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Toxicological Effects of Simultaneous Exposure to Toluene and Noise on some Sexual and Stress Parameters in New Zealand White Rabbits

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ABSTRACT

Noise and toluene are among the numerous physical and chemical pollutants that can induce adverse effects on different body tissues and systems; nevertheless, most studies have only experimented the auditory changes induced by co-exposure to them. The present in-vivo study aimed to examine the endocrine effects of co-exposure to toluene and noise on the testes and adrenal glands. In this experimental study, 24 healthy male New Zealand White rabbits were used. The noise intensity was 100 dB (white noise) and the toluene concentration was 1000 ppm for two consecutive weeks. The luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, cortisol and adrenocorticotropic hormone (ACTH) were measured using the enzyme-linked immunosorbent assay (ELISA) method. The hematoxylin and eosin stain method (H&E) was performed for the histopathological analysis. Comparing different parameters in different groups on post-exposure days was carried out using GEE (generalized estimating equations) method. The results indicated that noise and toluene increased cortisol, LH and FSH levels during different days after the exposure. Exposure to toluene and noise made vacuolization and reduction of primary spermatogonial cells in the testes. Moreover, lymphocyte infiltration, congestion, swelling and vacuolization were detected in adrenal glands through exposure to toluene and noise. Toluene and noise induced different destructive effects on the endocrine system. More studies are required to elucidate other endocrine changes induced by exposure to toluene and noise.

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INTRODUCTION

Nowadays, noise generation is an unavoidable phenomenon in different activities. People are exposed to noise both in the living environment and at the workplace all over the world (Wang et al., 2010; Abouee-Mehrizi et al., 2020a). Numerous harmful factors are physically or chemically generated at the workplace and in the living environments (Mader et al., 2004). Noise is one of the most common harmful physical factors (Licitra et al., 2018). Data from several studies demonstrate that exposure to noise affects the auditory system and visual organs, and induces physiological responses such as increased blood pressure and heart rate (Rosenlund et al., 2001; Bluhm et al., 2007; Jarup et al., 2008). It has also been reported that noise exposure induces mental disorders (Stansfeld et al., 2000; Haines et al., 2001), nervous problems (Lenzi & Bonsanto, 2018), interference with conversations (Kryter et al., 2013), sleep disturbances (Kryter et al., 2013) and social problems and discomforts (Kryter et al., 2013). Noise as a psychological and physical stressor also reduces people's productivity (Dolan, 2006).

Toluene as an aromatic hydrocarbon is widely used in the industry and many people are directly or indirectly exposed to it (Heibati et al., 2018). As an industrial solvent, it is utilized in many chemical products and industries, including petrochemical, plastic, agriculture, textile and pharmaceutical. Exposure to toluene often occurs through the respiratory system because of the volatility of this formulation (Heibati et al., 2018). Previous studies have demonstrated some toxic effects of toluene through long exposure or high concentrations (Prell et al., 2011; Abouee-Mehrizi et al., 2021). These effects include disorders in the auditory system (Prell et al., 2011), nervous system degeneration (Brown, 2008; Dobbs, 2009), adverse psychiatric effects as well as kidney and liver tissue damage (Abouee-Mehrizi et al., 2020b).

A much-debated question in the field of toxicology is investigating the interaction of different physical and chemical factors (McQueen, 2010; Abouee-Mehrizi et al., 2022). Many people exposed to noise are additionally exposed to toluene as a hazardous and harmful chemical substance; consequently, they are exposed to these factors simultaneously at various wood, chemical, plastic and pharmaceutical industries (Schäper et al., 2008; Board, 2017). While some studies have focused on sexual or endocrine changes induced by non-simultaneous exposure to noise or toluene, no study has been performed on the simultaneous exposure to noise and toluene as well as its effects on the sexual and endocrine systems. Accordingly, this project provides a unique opportunity to advance our understanding of the effects of co-exposure to noise and toluene on testicular and adrenocortical tissues, some sexual and stress parameters and a number of biochemical factors.

MATERIALS AND METHODS

In total, 24 healthy adult male New Zealand White rabbits (from Pasteur Institute of Tehran) weighing 2.83±0.41 kg were handled in this study. The animals were placed in an animal laboratory room with controlled temperature (21± 2 °C), relative humidity (50–70%) and a 12:12 hour light/dark cycle. The animals were free to access standard rabbit pellets and treated water. Fourteen days before the experiments, the animals were given time to adapt to the conditions of the laboratory environment. They were randomly grouped into four groups (n = 6 per group): control group (group 4), noise exposure group (group 1), toluene exposure group (group 2) and co-exposure to toluene and noise group (group 3). The exposure duration was 14 consecutive days, 8 hrs/day from 9:00 a.m to 5:00 p.m. for all the groups (similarly, for the control group). Initially, the control group was placed in the chamber and exposed to fresh air (toluene concentration = 0 ppm). Afterwards, groups 1 and 2 were placed in the exposure chamber. Finally, the co-exposure to toluene and noise group was located in the exposure chamber and exposed to similar specifications of the noise and toluene of groups 1 and 2. The

relative humidity was 60-80% and the temperature was 23±5 °C inside the exposure chamber for the period of exposure for all the groups. This experimental study was accepted by the National Ethics Committee in Iran's biomedical research and certification code is IR.TBZMED.VCR.REC.1397.083.

All the exposure groups were exposed in the same chamber, with the size of 50×60×90 cm^3. The exposure chamber was built with clear and transparent polycarbonate plates with the inside surfaces coated with transparent PET (polyethylene terephthalate) adhesive tape. The characteristics of the exposure chamber were designated considering the reverberant chamber features (Cobo et al., 2009) to produce similar noise at different distances in the exposure chamber and based on the animals' safety requirements (Gad, 2006). A mixer chamber was chosen and built with similar materials to the exposure chamber and with dimensions 50×50×20 cm^3.

White noise at 100±5 dBA was generated by the noise generator software (Audacity® Software 1.3.12 Beta) and checked by the monitoring software (Cool Edit Pro 2.1 ©1992-2003 Syntrillium Software Corporation) amplified using a power amplifier (3030 W, MULTI TONE) and transported to a speaker into the exposure chamber. The speaker was located directly above the middle zone of the exposure chamber roof. Specifications of the noise (SPL and bandwidth) were constantly measured by a sound level analyzer (TES 1358 Sound Analyzer Real-Time 1/1 & 1/3 Octave band Analyzer) in the 1/3 octave band. The microphone of the sound level meter was placed at the level of the animals' ears (through designed control outlets made with rubber tape and covered by PET adhesive tape) and using linear weights. The basic bandwidth of the noise made by the speaker was 70 - 80000 Hz (1/3 octave band).

Exposure to 1000 ±50 ppm toluene was made by the principle of liquid surface evaporation. Continuous toluene vapors were produced using an impinger (capacity of 250 ml) which at first contained 100 ml Merck KGaA toluene liquid (extra pure). Then, 20 ml toluene was injected into it every 90 min during exposure to toluene. The clean airflow rate was 30 lit/min and the toluene vapor flow rate was 3 lit/min to continuously receive the intended toluene concentration. A humidity-resistant real-time tool (Phocheck, Ion Science Ltd, Cambs UK 07-01782) which had already been calibrated was utilized to check and monitor toluene concentration during the exposure. Air samples were collected from the inside air of the exposure chamber through the designed control outlets. Toluene vapor flow rate, clean air flow rate, and level of toluene liquid in the impinger had been checked before the real exposure using the same calibrated real-time tool employed in real exposure as well as by charcoal absorbents through gas chromatography (Model Agilent 7890A GC System) based on the 1501 NIOSH method. Before commencing the animal exposure, toluene purity was demonstrated through air sampling of the exposure chamber by charcoal absorbents and via a gas chromatography-mass spectrometry device (Agilent 6890 / 5973 GCMS).

The rabbits were anesthetized using 35 mg/kg ketamine and 5 mg/kg xylazine through intramuscular injection and, then, sacrificed. The tissues of the testicles and the adrenal glands were removed and fixed in 10% formalin (formaldehyde solution, Merck KGaA, 1.04002.2500, 64271 Darmstadt, Germany) at pH=7.2. The drying up, clearing up and paraffin impregnation of the samples were performed using an Autotechnicon device. Subsequently, paraffin blocks were cut into 5-micron-thick sections using a microtome. Finally, the prepared slides were stained with hematoxylin and eosin (H&E). A Nikon Eclipse E100 light microscope (Nikon, Japan) used for viewing the slides.

The marginal vein of the rabbits' ears was used for blood collection which was performed in three phases: before exposure and on the 3rd, 7th and 14th days post-exposure (Fig. 1). The blood samples were centrifuged at 3000 rpm for 15 min, 1 hour after blood collection, to make serum samples. The serum samples were kept in a freezer at -80 °C up to starting biochemical and ELISA experiments.

The serum levels of ACTH, testosterone, cortisol, FSH and LH were determined by ELISA

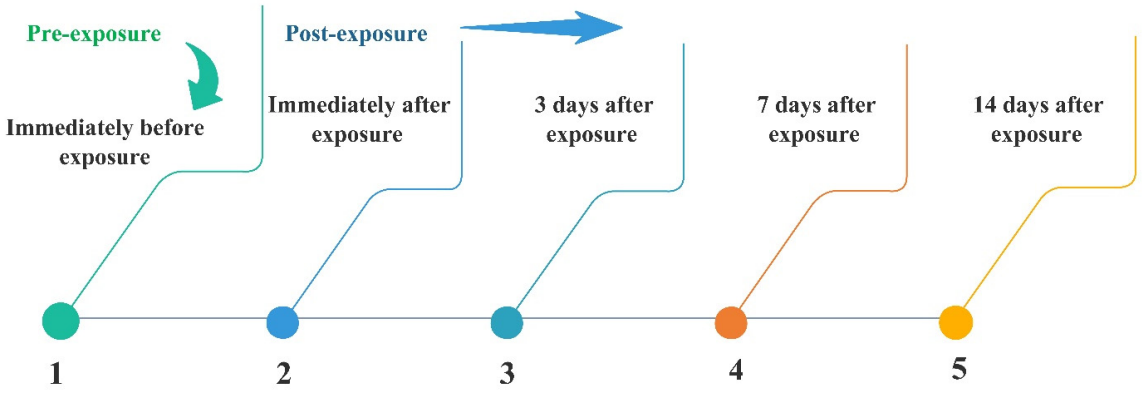

Fig. 1. Different phases of blood sampling

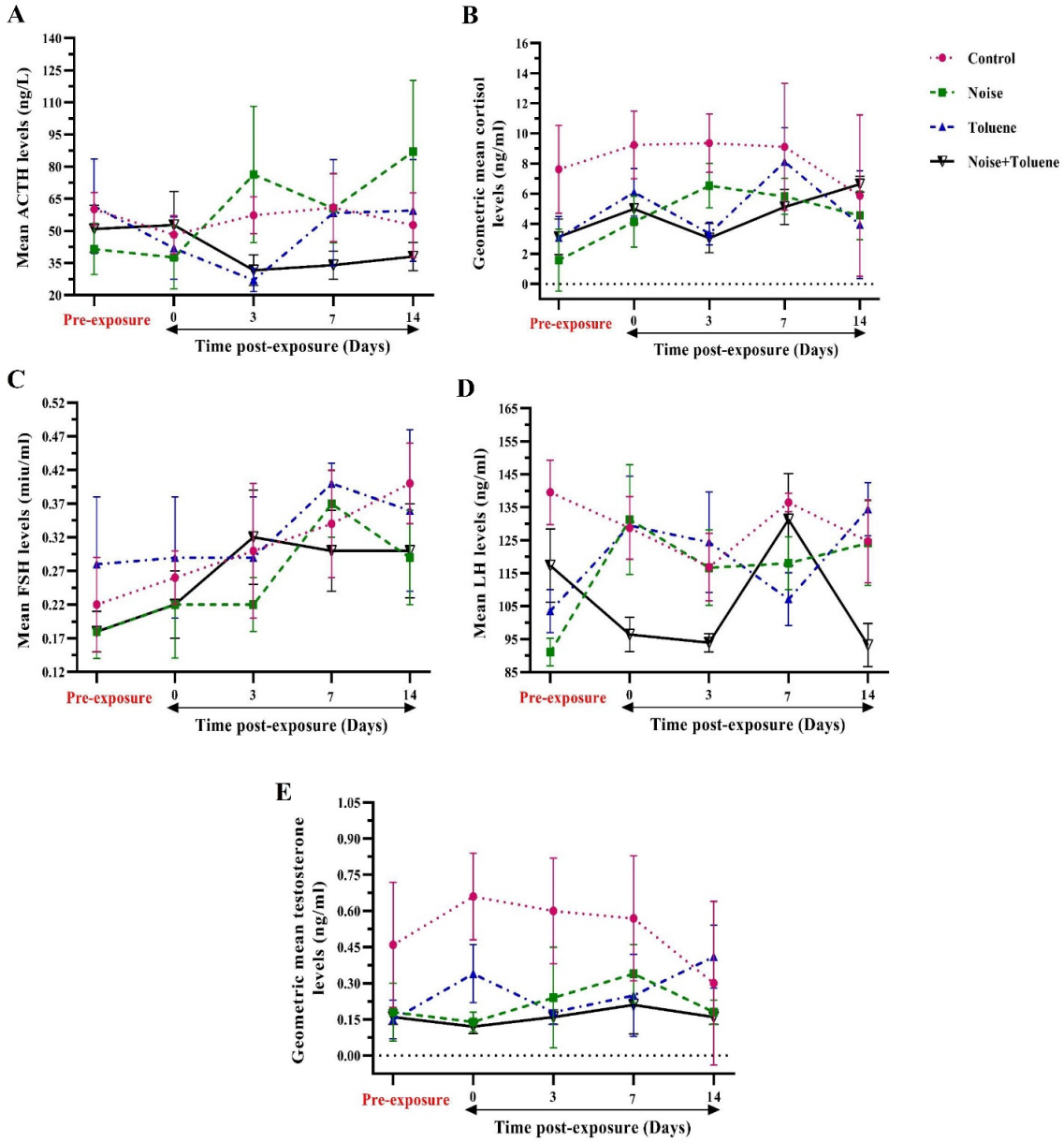

Fig. 2. Serum levels of different hormones in the exposure groups at different times. ACTH (A), cortisol (B), FSH (C), LH (D) and testosterone (E). Points represent mean ± SE (n=6). See text and Appendices 1 to 5 for significant changes.

kits (HANGZHOU EASTBIOPHARM CO., LTD, Hangzhou, China) and the experiments were conducted according to the manufacturer's guidelines (Cat. No (ACTH): CK-E80162, Cat. No (cortisol): CK-E80139, Cat. No (FSH): CK-E91856, Cat. No (LH): CK-E91857, Cat. No (testosterone): CK-E91858). 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 in these experiments.

The GEE (generalized estimating equations) statistical method was adopted to compare the changes and differences in ACTH, cortisol, LH, FSH and testosterone levels between the repeats per group using IBM SPSS 25. Analysis of variance (ANOVA) followed by Tukey's pairwise comparisons was used to compare tissue weight/body weight across the groups in Minitab 18. The statistical significance level was 0.05. All the indices measured in this study had a normal distribution, except for testosterone and cortisol levels.

RESULTS AND DISCUSSION

The serum level of ACTH in group 2 was significantly lower than that in group 4 in phase 3. The ACTH level in group 3 was significantly lower than that in group 4 in phase 3; in addition, the ACTH level in group 3 was significantly lower than that in group 1 in phase 4 (Fig. 3-A, Appendix 1).

The serum cortisol level was significantly lower in group 1 than group 4 in phase 2, significantly lower in group 2 than group 4 in phase 3, significantly lower in group 3 than group 1 in phase 3 and significantly lower in group 3 than in group 4 in phases 2 and 3 (Fig. 3-B, Appendix 2).

Based on the results of changes at the serum level of FSH across the groups and repeats, the repeats and the groups significantly changed during the study. In terms of the repeats, the FSH level significantly increased in phase 4 compared to phase 2 in all the groups. Furthermore, it

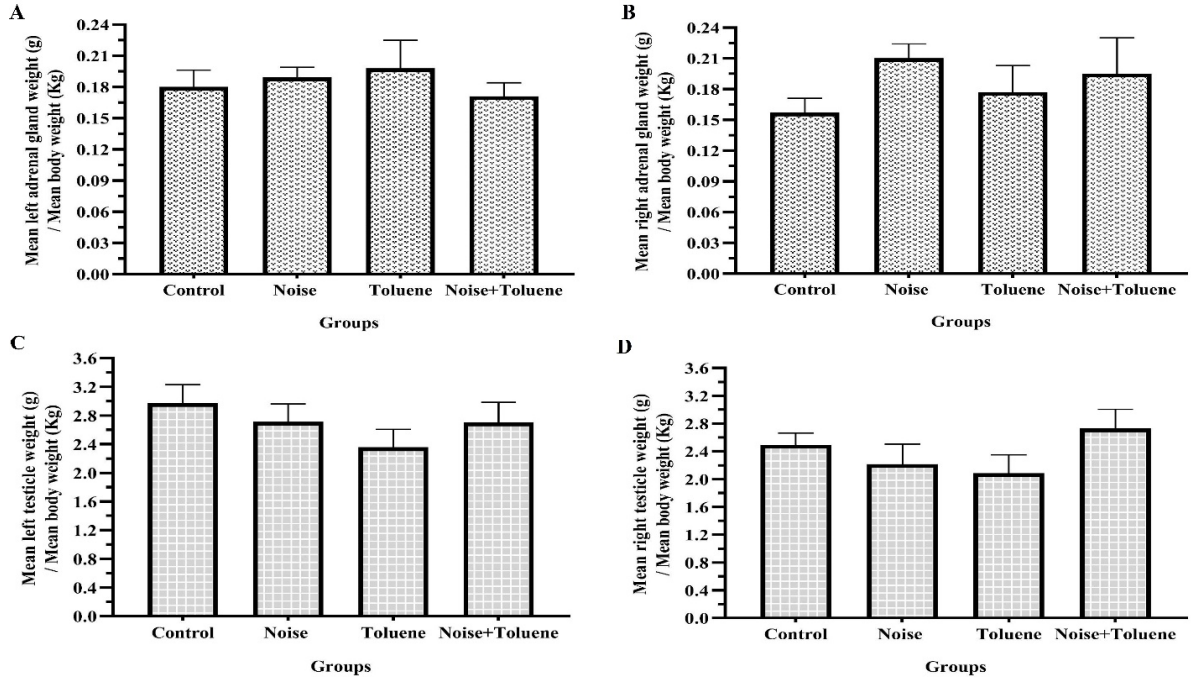

Fig. 3. Mean of tissue weights/body weights in the exposure groups. Left adrenal glands/body weight (A), right adrenal glands weight/body weight (B), left testicle weight/body weight (C), left testicle weight/body weight (D). Bars represent mean ± SE (n=6).

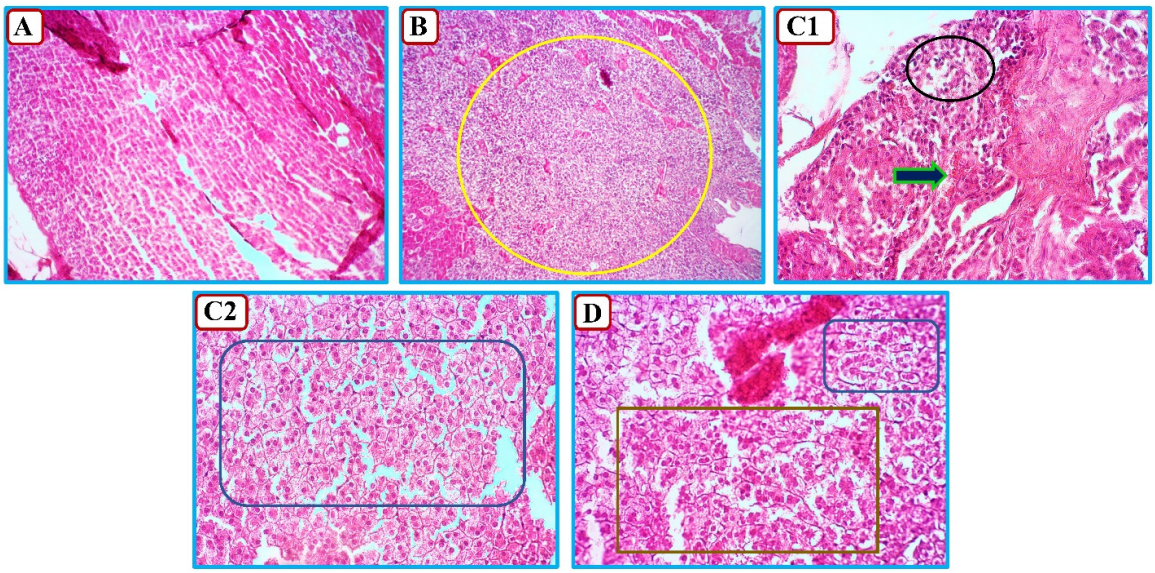

Fig. 4. Photomicrographs of rabbit adrenal glands (H&E) at 10× (A, B and C1) and 40× (C2 and D); (A): control without any significant damage; (B): exposed to 100dB noise; (C1 and C2): exposed to 1000ppm toluene; and (D): simultaneously exposed to 100dB noise and 1000ppm toluene. Signs denote medulla ( ), lymphocyte infiltration ( ), congestion ( ), swelling and vacuolization ( ) and severe tissue damage ( ).

significantly rose in phases 4 and 5 compared to phase 1 in all the groups. In terms of the groups, group 2 was significantly higher than group 1 in the overall repeats (Fig. 3-C, Appendix 3).

The serum LH level was significantly lower in group 2 than group 4 in phase 4, significantly lower in group 3 than group 2 in phases 3 and 5, significantly higher in group 3 than group 2 in phase 4, significantly lower in group 3 than group 1 in phase 5 and significantly lower in group 3 than group 4 in phases 2 and 3 (Fig. 3-D, Appendix 4).

The serum testosterone level was significantly lower in group 1 than group 4 in phases 2, 3 and 5, significantly higher in group 2 than group 1 in phase 5, significantly lower in group 2 than group 4 in phase 3, significantly lower in group 3 than group 2 in phases 2 and 5 and significantly lower in group 3 than group 4 in phases 2 and 3 (Fig. 3-E, Appendix 5).

According to Fig. 3, there was no significant difference in tissue weight/body weight among the groups. The histologic slides of rabbit adrenal glands stained with H&E had a normal structure in the control group (Fig. 4). The capsule, cortex and medullar layer of the adrenal glands were in normal conditions in the control group. The medullar layer was obviously expanded in group 1 compared to the control group, which may be due to the stress induced in group 1 and, consequently, the increased adrenaline level in this area. In other words, the medullar area of the adrenal glands was larger and more activated probably because of the elevated adrenaline level in group 1. Lymphocyte infiltration was observed in the capsule and zona glomerulosa in the adrenal glands of group 2. Cellular swelling, vacuolization and congestion were also detected in group 2. It seems that, overall, noise induced destructive effects on the medullar layer and toluene induced pathological alterations in cortex layers. More destructive effects were observed in group 3 compared to other groups. Lymphocyte infiltration was visible in the capsule area and the capsule was full of blood vessels in group 3. Furthermore, cellular swelling, congestion and vacuolization were noticeable in the zona fasciculata in group 3 (Fig. 4).

The analysis of H&E-stained sections revealed the normal structure of the testis tissue in

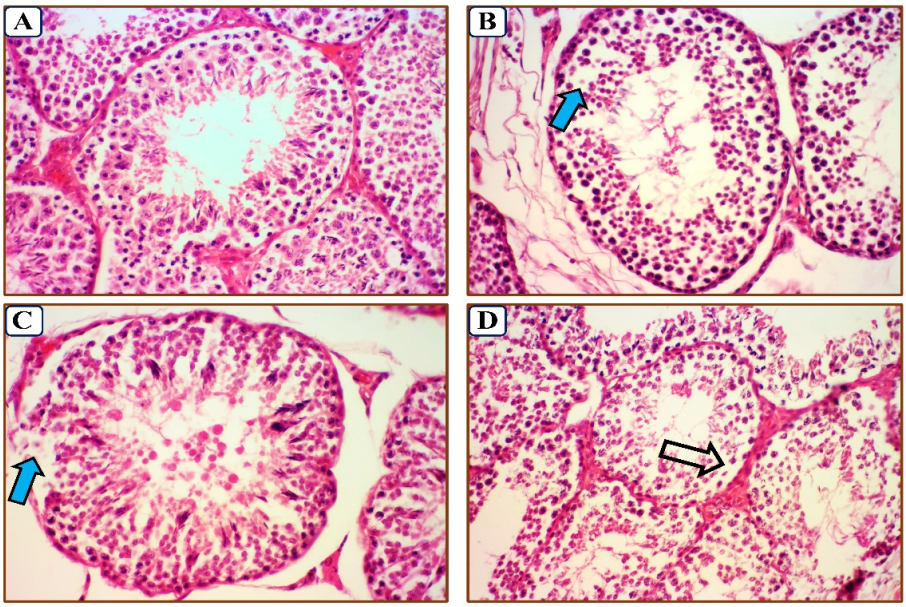

Fig. 5. Photomicrographs of rabbit testicular tissue (H&E, 40×); (A): control without any significant damage; (B): exposed to 100dB noise; (C): exposed to 1000ppm toluene; and (D): simultaneously exposed to 100dB noise and 1000ppm toluene. Signs denote vacuolization (⇨), and reduction of primary spermatogonial cells and spermatocytes (⇨).

the control group (Fig. 5). Nevertheless, vacuolization was observed in seminiferous tubules in the germinal parenchyma in group 1 compared to the control group, even more than in group 2. Moreover, the cellular regularity and number of spermatogonial cells in some seminiferous tubules in group 1 were lower than those of the control group; however, the parenchymal thickness of the tubules showed no obvious changes. Vacuolization was observed in the seminiferous germinal parenchyma in group 2, similar to group 1. Furthermore, spermatogonial cells in some seminiferous tubules in group 2 appeared to be more disordered and fewer in number than those in the controls and even group 1. Thickness of the parenchymal tubules in group 2 displayed no obvious differences compared to the control group. In addition, it seems that the number of seminiferous tubules containing stage 8 sperms in group 2 was lower than those in groups 1 and 4. According to Fig. 5-D, it seems that the degenerative effects of noise and toluene were more severe, and the seminiferous tubules were free of stage 8 sperms in group 3. In addition, fewer spermatogonial cells and primary spermatocytes appeared in the tubules in group 3 compared to the other groups. Therefore, more severe destructions were observed in group 3 compared to the other groups (Fig. 5).

Inducing various stresses, such as physical and chemical stresses, can directly impact the secretion of various hormones in the body (Ranabir & Reetu, 2011); consequently, varying the level of different hormones causes different problems in the body (Natelson, 2004; De Kloet et al., 2005). Increasing exposure to various harmful physical and chemical agents such as noise and toluene can be a warning for conducting further studies for reducing the generation of/exposure to these environmental and occupational pollutants (Rider et al., 2018).

Results showed that stress hormones increased by exposure to noise; additionally, some histopathological alterations such as an expanded medullar layer in adrenal glands and vacuolization in the testicular tissue were demonstrated following the noise exposure. The

increased volume of the medulla and cortex of adrenal glands as well as the serum level of cortisol were explained by exposure to noise at 100 dB for 30 days in rats (Monsefi et al., 2015). Farzadinia et al. (2016) reported a decrease in the serum testosterone level, and an elevation in the serum cortisol and ACTH levels by exposure to 115dB noise stress for 60 days in rats (Farzadinia et al., 2016). In addition, increased serum levels of cortisol were found in another study upon exposure to 90dB white noise for 30 days in mice (Taban et al., 2017). Furthermore, in the study by Farzadinia et al. (2016), decreased thickness of the germinal epithelium and the mean diameter of the seminiferous tubules as well as increased ratio of the interstitial area of the testicular tissue were reported following exposure to noise (Farzadinia et al., 2016). Therefore, noise can arguably induce many endocrine disorders.

As for toluene exposure, the findings indicated that the ACTH level decreased and the cortisol level increased following exposure to toluene. Moreover, lymphocyte infiltration, cellular swelling, vacuolization, and congestion in adrenal glands, as well as vacuolization in the testicular tissue were some of the obvious histopathological changes caused by toluene exposure. Increased plasma ACTH levels had already been reported following exposure to 1500 ppm of toluene inhalation for 7 days in rats (Gotohda et al., 2005). Gotohda et al. (2005) proposed that toluene exposure induced adrenocortical hypertrophy by the hypothalamus-pituitary-adrenal gland (HPA) axis (Gotohda et al., 2005). Amsterdam et al. (1989) reported that abnormal adrenocortical responsiveness to adrenocorticotropic hormone (ACTH) may induce excessive secretion of cortisol (Amsterdam et al., 1989). Thus, toluene elevated the cortisol level by inducing adrenocortical hypertrophy and, therefore, it is a major risk factor for endocrine diseases.

The findings demonstrated that, overall, LH and FSH levels rose and testosterone level decreased by non-simultaneous exposure to noise and toluene compared to the control group, and in some days, after the exposure. The elevated serum levels of LH and FSH as well as the reduced serum level of testosterone had previously been reported by exposure to noise at 90-130 dB for 50 days in adult male rats (Fathollahi et al., 2013).

According to the examination of the toxicity effects of toluene on semen quality, apoptotic degeneration, and testis morphology in male rats, inhalation exposure to 1200 ppm of toluene for 6 h per day from the gestational day to postnatal day exhibited no significant changes in semen quality and testis tissue (Dalgaard et al., 2001). Nevertheless, reduced sperm motility, epididymal sperm count, and sperm quality were shown by inhalation exposure to 6000 ppm of toluene for 5 weeks in rats (Ono et al., 1999). Another study on the effects of 1500 ppm of toluene inhalation for 20 days in rats, toluene exhibited a slight increase in signals on in situ apoptosis detection (Ishigami et al., 2005).

Decreased testosterone and epididymal sperm counts were reported following toluene injection (50, 500 mg/kg) in male rats (Nakai et al., 2003). Moreover, Nakai et al. (2003) concluded that the reproductive toxicity of toluene was induced through direct oxidative impairment of the spermatozoa. Nakai et al. (2003) suggested that oxidative DNA destruction in the testis tissue played a pivotal role in reproductive toxicity caused by toluene exposure (Nakai et al., 2003).

Results of the present study indicated that co-exposure to toluene and noise exhibited an elevation in the serum levels of cortisol and FSH, and induced a reduction in the serum levels of testosterone, LH, and ACTH.

The anterior pituitary gland is an essential endocrine gland due to the secretion of several hormones such as ACTH and reproductive hormones (LH, and FSH) (Marti & Armario, 1998). Previous studies have reported that stress can produce ACTH by inhibiting the anterior pituitary hormones and inducing intermediates on the gonadotropin gene (Dorshkind & Horseman, 2001; Breen & Mellon, 2014). ACTH can raise the production of steroid hormones such as cortisol after directing signals to the adrenal gland (Folt et al., 1999).

Although toluene is categorized into group 3 of carcinogen classification (IARC, 2021), co-

Table 1. *Parameter estimates of GEE analysis for all the measured parameters of different groups in phases 2-5*

Phase	Parameter	Group 1 (noise exposure)	Group 2 (toluene exposure)	Group 3 (simultaneous exposure)	Interaction type
2 (*immediately post-exposure)*	ACTH	7.923±12.330	-7.782±28.322	13.680±15.275	+Synergism
	Cholesterol	-18.510[a]±8.687	-11.203[a]±4.236	-20.190[b]±4.719	-Antagonism
	Cortisol (log)	0.333±0.488	0.214±0.218	0.119±0.118	+Antagonism
	Glucose	2.450±8.999	11.867±10.682	14.950±9.949	+Synergism
	FSH	0.001±0.159	-0.024±0.123	-1.160E-16±0.063	-Antagonism
	LH	50.935[a]±17.291	36.635[a]±10.582	-10.140±19.818	-Synergism
	Testosterone (log)	-0.275[a]±0.099	0.189±0.311	-0.276[a]±0.106	-Synergism
	Triglyceride	-12.967±19.544	-16.383±15.385	36.133[b]±9.560	+Synergism
3 (*3rd day post-exposure)*	ACTH	37.485±34.360	-31.815±27.699	-16.560[b]±4.252	+Antagonism
	Cholesterol	-5.250±9.558	-15.863[b]±4.210	-26.950[b]±4.275	-Synergism
	Cortisol (log)	0.526±0.502	-0.049±0.249	-0.100±0.185	-Synergism
	Glucose	-11.667±6.900	-11.817±6.788	-1.483±6.788	-Antagonism
	FSH	-0.040±0.183	-0.065±0.239	0.060±0.092	+Synergism
	LH	48.236[a]±19.712	43.461±25.179	-0.800±18.849	-Synergism
	Testosterone (log)	0.004±0.271	-0.040±0.288	-0.115±0.116	-Synergism
	Triglyceride	-36.350±25.042	-23.900±28.504	20.883±19.644	+Synergism
4 (*7th day post-exposure)*	ACTH	18.155±22.729	-3.895±25.901	-17.760±16.373	-Synergism
	Cholesterol	-16.310±15.976	-1.093[b]±7.760	-12.110[b]±7.942	-Antagonism
	Cortisol (log)	0.487±0.544	0.345±0.343	0.136±0.153	+Antagonism
	Glucose	9.800±9.094	-21.900[a]±8.529	5.583±10.092	-Antagonism
	FSH	0.074±0.079	-0.001±0.134	-1.060E-16±0.049	+Antagonism
	LH	30.013[a]±13.679	6.563±16.661	16.940±28.450	+Antagonism
	Testosterone (log)	0.176±0.090	0.125±0.191	0.015±0.211	+Antagonism
	Triglyceride	5.933±18.139	-15.900±13.080	20.00±19.196	+Synergism
5 (*14th day post-exposure)*	ACTH	52.785±38.757	5.385±44.010	-5.620±11.766	-Synergism
	Cholesterol	-36.960[a]±11.361	-13.973[a]±6.433	-35.910[b]±6.142	-Antagonism
	Cortisol (log)	0.570±0.373	0.221±0.608	0.439[a]±0.173	+Antagonism
	Glucose	4.767±5.310	-21.350[a]±8.206	-14.567±9.660	-Antagonism
	FSH	-0.068±0.106	-0.093±0.251	-0.060±0.108	-Antagonism
	LH	47.921[a]±17.155	45.646[a]±16.858	-9.300±28.516	-Synergism
	Testosterone (log)	0.187±0.158	0.611[a]±0.199	0.187±0.113	+Antagonism
	Triglyceride	-11.700±19.649	-14.550±15.881	33.283±19.783	+Synergism

[a] Significant changes (p-value < 0.05), [b] Significant changes (p-value < 0.001)

All values are the β value ± SE (standard error)

Table 2. *ANOVA coefficients for tissue weight/body weight in different groups*

Tissue	Group 1 (noise exposure)	Group 2 (toluene exposure)	Group 3 (simultaneous exposure)	Interaction type
Adrenal gland (right)	0.0252±0.0214	-0.0073±0.0214	0.0101±0.0214	+Antagonism
Adrenal gland (left)	0.0045±0.0159	0.0134±0.0159	-0.0133±0.0159	-Synergism
Testicular (right)	-0.165±0.221	-0.296±0.221	0.347±0.221	+Synergism
Testicular (left)	0.025±0.225	-0.330±0.225	0.018±0.225	-Antagonism

All values are the coefficient value ± SE (standard error of coefficient).
All values are insignificant (p-value >0.05).

exposure to noise and toluene could have different carcinogenic effects. According to the results of the current study and according to the evaluation of several stressors' effects (Piggott et al., 2015; Gannouni et al., 2014), toluene and noise had synergistic and antagonistic changes on the measured parameters (Tables 1 and 2).

Oliveira et al. (2009) reported that decreased zona fasciculata (ZF) and increased zona reticularis volumes in the adrenal glands of rats were induced by exposure to 92dB noise for 1 to 7 months (8 h/day and 5 days/week) (Oliveira et al., 2009). Zidan and Elnegris (2013) concluded that the loss of architecture of the zona glomerulosa and fasciculata through cellular infiltration occurred following exposure to 100dB noise in male guinea pigs (Zidan & Elnegris, 2013).

According to some studies, noise exposure can induce the production of nicotinamide adenine dinucleotide phosphate oxidase (NOX) (Vlajkovic et al., 2013) and increase reactive oxygen species (ROS) levels (Nicotera et al., 1999). Furthermore, some aromatic compounds such as toluene can increase ROS production in different organs (Revilla et al., 2007; Verma & Rana, 2008; Singh, 2010). Consequently, ROS can induce apoptosis through the expression of different apoptosis genes (Redza-Dutordoir & Averill, 2016).

As found in this study, noise and toluene significantly reduced the testosterone level on the 3rd day after the exposure. Furthermore, exposure to noise and co-exposure to toluene and noise significantly decreased the testosterone level on the day of tissue extraction. Therefore, the destructive effects of noise and toluene on testicles supported by the pathological evidence in this study decreased the testosterone levels.

The generalizability of these results is subject to certain limitations. The restricted budget led to a limited exposure time, a limited sample size, a similar toluene concentration, a similar noise intensity and lack of histochemical and specific staining of the studied tissues.

CONCLUSION

The current study identified the toxicological effects of co-exposure to toluene and noise on the testes and adrenal glands of New Zealand White rabbits. Toluene and noise are able to make antagonistic and synergistic changes on the adrenal glands and testes. Although insightful results were obtained in this study, more studies are required to elucidate the specific endocrine effects of co-exposure to toluene and noise in various study designs.

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

Experiments in this study were performed according to the "guidelines for keeping and protecting laboratory animals" of Ethics Committee of Tabriz University of Medical Sciences. This experimental study was accepted by National Ethics Committee in Iran's Biomedical Research under the certification code IR.TBZMED.VCR.REC.1397.083.

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