Iran Red Crescent Med J. 2018 December; 20(12):e69779. doi: 10.5812/ircmj.69779.

Published online 2018 December 12. **Research Article**

The Effect of Noise Stress on Adult Male Rat Sperm Parameters and the Protective Effect of Hydroalcoholic *Cinnamomum verum* Extract: An Experimental Study

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Received 2018 April 23; **Revised** 2018 October 11; **Accepted** 2018 October 14.

Abstract

Background: Noise is one of the harmful environmental factors and an inevitable phenomenon in workplaces. Noise stress can lead to endocrine and reproductive system disorders.
Objectives: This experimental study was conducted to investigate the effect of noise stress on sperm parameters and the protective effect of hydroalcoholic *Cinnamomum verum* extract in adult rats.
Methods: A total of 32 male Wistar rats were randomly assigned to four groups (n = 8). Group 1 was treated with distilled water (control), Group 2 was treated with 75 mg kg^{-1} hydroalcoholic *C. verum* extract, Group 3 was exposed to noise (100 dB) for the eighth/day, and Group 4 was exposed to noise and treated with 75 mg kg^{-1} hydroalcoholic *C. verum* extract by gavage. After 50 days, the rats were anesthetized, blood samples were collected, and the cauda epididymis was removed to examine sperm parameters. Data analysis was performed using SPSS.
Results: In Group 3, noise stress significantly decreased the levels of sex hormones (LH, FSH, and testosterone), sperm viability, and the percentage of morphologically normal sperm compared to the control group. In Group 2, the levels of sex hormones and sperm parameters increased significantly compared to the control group. Comparison of the results of Groups 3 and 4 showed the protective effect of *C. verum* extract on the levels of sex hormones and sperm viability.
Conclusions: It is recommended to investigate the action mechanism of *C. verum* effect on the male reproductive system of animal models and humans who work in noisy environments.

Keywords: *Cinnamomum verum*, Endocrine, Extract, Noise, Reproductive, Sex Hormone, Sperm

1. Background

Infertility is defined as an inability of a couple to become pregnant after unprotected sexual intercourse for 12 months or more (1). Infertility affects an estimated 8% - 12% of couples worldwide (2). Male factors account for 30% - 40% of the causes of infertility (3). A study demonstrated that the sperm number of European men has decreased by 32.5% within the past 50 years, which is mainly due to lifestyle and environmental factors (4). Noise is one of the harmful environmental factors and an inevitable phenomenon in workplaces. An estimated 22.4 million workers in the United States are exposed to noise levels higher than the standard threshold (85 dB) (5). Noise is defined as an unpleasant sound and is one of the most common risks in the workplace and the environment (6). Noise stress can affect the human body, for example, hearing loss, neurological, and psychological disorders, as well as sound, can affect the visual system, electrolytes, and the hormonal system, and lead to the development of mental disorders (7). The results of one study showed that exposure to 110 dB noise for seven days (daily for half an hour) caused significant damage in their DNA in newborn rats (aged three to six days) (8). Noise-induced stress suppresses testosterone synthesis in the testes (9). A study indicated that exposure to 119 dB noise significantly decreased the blood levels of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) in the workers (10). The study of Ruffoli et al. showed that noise stress led to fat accumulation in mouse testes and decrease of testosterone production (11). The study by Saki et al. showed that noise

adversely affected the levels of LH and FSH (12). Exposure to 100 dB noise significantly decreases testosterone levels in male rodents (13). The study of Vosoughi et al. showed that sperm count, the percentage of progressive motility sperm, and sperm survival in exposed mice to 100 dB noise for 35 days were significantly reduced compared to the control group (14). However, the study of Ghanbari et al. with the same level of sound in a 14-day exposure period, did not show a significant difference between the above variables with the control group of rats (15). In addition, the study by Bisong et al. showed that exposure to the sound level of 120 - 90 dB significantly reduced sperm count, percentage of survival, and sperm motility in comparison with the control group (16). An imbalance between reactive oxygen species (ROS) production and the capacity to immediately detoxify reactive intermediates lead to oxidative stress in different tissues such as the testicular tissue (17). The study of Ghanbari et al. showed that exposure to 100 dB noise significantly decreased the total antioxidant capacity in rats compared to the control group (15). To counteract oxidative stress, the testes need antioxidant compounds (18). Different studies have investigated the protective effects of antioxidant compounds including vitamins C and E (12), and pomegranate juice (19) against male fertility parameters. In many developed countries, herbal drugs, most of which are rich sources of minerals and antioxidants, are used to treat certain diseases (20). Cinnamon (*Cinnamomum verum* or *Cinnamomum zeylanicum*) is a small and evergreen tree from the Lauraceae family. This plant reaches a height of 10 - 15 m, has a potent antioxidant activity, and is native to Sri Lanka and Southern India (21). The bark of different species of the *Cinnamomum* genus is one of the most important and widely used substances not only in foods but also in traditional and modern medications worldwide (22). *C. verum* contains essential oils, cinnamaldehyde, cinnamic acid, and cinnamate (23). *C. verum* has many therapeutic properties including potency-enhancing (24). *C. verum* extract is a frequently used, inexpensive, and easily accessible antioxidant substance (25). The study of Khaki showed that cinnamon administration caused a significant increase in sperm parameters and total serum level of testosterone in rats (20).

2. Objectives

Given the prevalence of noise pollution in workplaces (two million workers are exposed to noise of higher than 85 dB in Iran) and contradictory to the findings on the effect of noise on male fertility, as well as the limitations of previous studies, the aim of this study was to evaluate the effects of administration of *C. verum* (as an antioxidant substance) on sperm parameters and level of sex hormones in rats exposed to noise. Unlike previous studies, laboratory conditions in this study are roughly the same as the real conditions of the working environment of the workers.

3. Methods

3.1. Chemicals

Methanol (96%) was procured from Merck Co., Germany. Trypan blue and xylol were procured from Sigma Co., USA, and Ham's F10 culture medium was purchased from Gibco Co., UK.

3.2. Preparation of Extract

Dried *C. verum* bark was purchased from traditional drugstores in Tehran, Iran, and then was identified as *C. verum* from the Lauraceae family (herbarium no. PMP-906) at the Pharmacognosy Department of the Faculty of Pharmacy of Tehran University of Medical Sciences. Hydroalcoholic *C. verum* extract was prepared by maceration (26). For this purpose, 250 g of pulverized *C. verum* (by an electric mill) was dissolved in 2500 mL of 80% methanol at 25°C temperature for 72 hours. In addition, the container (covered by an aluminum sheet) was shaken several times every day to completely dissolve the *C. verum* in the solvent. Then, the resulting solution was filtered with Whatman paper grade 1. The filtrate was concentrated in a rotary evaporator (Heidolph Co., Germany) under 40°C. The final weight of the dried extract was 17.5 g. To prevent contamination, the dried extract was stored in the refrigerator until needed.

3.2.1. Cinnamaldehyde Determination

Studies have reported that cinnamaldehyde (3-phenyl-2-propanol) is the main aromatic compound of *C. verum* (20, 27). Therefore, high performance liquid chromatography (HPLC) was used to measure the amount of cinnamaldehyde (27, 28). For this purpose, HPLC (Knauer) Teknokroma-c_{18} column (with length and an internal diameter of 25 and 0.46 cm, respectively), a pump (Knauer-K1001), and a detector (K250 Knauer-UV) at 210 nm wavelength were used. The cinnamaldehyde concentration in *C. verum* extract was 1.2 mg g^{-1}. The no observable effect level (NOEL) of cinnamaldehyde was determined as 620 mg kg^{-1} (29). The US Environment Agency has determined LD50 2.25 - 3.35 g kg^{-1} for acute toxicity of oral cinnamaldehyde in rats (according to Cinnamaldehyde Fact Sheet). The cinnamaldehyde concentration in 75 mg kg^{-1} of rat body weight (BW) of *C. verum* extract (assuming that the average weight of a rat is 250 g) was derived 0.0225 mg; therefore, the administered dose could not cause toxic effects in rats.

3.3. Animals Housing

This experimental study was conducted in the Tarbiat Modares University, Tehran in June-November, 2016. A total of 32 healthy male adult Wistar rats aged over eight weeks (from 10 to 20 week) and weighing between 175 and 200 g were procured from the Pasteur Institute of Iran, Tehran, Iran. The rats were housed under 12-hour dark (7 pm to 7 am)/12-h light cycles (7 am to 7 pm), (22 ± 2°C) temperature, and 30% - 70% humidity to acclimatize to the laboratory environment. Animals were maintained under pathogen-free conditions. The cage floor was covered with sawdust. The animals' cages were cleaned and disinfected twice a week. The rats were weighed before the experiments and then once a week, and given fresh food (100 g kg^{-1} BW) and water. Rats had free access to plates (prepared from Behparvar Animal Chow Company (Iran)) containing raw protein, raw fibers, raw fat, calcium, phosphorus, salt, and humidity at specific proportions without any antioxidant intervening in the experiment. The experimental design and procedures of the study were in complete compliance with the international guidelines (World Medical Association Declaration of Helsinki) and approved by the Ethics Committee of the School of Medicine of the Tarbiat Modares University (Iran) (code 52D/9290, March 2016). Every effort was made to minimize the number of animals used and their suffering. Prior to the beginning of the study, the researcher had acquired the necessary skills in performing the technique of gavage for laboratory animals in the workshops. Adequate measures were taken to minimize animal's pain or discomfort.

3.4. Animal Grouping

The rats were randomly divided into four groups (n = 8). For this purpose, each rat was assigned a number and then divided into four groups using the random number table. Group 1 (control) was treated with distilled water daily; Group 2 was treated with 75 mg kg^{-1} of hydroalcoholic *C. verum* extract daily; Group 3 was exposed to 100 dB noise for eight hours per day along with receiving distilled water; and Group 4 was exposed to 100 dB noise for eight hours per day along with receiving 75 mg kg^{-1} of hydroalcoholic *C. verum* extract for 50 consecutive days. The dried extract (75 mg kg^{-1} BW dissolved in distilled water) was administered to the animals by gavage (20). To ensure that gavage stress would not affect the results, the control group was gavaged with 2 mL of distilled water every day for 50 consecutive days (the duration of spermatogenesis in rat) (12, 30, 31) between 06:30 am and 07:00 am (11).

3.5. Noise Exposure

Noise chamber was made up of transparent Plexiglass with a thickness of 5 mm. The dimensions of the noise chamber (59 × 49 × 30 cm) were determined based on Bolt's Chart (32) and welfare and physiological requirements of the rats so that eight rats could simultaneously live inside it comfortably. In this study, the rats in Groups 3 and 4 were exposed to 100 dB noise of 700 - 5700 Hz frequency (combination of three octave-band sound, 1000, 2000, and 4000 Hz (14,15). In addition to being within rat hearing spectrum, the selected frequency range is the predominant frequency in most industrial sites. The calculated room constant (R = 0.408) in this study demonstrated that the chamber had reverberant conditions (chamber's height: 30 cm, the Plexiglass's mean sound absorption coefficient: 0.25). (100 ± 1) dB noise with the above frequency range was produced by the *Signal* software and delivered by the *Cool Edit Pro* (Syntrillium Software Corporation, 1999 - 2003). The noise was amplified and broadcast by using four speakers attached to the chamber ceiling at equal distances. The level and frequency of noise in the chamber were monitored once an hour using a calibrated Cel-450 sound level meter (SLM) equipped with a frequency analyzer through holes created around the chamber at a distance of 8 cm (the approximate distance between the chamber ground and the rats' head). Humidity and temperature inside the chamber were monitored and the chamber was aerated using two axial fans (with noise frequency of 38 - 42 dB) 12 times an hour. Space inside the chamber was divided into four equal parts separated by metal wires, and two rats were placed inside each part to prevent loneliness-induced stress (33). Groups 3 and 4 were kept in the chamber for 8 hours (07:00 - 15:00) per day for 50 consecutive days as the chamber was exposed to noise. Groups 1 and 2 were kept in similar conditions to those in which Groups 3 and 4 were kept, however, the noise did not exceed 66 dB (34).

3.6. Determination of the Levels of Testosterone, LH, and FSH

After 50 days, the rats were weighed and anesthetized by intraperitoneal injection of ketamine (90 mg kg^{-1}) and xylazine (10 mg kg^{-1}) (35) between 09:00 and 11:00 o'clock (31), and then 5 mL blood samples of the left ventricles were collected. The blood samples were centrifuged at 3000 rpm for 12 - 15 minutes, and then serum was isolated and stored at -20°C until needed. The levels of LH, FSH, and testosterone were measured by rat LH, FSH, and testosterone ELISA kits (ZellBio GmbH, Ulm, Germany; catalogue no. ZB-0179-R9648, ZB-0182-R9648, and ZB-0259-9648, respectively) using the ELISA technique, according to the manufacturer's instructions. The sensitivity of the rat FSH, LH, and testosterone ELISA kits was 0.12 mIU mL^{-1}, 0.05 mIU mL^{-1}, and 2.5 nmol L^{-1}, respectively.

3.7. Epididymal Sperm Count, Viability and Morphology

After collection of the blood samples, the intraperitoneal cavities were opened by transverse incision, and the testes and epididymis were carefully removed and washed with normal saline (0.9%). Epididymal sperm was analyzed according to the WHO guidelines (36). The cauda epididymis (where mature sperm is stored) of each rat's right testis (30) was finely sliced by scissors in 5 mL of Ham's F10 and incubated at 37°C for 20 minutes to remove the sperm from the epididymal tissues and spread it in the medium. To determine the sperm viability, 20 μL of the sperm suspension was combined with an equal amount of Trypan blue. After an incubation of five minutes at room temperature, the slide was observed using an optical microscope (LABOMED equipped with CCD Camera) at 400× magnification. The dead sperm appeared as dark blue while the viable sperm appeared as colorless. Sperm viability was determined by calculating the proportion of the viable sperm count to the total sperm count (37). To determine the sperm count, the sperm suspension was diluted by addition of an equal amount of distilled water. Neubauer counting chamber (HBG, Germany) was filled with 10 μL of seminal fluid, and then the sperm count was carried out by using an optical microscope at 400× magnification in four 16-well squares. The sperm count was expressed as the number of sperm per mL (37). To determine the percentage of morphologically normal sperm, a smear of the sperm suspension, diluted to 1:10 with normal saline, was prepared and fixed with acetone after drying. After fixation with the Diff-Quick kit (Ibn Sina, Iran), the parts of the sperm such as head, neck, and tail were investigated using solutions A, B, and C, and the defects in normal and abnormal sperm were expressed as percentage. All of the observations were performed by a single observer.

3.8. Sample Size

The sample size was determined by a formula to compare the mean values of the two groups with a type 1 error of 0.05 and a power of 80%. Due to the fact that the studies have not yet investigated the effect of coincidence of noise and *C. verum*, the sample size was determined according to previous studies. In a study, the effect of cinnamon on sperm quality parameters (number, motility, and survival) was investigated in comparison to the control group, (20) and in the other study, the effect of noise on sex hormones was studied in comparison to the control group (12). Maximum sample size in each group, according to the above studies, was determined to be 6.22 rats. To deal with mortality during the study, eight rats were enrolled in each group.

3.9. Statistical Analysis

Distribution normality was tested by Kolmogorov-Smirnov test. All of the variables naturally follow normal distribution. One-way analysis of variance and Tukey's test in the SPSS version 19 were used to conduct intergroup comparisons. The experiments were conducted at least in triplicate and data were presented as mean (standard deviation) value of the three measurements. $P < 0.05$ was considered as the significance level.

4. Results

4.1. Hormones

The mean serum levels of testosterone, LH, and FSH are shown in Table 1. There were significant differences in the LH, FSH, and testosterone levels between Group 3 (noise-exposed) and the control group. The levels of these hormones were significantly higher in Group 2 (extract-treated) than in the control group. Comparison of the results in the noise-exposed groups showed that the levels of sex hormones were significantly higher in the extract-treated group (Group 4) when compared to Group 3, which was not treated with the extract.

4.2. Sperm Parameters

The data on the sperm count and the viability and normal morphology of sperm are shown in Table 2. The sperm count increased significantly in Group 2 when compared to the control group. In addition, the sperm count was significantly higher in Group 4 than in Group 3; however, the difference was not statistically significant. The sperm viability increased significantly in Group 2 when compared to the control group, however, it decreased significantly in Group 3, which was only exposed to noise. The sperm viability also increased significantly in Group 4 when compared to Group 3. The percentage of the morphologically normal sperm increased significantly in Group 2 when compared to the control group, however, it decreased significantly in Group 3, which was only exposed to noise. The percentage of the morphologically normal sperm also increased significantly in Group 4 (the extract-treated and noise-exposed) when compared to Group 3 (Table 2).

5. Discussion

Noise stress can lead to hearing loss, neurological and psychological disorders, adverse effects on the visual system, electrolytes, and endocrine system as well as mental disorders (7). In the present study, the level of testosterone in the noise-exposed group (Group 3) decreased significantly compared to the control group, which is consistent with other studies (11, 12). Noise exposure leads to

Table 1. The Mean ± SD Levels of Luteinizing Hormone, Follicle Stimulating Hormone, and Testosterone in Different Groups of Male Rats[a]

Hormones	Groups			
	1	2	3	4
LH, mIU mL^{-1}	1.77 ± 0.023	1.96 ± 0.016 [b]	1.42 ± 0.0305 [b,c]	1.63 ± 0.040 [b,c,d]
FSH, mIU mL^{-1}	1.59 ± 0.038	1.74 ± 0.017 [b]	1.33 ± 0.034 [b,c]	1.47 ± 0.033 [b,c,d]
Testosterone, nmol L^{-1}	14 ± 0.17	15.55 ± 0.17 [b]	9.51 ± 0.45 [b,c]	11.76 ± 0.173 [b,c,d]

[a]Values are expressed as mean ± SD.
[b]P < 0.05, vs. control group.
[c]P < 0.05, vs. *Cinnamomum verum* group.
[d]P < 0.05, vs. noise group.

Table 2. The Count, Viability, and Normal Morphology of Caudal Epididymal Sperm in Different Groups of Male Rats[a]

Parameters	Groups			
	1	2	3	4
Count, 10^6 mL^{-1}	59.66 ± 5.13	84 ± 5.29 [b]	52.66 ± 3.05 [c]	59 ± 3.60 [c]
Viability, %	80 ± 0.5	92.69 ± 2.81 [b]	58.70 ± 1.58 [b,c]	75.41 ± 1.30 [c,d]
Normal morphology, %	80 ± 1	87.33 ± 2.08 [b]	71.33 ± 1.52 [b,c]	78 ± 1 [c,d]

[a]Values are expressed as mean ± SD.
[b]P < 0.05, vs. control group.
[c]P < 0.05, vs. *Cinnamomum verum* group.
[d]P < 0.05, vs. noise group.

decreased biosynthesis of testosterone and the accumulation of cholesterol in the testes (38). We observed that the serum LH and FSH levels decreased significantly in Group 3 (noise-exposed group) compared to the control group. This finding is consistent with the study of Saki et al. (12), and Bisong et al. (16), and inconsistent with the studies of Fathollahi et al. (31), Ghanbari et al. (15), and Vosoughi et al. (14). In our study, the levels of LH, FSH, and testosterone increased significantly in the extract-treated group (Group 2) compared to the control group. This important finding indicates that hydroalcoholic *C. verum* extract is effective on the secretion of these hormones. The study of Khaki showed that *C. verum* treatment significantly increased serum testosterone and superoxide dismutase (SOD) levels and decreased malondialdehyde (MDA) level (20). The significant difference in LH, FSH, and testosterone levels between the noise-exposed groups (Groups 3 and 4), indicated that *C. verum* extract counteracted the declining effect of noise on sex hormones. Jalali et al. (30), and Bisong et al. (16), observed that exposure to 90 to 120 dB noise (300 to 350 Hz frequency) for 50 days decreased sperm count significantly in rats. In the present study, the sperm count decreased in the rats exposed to 100 dB noise; however, this decrease was not statistically significant. These contradictory findings can be attributed to exposure duration and different noise levels and frequencies. Khaki reported that *C. zeylanicum* treatment increased sperm count in rats (20). In our study, the sperm count increased significantly in Group 2 (hydroalcoholic *C. verum* extract-treated group) compared to the control group. The number of the sperm per mL increased in Group 4 compared to Group 3, however, this increase was not significant. The study of Farzadinia et al. demonstrated that the testosterone levels and spermatogenesis decreased and cell apoptosis increased in the rats exposed to 115 dB noise. These changes lead to a decreased thickness of germinal epithelium and the diameter of seminiferous tubules. Besides that, the maturation of certain stem cells (spermatogonia, spermatocytes, and spermatid) may be discontinued due to long-term exposure to noise and a decrease in testosterone levels (33). Oxidative stress refers to the disrupted balance of ROS and reactive nitrogen species (RNS) with the body's antioxidant defense system (39). Oxidative stress is diagnosed in approximately half of the infertile men (40). A study on rats demonstrated that exposure to traffic noise for 30 and 60 days caused a significant increase in the morphologically abnormal sperm count when compared to the control group (38). In our study, there was a significant difference in morphologically normal sperm percentage between Group 3 (the noise-exposed group) and the control group (71.33% ± 1.52% vs. 80% ± 1%). Comparison of the results in Groups 3 and 4, indicated that *C. verum* extract caused an increase in the morphologically normal sperm percentage, such that its percentage increased from (71.33% ± 1.52%) to (78% ± 1%). Methanol *C. verum* bark extract contains certain antioxidant compounds that can effectively

eliminate ROS, including superoxide anions and hydroxyl radicals (41). One study showed that treatment with *C. zeylanicum* bark oil caused a significant decrease in MDA levels and a significant increase in the weight of the testes and epididymis, the concentration of epididymal sperm and the diameter of the seminiferous tubules (42). Shah et al. reported that *C. zeylanicum* extract treatment significantly increased sperm count compared to the control group (43). Khaki reported that *C. zeylanicum* treatment increased sperm viability significantly (20). Consistently, there was a significant difference in the sperm viability between Group 2 and the control group in our study. Noise stress in Group 3 significantly decreased the sperm viability when compared to the control group, which is consistent with the study of Vosoughi et al. (14). In group 4, the sperm viability increased yet insignificantly compared to the control group.

5.1. Conclusions

As far as we searched, no study has yet investigated the protective effect of *C. verum* extract (75 mg kg^{-1}) against sperm parameters and damage to the testicular tissue after noise exposure. *C. verum* extract exerts protective effects on the testes against noise by enhancing sperm parameters (including count, viability, and morphology) and increasing effective hormones on spermatogenesis. It is therefore recommended to conduct additional studies on humans and animals to determine the precise action mechanism of *C. verum* on the male reproductive system as well as to prescribe *C. verum* for workers due to the fact that it can be used as an inexpensive and widely available drug to prevent noise-induced adverse effects on the reproductive system. Occupational parameters should be an important part of history taking in patients referred to infertility clinics. To supplement the information, it is suggested that this research be repeated on humans as well.

Acknowledgments

This article was derived from the Ph.D. thesis of Farshad Nadri that received a funding from the Research Technology of Tarbiat Modares University, Tehran, Iran.

Footnotes

Authors' Contribution: Study concept and design: Ali khavanin, analysis and interpretation of data: Zohreh Mazaheri, drafting of the manuscript: Farshad Nadri, critical revision of the manuscript for important intellectual content: Farshad Nadri and Farahnaz Khajehnasiri, statistical analysis: Farshad Nadri and Zohreh Mazaheri.

Conflict of Interests: None declared.

Funding/Support: This article and thesis supported by Deputy of Research and Technology in Tarbiat Modares University

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