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Protective Effects of Pomegranate Juice on Testicular Histopathology and Sex Hormone Levels in Noise-Exposed Rats

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Abstract

Background: Occupational noise exposure represents the second most prevalent occupational hazard and is known to induce both auditory and extra-auditory health consequences. This study investigated the potential protective effects of pomegranate juice (PJ), as a natural antioxidant, on sex hormone levels and testicular histopathological alterations in noise-exposed rats.

Materials and Methods: In this experimental study, 64 adult male Wistar rats were randomly divided into eight groups (n = 8). After defining the control and noise groups, the remaining six groups received fresh PJ at doses of 0.5, 1, and 1.5 mL under two conditions, with and without noise exposure (100 dB). Sex hormone levels (testosterone, LH, and FSH) and testicular histopathology were assessed 50 days after treatment.

Results: Noise exposure significantly reduced the levels of all three hormones (testosterone, LH, and FSH) compared with the control group ($p < 0.05$). Administration of PJ at all three doses significantly increased testosterone and FSH levels in the noise-exposed groups ($p < 0.05$), whereas only administration of 1 and 1.5 mL of PJ significantly increased LH levels ($p < 0.05$). Histopathological analysis revealed that pomegranate juice (PJ) supplementation attenuated noise-induced testicular damage in the exposed groups.

Conclusions: The results indicate that noise exposure was associated with hormonal disruption and testicular damage, while PJ exerted protective effects on both parameters. Future studies should investigate the effects of pomegranate juice at multiple doses and exposure durations.

Keywords: Noise, Pomegranate, Testicular Tissue, Sex Hormones

Introduction

Chronic stress can lead to alterations in testosterone production, sperm parameters, and erectile function [1]. According to previous studies, people at the community level are exposed to noise from public transportation [2-3], agricultural land [4-6], and industrial sources [7]. Noise exposure, as a common stressor in industrial and social environments, leads to a significant decrease in sperm number, viability, and motility compared with the control group [8]. Previous studies have reported that noise exposure in rats results in reduced serum levels of LH, FSH, and testosterone, as well as impaired reproductive function [9, 10]. In a study conducted by Chamkori et al. investigating the impact of noise pollution on endocrine function among industrial workers in Bushehr, Iran, researchers found that prolonged exposure to 119 dB noise significantly suppressed serum concentrations of testosterone, LH, FSH, prolactin, and thyroid hormones (T3, T4, and TSH) compared with unexposed controls [11].

Pomegranate is a fruit with the botanical name Punica granatum, belonging to the family Punicaceae, and is widely cultivated in tropical and subtropical regions of the Middle East and India [12]. Pomegranate juice has a greater antioxidant capacity than other polyphenol-rich beverages [13]. Commercial pomegranate juice is obtained by completely squeezing the pomegranate fruit and its peel. The antioxidant potential and concentration of phenolic compounds in pomegranate juice are significantly higher than those of grape, cranberry, grapefruit, and orange juices [14, 15]. Pomegranates are rich in phenolic compounds, including flavonoids (such as anthocyanins), other complex flavonoids, and hydrolyzable tannins (e.g., punicalagin, gallic acid, and ellagic acid). These bioactive compounds exhibit strong antioxidant activity, which may contribute to their potential health benefits [16].

Previous studies have shown that pomegranate juice prevents damage to spermatogenesis caused by lead [17, 18] and carbon tetrachloride [19]. In a study by Bououza et al. in 2022, administration of the fungicide methylthiophane was found to reduce sperm concentration, viability, and motility in the study groups, whereas administration of 1.5 mL of pomegranate juice attenuated the effects of the fungicide on sperm concentration and motility [20]. In another study, aluminum administration resulted in loss of body weight and sexual organs, decreased sexual behavior, reduced sperm count, altered sperm morphology and progressive motility, decreased testosterone levels, and reduced seminiferous tubule epithelial diameter. Administration of pomegranate juice reduced these adverse effects [21].

Industrial development has elevated noise pollution to a predominant position among environmental pollution types. Given the relatively high prevalence of occupational exposure to industrial noise in developing countries, including Iran, and the natural cultivation of pomegranate in this country, the present study was designed to investigate the potential antioxidant and protective effects of pomegranate juice on sex hormone levels and testicular changes in rats exposed to noise.

Materials and Methods

This experimental and interventional study was conducted on 64 adult male Wistar rats weighing between 170 and 205 g, purchased from the Pasteur Institute in Tehran. For acclimatization, the rats were transferred to the animal facility 10 days before the start of the experiment and maintained at an ambient temperature of 20–24 °C, relative humidity of 30–70%, and a 12-hour light cycle (7:00–19:00) followed by 12 hours of darkness (19:00–7:00). The animals had free access to sufficient food and water.

At the beginning of the study, the rats were weighed and randomly divided into eight groups based on previous studies and according to Table 1. Groups P 0.5 and NP 0.5 received 0.5 mL, groups P 1 and NP 1 received 1 mL, and groups P 1.5 and NP 1.5 received 1.5 mL of fresh pomegranate juice daily by gavage [19]. To eliminate the stress associated with gavage and prevent its potential influence on the study results, the control and noise groups were also administered 1 mL of distilled water daily by gavage.

Table 1. Characteristics of the different study groups according to the interventions performed (n = 8)

Groups	Receive Pomegranate Juice			Exposed to Noise(100dB)
	0.5 ml	1 ml	1.5 ml	
Control (C)	-	-	-	-
Noise (N)	-	-	-	*
Pomegranate Juice (P 0.5)	*	-	-	-
Noise + Pomegranate Juice (NP 0.5)	*	-	-	*
Pomegranate Juice 1 (P 1)	-	*	-	-
Noise + Pomegranate Juice (NP 1)	-	*	-	*
Pomegranate Juice (P 1.5)	-	-	*	-
Noise + Pomegranate Juice (NP 1.5)	-	-	*	*

Preparation of pomegranate juice: Since Saveh pomegranate is considered one of the well-known pomegranate varieties in Iran and worldwide, healthy pomegranates were procured from Saveh city (Markazi Province, Iran) for this study. The fruits were stored in a refrigerator and administered daily to the experimental groups by gavage throughout the study period. The Saveh region was selected due to its importance as a pomegranate-growing area and its cultivation potential [22].

Noise exposure chamber: The noise exposure chamber was constructed from transparent Plexiglas polycarbonate with a thickness of 5 mm and dimensions of 30 × 59 × 49 cm (Fig. 1), based on the information provided in the Bolt chart and previous studies [23-25]. A sound pressure level of 100 dB within the frequency range of 700–5700 Hz, consisting of the central frequencies of 1000, 2000, and 4000 Hz, was generated using Cool Edit software and propagated through four loudspeakers installed on the ceiling of the chamber.

The noise level was monitored regularly every hour using a calibrated sound level meter (CEL-450) through openings in the chamber walls. The noise frequency was also monitored during exposure, and necessary corrections were made using Cool Edit software in the event of any mismatch. To control the temperature and humidity inside the chamber, the air was exchanged 12 times per hour using two fans.

Rats in groups N, NP 0.5, NP 1, and NP 1.5 were placed inside the noise exposure chamber for 8 consecutive hours per night over 50 consecutive nights (23:00 to 07:00). The control groups, P 0.5, P 1, and P 1.5, were maintained under the same conditions as groups N, NP 0.5, NP 1, and NP 1.5, except that the ambient noise level was maintained below 6 [unit not specified].



Fig. 1. Noise exposure chamber

Analysis of sex hormone levels: One day after the last exposure (day 50), rats from the different groups were deeply anesthetized with ketamine–xylazine, and blood samples were collected from the left ventricle of the heart. The blood samples were then centrifuged at 3000 rpm for 12–15 minutes. The separated serum was stored at −20 °C for subsequent measurement of LH, FSH, and testosterone levels. Finally, the concentrations of these hormones were determined using the ELISA technique.

Determination of histopathological changes: Following blood collection from the heart, the right testicle was removed for histopathological examination and immediately placed in 10% formalin solution. After 48 hours, the testicular tissue was dehydrated through an ascending series of ethyl alcohol concentrations (70–100%), cleared in xylene, and embedded in molten paraffin. Sections with a thickness of 5 µm were then prepared using a rotary microtome (Leica, USA).

Four slides were prepared from each testis and stained with hematoxylin and eosin (H&E), which stained the nuclei blue and the cytoplasm pink. The sections were examined under a light microscope (LABOMED, USA) equipped with a digital camera (CCD camera, Ziss) at 400× magnification [26, 27].

The Johnsen scoring system was used to assess the maturity and quality of the seminiferous tubules. A cross-section of 100 seminiferous tubules from each animal was evaluated, and each tubule was assigned a score from 1 to 10 according to the aforementioned criteria under light microscopy [28, 29].

All values were expressed as mean ± SD. Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test in SPSS version 21. Statistical significance was set at $P < 0.05$.

Results

No significant differences were observed in the mean body weights of the different study groups at the beginning or end of the study. The testis-to-body weight index (%) was also evaluated across all experimental groups; however, no statistically significant correlations were observed.

Sex hormone results: Table 2 presents the mean and standard deviation of sex hormone concentrations in the different study groups. According to the results, noise exposure significantly reduced the levels of all three hormones compared with the control group ($p < 0.05$).

Administration of PJ at a dose of 0.5 mL resulted in a significant difference in LH and FSH levels compared with the control group ($p < 0.05$), whereas no significant difference was observed in testosterone levels ($p > 0.05$). Comparison of testosterone and FSH levels between the noise group and the noise-exposed PJ groups showed that PJ administration at all three doses significantly increased testosterone and FSH levels in the noise-exposed groups ($p < 0.05$). In contrast, a significant increase in LH levels was observed only in the groups receiving 1 and 1.5 mL of PJ ($p < 0.05$).

Table 2. Mean and standard deviation of sex hormone concentrations in different study groups.

Groups	Testosterone (ng/ml)	LH (mIU/ml)	FSH (mIU/ml)
Control	5.66±0.206[d, e]	2.11±0.02[b, c, d, h]	1.54±0.04[b, c, d, e, f]
Pomegranate juice (PJ 0.5)	6.03±0.184[d, e]	2.45±0.06[a, d , e, f, g]	1.80±0.019[a, d, e, f, g, h]
Pomegranate juice 1 (PJ 1)	5.96±0.80[d, e]	2.57±0.145[a, d, e, f, g]	1.76±0.009[a, e, f, g, h]
Pomegranate juice (PJ 1.5)	7.69±0.33[a, b, e, f, g, h]	2.88±0.07[a, b, c, e, f, g, h]	1.72±0.04[a, b, e, f, g, h]
Noise (N)	3.38±0.22[a, b, c, d, f, g, h]	1.85±0.08[a, b, c, d, g, h]	1.17±0.030[a, b, c, d, f, g, h]
Noise + Pomegranate juice (NPJ 0.5)	5.87±0.42[d, e]	2.10±0.04[b, c, d, f, h]	1.39±0.015[a, b, c, d, e, g, h]
Noise + Pomegranate juice (NPJ 1)	5.97±0.58[d, e]	2.14±0.038[b, c, d, e, h]	1.52±0.015[b, c, d, e, f, h]
Noise + Pomegranate juice (NPJ 1.5)	6.19±0.61[d, e]	2.48±0.173[a, d, e, f, g]	1.60±0.01[b, c, d, e, f, g]

[a] Significant difference with the control group, [b] Significant difference with pomegranate juice group (PJ 0.5), [c] Significant difference with pomegranate juice group (PJ 1), [d] Significant difference with pomegranate juice group (PJ 1.5), [e] Significant difference with noise group, [f] Significant difference with NPJ 0.5 group, [g] Significant difference with NPJ 1 group, [h] Significant difference with NPJ 1.5 group. (The significance level of the tests was considered $P < 0.05$).

Results of histopathological changes: The results of Johnsen's scoring system showed that there is a significant difference between the group exposed to noise and the control group ($P<0.05$) (maturity and quality of the seminiferous tubules), and this difference was not significant among other groups compared to the control group ($P>0.05$). Prior to the examination of the seminiferous tubules, the structure of a seminiferous tubule is presented in Fig. 2. The pathologist's assessment was also conducted based on this same image.

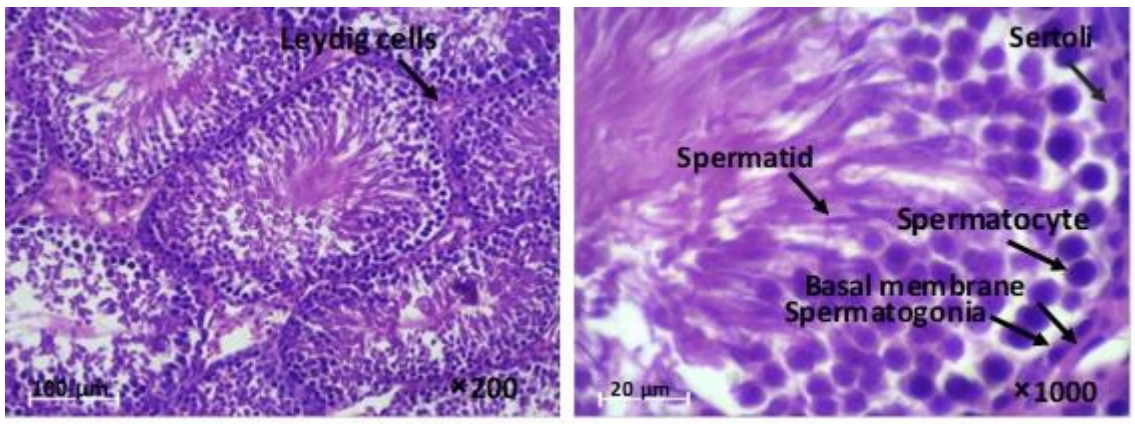


Fig. 2. The structure of a seminiferous tubule

Fig. 3 shows the comparison of the tissue changes of the control group with the groups receiving PJ (different doses), while Fig. 4 shows the comparison of the tissue changes of the noise group with the groups exposed to noise and receiving PJ (in different doses).

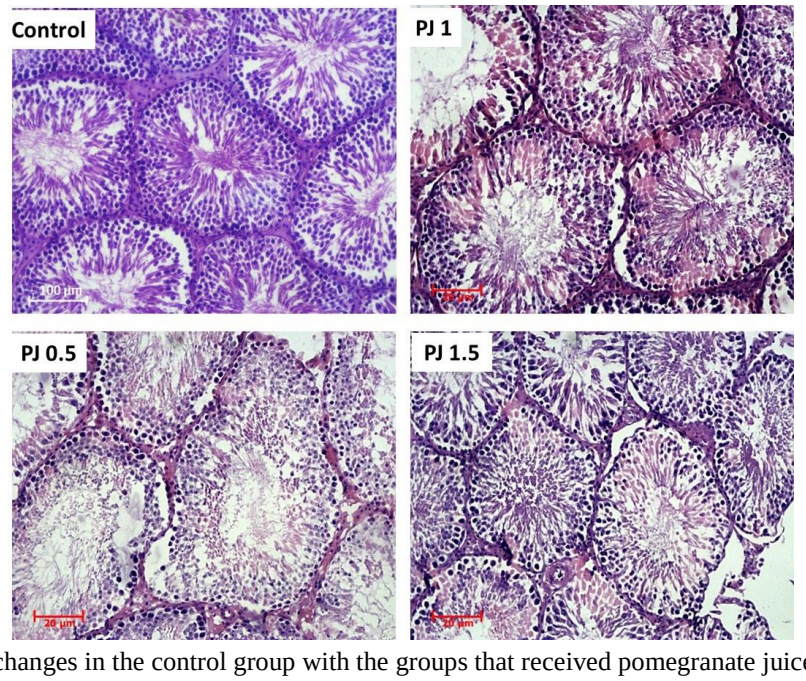


Fig. 3. Comparison of tissue changes in the control group with the groups that received pomegranate juice (different doses)

In the control group, the integrity of the seminiferous tubules was preserved. The thickness of the germinal epithelium of the seminiferous tubules is normal. The tissue between the seminiferous tubules was perfectly regular and even. While in the groups that received PJ, all seminiferous tubules were preserved and seminiferous tubules with structural and cellular organization were visible. The spermatogonial cell lineage is based on the basement membrane and is closely related to the supporting Sertoli cells. The number of Leydig cells was higher compared to the control group, resulting in higher testosterone secretion (especially in the group that received 1.5ml PJ). A decrease in the interstitial space between the tubes is observed.

In the groups exposed to noise without receiving pomegranate juice, the interstitial space between the seminiferous tubules increased. The density of Leydig cells in the intertubular space was reduced, which may lead to decreased testosterone secretion. The seminiferous tubules exhibited moderate damage due to noise exposure. Sertoli cells were difficult to detect. The diameter of the seminiferous tubules and the thickness of the germinal layer decreased. In the groups exposed to noise and receiving PJ, the spacing between the tubules increased, accompanied by a reduction in Leydig cell density. On the other hand, the cohesion of germ cells and cell lines was clearly evident (Fig. 4).

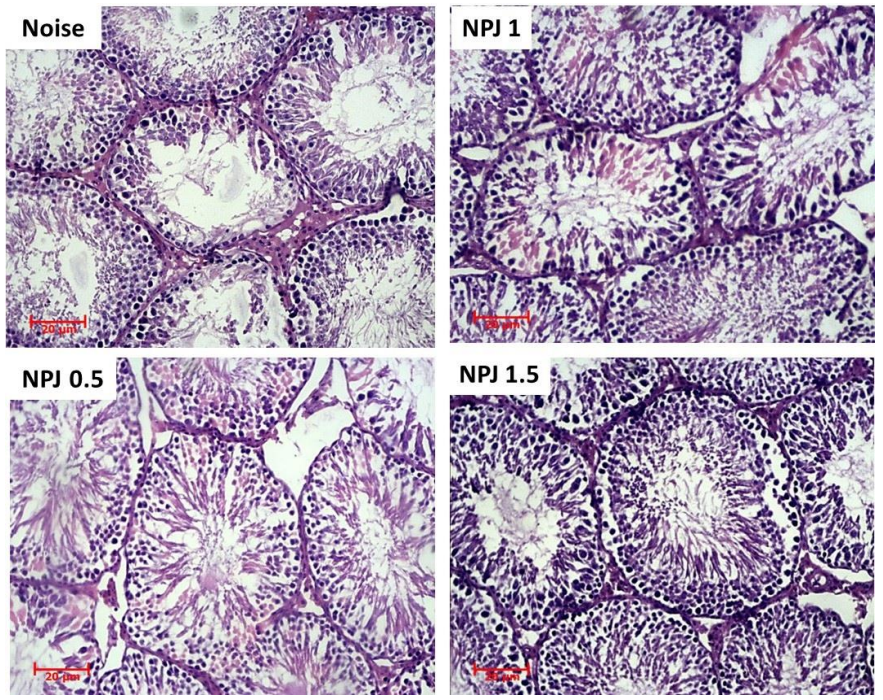


Fig. 4. Comparison of tissue changes in the noise-exposed group with the noise-exposed groups that received pomegranate juice (at different doses)

Discussion

Noise, as one of the major sources of occupational and environmental pollution, has attracted considerable attention. In this study, body weight did not show a significant decrease due to noise exposure, which is consistent with the findings of Dehghani et al. [30]. The results showed that noise exposure led to a decrease in testosterone levels, consistent with the findings of Farzadinia et al. [31], Saki et al. [12], and Bisong et al. [10]. As a result of reduced testosterone levels, sperm production may decrease and cellular apoptosis may occur [31]. In addition, reduced testosterone levels may be associated with Leydig cell dysfunction [32]. In the present study, the density of Leydig cells in the intertubular space was reduced, which may have contributed to the decrease in testosterone levels [32]. Farzadinia et al. reported that exposure to 115 dB noise reduced testosterone levels, decreased sperm production, and increased cellular apoptosis. These changes resulted in reduced germinal epithelial thickness and seminiferous tubule diameter. In addition, the maturation of some stem cells may be halted following long-term noise exposure due to decreased testosterone levels [31]. A decrease in testosterone levels may also result from Leydig cell dysfunction [32], which is consistent with the findings of the present study.

In this study, noise exposure reduced LH levels, consistent with the findings of Diab et al. [33] and Kenani et al. [34], whereas Fathollahi et al. [9] reported that noise increased the level of this hormone. These variations may be attributable to differences in sound pressure levels (100 dB vs. 90–130 dB) and frequency ranges (5700–7000 Hz vs. 300–350 Hz). Several studies have investigated the effects of antioxidant substances on sex hormone levels and sperm parameters. For example, one study used hydroalcoholic cinnamon extract to counteract the reactive oxygen species

produced by exposure to oxidants, such as noise and vibration, because of its low cost, accessibility, and popularity [24]. Previous studies have indicated that noise reduces FSH levels [8, 10], which is consistent with the findings of the present study, whereas Ghanbari et al. reported that noise had no effect on this hormone [35]. This discrepancy may be related to differences in the duration of noise exposure between the studies (28 days vs. 50 days).

In the present study, administration of PJ at a dose of 1.5 mL significantly increased testosterone levels compared with the control group. In addition, PJ significantly increased LH and FSH levels at all three selected doses. The high antioxidant activity of different parts of pomegranate, including the juice, peel, and seeds, may be attributed to their phenolic compounds, including anthocyanins, anthocyanidins, ellagic acid, ellagitannins, punicic acid, flavonoids, flavonols, and flavones [36].

This study had several limitations. Only a single noise level (100 dB) was applied; therefore, future studies should consider a wider range of noise levels or recorded industrial noise, particularly from high-noise industries such as cement and textile factories. In addition, pure pomegranate juice was administered; thus, future research should evaluate the effects of bioactive compounds extracted from pomegranate juice. The study duration was limited to 50 days, and longer-term investigations are recommended to better assess the sustained effects. Moreover, sperm quality parameters were not evaluated due to financial constraints. Therefore, future studies should include a comprehensive assessment of sperm quality parameters and investigate their potential effects on subsequent generations.

Conclusion

This study demonstrated the detrimental effects of noise exposure on sex hormone concentrations, including testosterone, LH, and FSH, as well as on testicular tissue in adult rats, whereas pomegranate juice partially ameliorated these adverse effects. Further studies are recommended to investigate the mechanisms underlying the effects of PJ on the spermatogenesis process in rats. Furthermore, given the availability and low cost of PJ, future studies may evaluate its potential as a health intervention in noisy environments by monitoring sex hormone levels and other relevant reproductive parameters.

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Conflict of interest

None declared.

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Ethical Considerations

All testing procedures were performed in accordance with the principles of the Declaration of Helsinki.

Code of Ethics

This study was approved by the Ethics Committee of Kermanshah University of Medical Sciences under code IR.KUMS.REC.1399.971.

Authors' Contributions

Farshad Nadri: Study concept and design; Ali Tajpoor: Data collection and experimental procedures; Hamideh Pirami: Data collection and experimental procedures; Hamed Nadri: Data analysis and critical revision of the manuscript; Elaheh Tardideh: Proposal writing and drafting of the initial manuscript.

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