrus) ( ) and dark cells ( ). Exposure routes: Inhalation exposure to toluene and hearing noise.

by exposure to noise at 100 dB in the cerebral cortex of the rats' brain (Samson et al., 2007); however, increased anti-oxidant factors after exposure to high levels of noise may be due to induced oxidative stress during noise exposure. Moreover, activating protective mechanisms in the body, such as producing TAC, GPx, SOD, and CAT as anti-oxidant factors and decreasing MDA levels as an oxidative factor are predictable during oxidative stress to prevent the excessive generation of free radicals (Rosenbaum et al., 1994). Furthermore, some of the previous studies have shown that toluene induces oxidative stress in several brain regions by producing reactive oxygen species (ROS) (Kodavanti et al., 2011). As a result, ROS can induce apoptosis in the brain (Redza-Dutordoir & Averill-Bates, 2016). Therefore, co-exposure to toluene and noise changed oxidant and anti-oxidant parameters in the brain tissue.

BDNF is a protein encoded by a gene called *BDNF*. This protein promotes the growth and development of the central and peripheral nervous system. It also triggers nerve synapses and establishes neural connections. It is most active in the hippocampus and the upper part of the brain. The highest production of BDNF is in the brain, the amount of which is different in brain damage, especially with environmental stress.

The current study revealed that co-exposure to toluene and noise increased the serum levels of BDNF; however, exposure to noise decreased BDNF serum levels compared to the control group. In the study on the effects of toluene inhalation exposure (5 ppm) for five days, a decrease in the relative mRNA expression of BDNF, and an increase in the mRNA expression levels of *NF-κB* were reported in the hippocampus of the infant mice during brain development (Win-Shwe et al., 2012). In a study on the effects of chronic stress on BDNF levels, inducing 80 dB noise stress for five weeks made a significant reduction in hippocampal BDNF levels in rats (Taliaz et al., 2011). Moreover, another study demonstrated that acute stress caused oxidative stress and consequently, produced an increase in BDNF levels (Hacioglu et al., 2016). This means that there is a direct relationship between BDNF levels and oxidative stress indicators (Hacioglu et al., 2016). On the other hand, previous studies have shown that some stressors, such as noise initiate an increase in corticosterone levels (Laugero & Moberg, 2000; Lehmann et al., 2002; Şahin & Gümüşlü, 2007). Consequently, corticosterone can induce a decrease in BDNF levels (Jacobsen & Mork, 2006; Schaaf et al., 2000).

This study provides considerable insight into the combined effects of noise and toluene on neurological disorders. Based on the results of the present study related to oxidative stress induced after co-exposure to toluene and noise and according to the multiple combined stressors (Piggott et al., 2015), different synergistic and antagonistic effects induced by exposure to noise and toluene are provided in Appendices 1 and 2.

This study demonstrated that co-exposure to noise and toluene can induce different histopathological changes in the brain tissue, such as lymphocyte infiltration, a relative reduction in pyramidal cells, chromatolysis, and vacuolization. There were remarkable signs of inflammation in the co-exposure group, even more severe than the noise and toluene exposure groups. Based on the results obtained, it seems that even though there were no significant changes induced by noise exposure, there were some changes in the brain tissue, which if persisted, can be an important alarm. Induced mild atypia, edema, hyperemia, expansion of blood vessels, and glial cell hyperplasia were reported in several areas in the brain tissue in female rats by exposure to noise at 95 dB (4000 Hz) for two weeks (Xue et al., 2014). Makhlouf et al. (2014) reported a decrease in the thickness of the pyramidal cell layer of CA1 (cornu ammonis area 1), CA3 (cornu ammonis area 3), and also the granular cells, which exhibited degeneration and loss of many cells after exposure to 100 dB noise during four weeks (4 h/day) in male albino rats (Makhlouf et al., 2014). Abousetta et al. (2014) indicated that exposure to 100 dB white noise for 6 h/day during four consecutive weeks induced a decrease in the thickness of dentate gyrus (DG) granular cells and pyramidal cells with the loss and degeneration of numerous cells in male albino rats (Abousetta et al., 2014).

Enhanced glial fibrillary acidic protein (GFAP)-immunoreactive cytoplasm was demonstrated in the white matter of the cerebellum and in the dentate gyrus of the hippocampus by inhaling 1500 ppm toluene for four and seven days in the brain tissue of the rats (Gotohda et al., 2000). In addition, Gotohda et al. (2000) showed that chronic exposure to 3000 ppm toluene for 12 weeks caused severe degenerative changes, dark pyknotic nuclei, and small cytoplasm in neurons of different hippocampal regions in rats (Gotohda et al., 2000). Kanter (2007) demonstrated that exposure to 3000 ppm toluene for 12 weeks (8 h/day) induced dilated cisternae of endoplasmic reticulum, small cytoplasm, evidently inflamed mitochondria with deteriorated cristae in the hippocampus in male Wistar albino rats (Kanter, 2008). Tas et al. (2012) reported that exposure to a high dose of toluene (5200 mg/kg/gavage) for two weeks caused a significant

increase in the immune reactivity of Bax in the cerebellum and the brain cortex (Tas et al., 2012). Demır et al. (2017) demonstrated that acute exposure of New Zealand rabbits to toluene by a single dose (876 mg/kg) intraperitoneal (IP) injection into the brain caused severe dilation of blood vessels, diffuse cell borders, vacuolar degeneration, severe degeneration of the compensation, perivascular demyelination, gliosis, and several necrosis and pyknotic cells (Demır et al., 2017). Moreover, a noticeable enhancement in the count of apoptotic cells in the cerebral granule layer was indicated by inhaling 1200-1800 ppm toluene for 6 h per day in rats (Dalgaard et al., 2001). Nevertheless, Seo et al. (2009) indicated that exposure to toluene at the dose of 500 mg/kg did not cause apoptosis in the hippocampus in mice (Seo et al., 2010). Considering the findings proven by previous studies and the results obtained from this study, noise, and toluene are some of the noticeable neurotoxic pollutants.

5. Conclusion

This study indicated that co-exposure to toluene and noise might induce neurotoxicity in white New Zealand rabbits. Histopathological experiments indicated that co-exposure to toluene and noise could aggravate neurotoxic effects. Co-exposure to noise and toluene induced some pathological changes in the cerebellum, hippocampus, and frontal section. Nonetheless, further studies are needed to determine the definite neurotoxic effects induced by co-exposure to toluene and noise.

Limitations

Although this study showed non-auditory changes induced by exposure to toluene and noise, neurotoxic effects induced by chronic exposure to noise and toluene were not identified. Furthermore, this study did not evaluate other biological and pathological factors during or after exposure. Therefore, further studies are necessary in order to enhance the results obtained from this work.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the National Ethics Committee of Tabriz University of Medical Sciences, for biomedical research in Iran (Code: IR.TBZMED.REC.1396.953).

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Authors' contributions

Conceptualization, supervision, investigation, data analysis and writing: Amirreza Abouee-Mehrizi and Yahya Rasoulzadeh; Methodology: Amirreza Abouee-Mehrizi, Yahya Rasoulzadehand, and Masoud Motallebi-Kashani; Data collection: Amirreza Abouee-Mehrizi, Yahya Rasoulzadeh, and Ahmad Mehdipour; Nafiseh Nasirzadeh, Omid Shatouei-Gharenjeh and Abbas Ebrahimi-Kalan.

Conflict of interest

The authors declared no conflict of interest.

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Appendix 1. Parameter estimates of GEE analysis (BDNF-α) and ANOVA analysis (Gpx, TAC, MDA (log), catalase, SOD)

Parameter	Group 1	Group 2	Group 3	Interaction Type
BDNF-α (0th day●)*	17.436±28.468	-30.314±24.750	5.280±22.915	-Antagonism
BDNF-α (3rd day●●)*	1.374±25.569	-36.676±21.176	39.340±23.573	+Synergism
BDNF-α (7th day●●●)*	-13.055±29.367	-16.63±17.939	-6.460±24.754	-Antagonism
BDNF-α (14th day●●●●)*	-11.782±19.406	-20.457±25.493	1.680±23.343	+Synergism
Gpx**	0.536±0.688	0.091±0.688	-0.175±0.688	-Synergism
TAC**	774.700±90.0[B]	-334.700±90.0[a]	-207.600±90.0[a]	+Antagonism
MDA (log)**	47.700±82.8	-91.300±82.8	119.700±82.8	+Synergism
Catalase**	1.215±0.784	-0.548±0.784	0.047±0.784	+Antagonism
SOD**	36.200±40.5	22.800±40.5	2.700±40.5	+Antagonism

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Abbreviations: GEE: Generalized estimating equations statistical method; ANOVA: analysis of variance statistical method; BDNF-α: Brain-derived neurotrophic factor-α; GPx: Glutathione peroxidase; MDA (log): Logarithm malondialdehyde; SOD: Superoxide dismutase; TAC: Total antioxidant capacity; Exposure routes: Inhalation exposure to toluene and hearing exposure to noise.

Notes: Group 1: Exposure to 100±5 decibel (dB) noise (n=6); Group 2: Exposure to 1000±50 part per million (ppm) toluene (n=6); Group 3: Co-exposure to 100±5 dB noise and 1000±50 ppm toluene (n=6); 0th day●: Measured immediately after the end of exposure; 3rd day●●: Measured on the 3rd day after the end of exposure; 7th day●●●: Measured on the 7th day after the end of exposure; 14th day●●●●: Measured on the 14th day after the end of exposure; Significant changes (P<0.05).

[b]Significant changes (P<0.001), *Measured in serum, values for these parameters are the β value±SE (standard error), **Measured in cortex on the 14th day after the end of exposure, values for these parameters are the coefficient value±SE (standard error of coefficient).

Appendix 2. Coefficient values of ANOVA analysis for logarithm brain weight/body weight

Groups	Coefficient	SE	95% CI	P
1	-0.0486	0.0155	-0.0809, -0.0162	0.005
2	0.0399	0.0155	0.0076, 0.0723	0.018
3	0.0134	0.0155	-0.0189, 0.0458	0.397
Interaction type	Antagonism			

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Group 1: Exposure to 100±5 decibel (dB) noise (n=6); Group 2: Exposure to 1000±50 part per million (ppm) toluene (n=6); Group 3: Co-exposure to 100±5 dB noise and 1000±50 ppm toluene (n=6); Exposure routes: Inhalation exposure to toluene and hearing exposure to noise; Values measured on the 14th day after the end of exposure.

Abbreviations: ANOVA: Analysis of variance statistical method; CI: Confidence interval; SE: Standard error.