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Simultaneous Effect of Noise Stress and Opium Addiction on Adult Rat Sperm Parameters: An Experimental Study

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Abstract

Background: There are considerable opium addicts, particularly among workers in Iran's industrial sectors. This study aimed to investigate the effects of simultaneous exposure to excessive noise and opium addiction on adult rat sperm parameters.
Materials and Methods: In this experimental study 42 adult Wistar male rats in 6 groups were studied as follows: group 1 control, group 2 exposed to noise (100 dB) 8 h/day, group 3 received daily 50 mg/case opium solution, group 4 received daily 100 mg/case opium solution, group 5 received daily 50 mg/case opium solution and exposed to noise 8h/day, and group 6 received daily 100 mg/case opium solution and exposed to noise 8h/day. After 50 days, cauda epididymis was removed for sperm parameters examination (WHO guidelines).
Results: In the groups receiving opium (50 and 100 mg/case), sperm count, sperm viability, and normal sperm morphology (%) significantly decreased compared to the control group. Exposure to noise and consumption of opium solution simultaneously significantly reduced the count, viability, and percentage of sperm with normal morphology in both selected doses, as well as a significant difference between groups 5 and 6 in count, viability, and normal sperm morphology(%) parameters($P<0.05$).
Conclusions: According to the results, noise exposure and taking opium would actively reduce the count, viability, and normal sperm morphology. It is therefore suggested that the mechanism of such effects should be investigated in animal and human studies.

Keywords: Sperm, Noise, Opium, Addiction

Introduction

Infertility is considered a global public health issue with multiple contributing risk factors. These risk factors may stem from individuals' lifestyle or occupational, physical, and psychological factors [1-4]. Approximately 30-40% of infertility cases can be attributed to men-related disorders [5]. Reducing the count and motility, as well as abnormal forms of sperm (morphology), are the

variables that affect male infertility [6]. Progress in all industrial fields and widespread use of industrial machinery and equipment has led to a dramatic increase in noise levels in workplaces, which endangers many workers, especially industrial workers exposed to the hazardous noise levels [7]. The World Health Organization (WHO) has identified noise as the second leading environmental stressor. Estimates show that in high-income countries such as Western Europe, with a population of about 340 million people, at least 1 million years of healthy life each year disappear due to environmental noise [8]. According to recent studies, at least 30 million people in the United States are exposed to noise above 85 dB [9]. A review study by Nadri et al. showed that noise exposure, in addition to affecting the levels of sex hormones, can affect sperm quality parameters as well [10]. Bisong and colleagues showed that in the exposed groups (90-120 dB), the sperm count and sperm viability were significantly lower than the control group. Also, in this study, the percentage of progressive and non-progressive motility and, finally, total mobility (%) were significantly reduced compared to the control group [11].

Narcotics abuse is one of the most important social and health problems in many parts of the world [12] and negatively affects societies' economic and cultural aspects [13]. Among the narcotics, opium and heroin account for the highest global consumption levels [14]. The United Nations Office on Drugs and Crime (UNODC) believes that in 2013, 32.4 million adults were consuming narcotics, of which 16.5 million people consumed opium and its derivatives (especially heroin). Asia is believed to be the most important narcotics market, having two-thirds of the world's total narcotics consumption [15]. Although opium abuse has significantly declined in recent years, in several societies such as Iran, it is still one of the most commonly used narcotics [16]. According to an estimate in 2011, in Iran, about 1,325,000 individuals aged between 15 and 65 had used unauthorized drugs and narcotics, of whom 1181,000 were opium users in the past 12 months [17].

Experts believe that one of the reasons for the extensive levels of drug abuse in Iran is the 900-kilometer (560-mile) common border with the neighboring country, Afghanistan, where almost 90 percent of the world's opiates are produced [18]. The oral (49.5%) and inhalational (Opium smoking) (59%) routes are the prevalent methods of taking opium in Iran [19]. Opium comprises more than 20 alkaloids and more than 70 other compounds [20-21]. Some people believe that long-term consumption of low-dose opioids may prevent chronic illnesses and prolong life [22]. It has been shown that opioids might have adverse physiological side effects on various body systems, including the reproductive system [23]. Much of the research on the effects of opioids on the male reproductive system has focused on the effects on the hypothalamus and the pituitary gland [24]. Opioids reduce the secretion of LH and FSH gonadotropins by inhibiting the release of gonadotropin released by the hypothalamus (gonadotropins are crucial for the survival of testicular cells, and their reduction leads to apoptosis of testicular cells) [25]. Several studies have shown the harmful effects of opioid compounds on sperm motility and morphology. The mean serum levels of LH, FSH, and sperm motility for addicted patients were significantly lower than the control group [26]. Cicero et al. showed that opioids greatly reduce serum testosterone levels in men. In the reduction of the level of testosterone, the three mechanisms can be effective. First, increased testosterone destruction; second, direct inhibition of testicular steroid genesis; and third, inhibitory hypothalamic-pituitary-luteinizing (LH) axis caused by the reduction of the testicular steroid genesis associated with the LH hormone [27]. Considering that, based on the searches, almost no study has investigated the effect of opium consumption and noise exposure simultaneously, this study was performed to investigate the combined effect of opium use and exposure to noise on sperm quality parameters of male rats.

Materials and Methods

This experimental study was carried out using an animal model [28] from January 2018 to January 2019. The selection of rats as the subjects of this study was carried out according to factors such as availability, reasonable price, small size and relatively submissive nature, metabolic similarities, and close spermatogenesis cycle with humans. In this study, 42 healthy adult Wistar rats [29] weighing 200-250 g were purchased from the Tehran Pasteur Institute. To adapt to the environmental conditions of the test location, rats were transferred to the animal house of Tarbiat Modares University ten days before exposure and placed in an animal house with an environmental temperature of 18-26 ° C and relative humidity of 30-70% [28]. During testing, rats had sufficient free access to enough food and enough water [30]. About working with laboratory animals, the Helsinki Declaration and the approvals of the Ethics Committee of the Tarbiat Modares University

(IR.TMU.REC.1396.646) were put on the agenda. During the study, efforts were made to minimize the suffering of laboratory animals.

Based on similar studies, 42 experimental rats, which were divided into 6 groups of 7 rats, were included as the subjects of this study. The grouping of laboratory animals was as follows:

Group 1: The control group, without receiving opium solution and noise exposure, only received 2 ml of distilled water by gavage for 50 consecutive days (to eliminate the effect of gavage stress).

Group 2: Exposed to noise (100 dB) 8 h/day and received 2 ml of distilled water daily by gavage for 50 consecutive days.

Group 3: Receiving daily oral doses of 50 mg of opium solution per rat by gavage for 50 consecutive days

Group 4: Receiving daily oral doses of 100 mg of opium solution per rat by gavage for 50 consecutive days

Group 5: Exposed to noise (100 dB) 8 h/day and receiving daily oral doses of 50 mg of opium solution per rat by gavage for 50 consecutive days.

Group 6: Exposed to noise (100 dB) 8 h/day and receiving daily oral doses of 100 mg of opium solution per rat by gavage for 50 consecutive days.

Hearing health of laboratory animals was confirmed by distortion product otoacoustic emissions DPOAE test before noise exposure [31].

This study exposed groups of 2, 5 and 6 to noise. Given that the human hearing spectrum is within the frequency range of 20 to 20 kHz, and the highest levels of human hearing damage due to excessive noise exposure occurs at 4 kHz, the rats were exposed to the sound pressure level of 100 dB [29, 32-35] in the frequency range of 700 to 5700 Hz (a combination of the central frequencies 1000, 2000, and 4000 Hz) [34-36]. The rat's hearing sensitivity of rats has also been reported to be dominant at 8 kHz [37-39]. Noise exposure was performed daily from 07:00 to 15:00. First, the noise with the defined frequency combination was created by the signal software. Then noise was run by the computer in Cool-Edit software. An amplifier amplified noise running in this software and sent to the built-in speakers (4 speakers) at the ceiling of the chamber. One of the features of the Cool Edit software is the ability to edit noise in terms of the noise intensity at various frequencies, so with constant monitoring of noise in the chamber, the noise intensity can be controlled at the defined frequencies. Inside the chamber, the rats' distance from the noise source (loudspeakers) did not affect the received noise level (the Chamber was reverberant and created based on Bolts chart [40], and monitoring the level and frequency of noise was performed by the sound level meter (Cel-490 model) in the holes that were installed on the four sides of the chamber(at the height of Rat's head) during the animal's exposure continuously (once every hour) and, if necessary, by editing the noise in the Cool Edit software, the exposure conditions were maintained in the defined range.

In this study, 155 grams of opium were obtained from the Counter Narcotics Police in Tehran under the supervision of the Iranian Food and Drug Administration. The required amount of opium was weighed daily,dissolved in distilled water, and administration by oral gavage based on previous studies; it was chosen to confirm the animal's addiction:

1) For groups 3 and 5, first, the oral dose of 25mg/case was prescribed, and then in a 7-day course, the dose increased to 50 mg/case, which was considered constant for 50 days.
2) For groups 4 and 6, first, the oral dose of 25mg/case was prescribed, and then in a 7-day course, the dose increased to 100 mg/case, which was considered constant for 50 days [41]

For groups addicted to opium, the dosage was dissolved in 1 cc distilled water and was provided to rats by gavage between 6:30 and 7:00 A.M. Since exposure to noise in groups 2, 5, and 6 started at 7:00 and during the time period from 7 to 15 the researcher did not have access to the exposure chamber, so the solution was given to the animal through gavage half an hour before the exposure. To ensure that the rats were totally addicted, at the end of the first week, from each group, one rat was randomly evaluated using naloxone (2mg/kg dose as an intraperitoneal injection). Animal hangover symptoms (such as isolation, gnashing of the teeth, severe weight loss, etc.) were considered confirmatory items of their addiction [42].

After 50 days, the rats were anesthetized applying a combination of ketamine and Xylazine. Their peritoneal cavity was then opened up through a transversal incision, and the testicles and epididymis were carefully removed and washed in normal saline solution (0.9%) prior to being weighted with a digital scale (Model PX6000). Epididymis sperm analysis was performed based on WHO guidelines [43]. Cauda epididymis (the storage site of adult sperm) [44] was placed in 5 ml of Ham's F10 medium. It was cut using scissors well and placed at 37 ° C in an incubator (MEMERT, Germany) for 20 minutes to remove spermatozoa from the epididymal tubes and release in the medium. To determine the sperm motility, one drop of sperm suspension was placed on the slide, and lamel placed on the droplet. At least 10 microscopic regions with a magnification of 400 were observed.

In this case, the number of fast-moving forward sperms (A), slow-moving forward sperm (B), non-progressive sperm (C), and non-moving sperm (D) were counted. Subsequently, the total of rapid and slow progressive sperm was considered as progressive sperm (A + B), and the total of progressive and non-progressive sperms was considered as total motility (A + B + C), then the results were expressed as percentages [47]. To determine the sperm viability, 20 µl of sperm suspension was combined with the same volume of trypan blue. After 5 minutes of incubation at room temperature (25° C), slides were observed with a light microscope (LABOMED, USA) equipped with a camera (CCD Camera, Ziss) with a magnification of 400. Dead spermatozoa were observed as dark blue, while viable spermatozoa were colorless. The viability of sperm was based on the percentage of viable spermatozoa relative to total spermatozoa counted [45]. To determine the sperm count, sperm suspensions were diluted with the same volume of distilled water. Neubauer counting chamber (model HBG made in Germany) filled with 10 µl Sperm fluid was, and then the number of spermatozoa was counted in in four 16-well squares. The number of sperm is expressed as the number of spermatozoa per milliliter [45]. To determine the number of spermatozoa with normal morphology, a smear was made of a sperm suspension (dilution 0.01) on slides, and after drying, the samples were fixed with acetone. After fixation, using the Diff-Quick kit (Ibn Sina, Iran) and with the solutions A, B, and C contained in the kit, different sperm areas, including acrosome, nucleus, overlapping acrosome, and nucleus and sperm neck, were investigated. The defects were reported in abnormal and normal sperm, based on percentages. After determining the normality of the data, results were analyzed by ANOVA and Tukey test using SPSS version 23 software. Data were expressed based on mean and standard deviation (mean ± SD), and differences between groups were considered significant at the level of $\alpha<0.05$.

Results

The mean and standard deviation of sperm count, viability, and percentage of sperm with normal morphology in the study groups are presented in Table 1.

Table 1. Mean and standard deviation of sperm count, viability, and percentage of sperm with normal morphology in the study groups

Parameters	Groups					
	1 (mean±SD)	2 (mean±SD)	3 (mean±SD)	4 (mean±SD)	5 (mean±SD)	6 (mean±SD)
Sperm count (106/ml)	54.89±4.38	39.96±2.14a	24.76±1.29a,b	16.50±1.57a,b, c	13.93±0.46a, b, c	12.17±2.72a, b, c, e
Sperm viability (%)	97±1	87±1a	79.66±2.51a	69.66±4.50a,b,c, d	61.66±2.88a,b, c, d	51.66±3.51a, b, c, d, e
Normal sperm morphology (%)	71.66±5.77	53.66±3.21a	52.66±2.51a	39±3.6a, b, c	40±5a, b, c,	23.33±2.88a, b, c,d, e

a P <0.05, vs. control group, b P <0.05, vs. noise group, c P <0.05, vs. opium(50 mg/case) group, d P <0.05, vs. opium(100 mg/case) group, e P <0.05, vs. opium(50 mg/case) group+ noise exposure. (According to ANOVA test (Tukey))

In this study, there was a significant difference between the mean sperm count in groups 2(Exposed to noise), 3(Receiving daily oral doses of 50 mg of opium solution), 4(Receiving daily oral doses of 100 mg of opium solution), 5 and 6 with the control group (P <0.05). The lowest sperm count was found in group 6, which was exposed to the noise and received the opium solution (100 mg/case dose). The mean sperm count between group 5 (exposed to noise and receiving 50 mg/case opium solution) showed significant differences with groups 2 and 3, implying a booster effect in decreasing when simultaneously exposed to these two factors (noise and opium). Also, there was a significant difference between group 6 (exposed to noise and receiving 100 mg/case opium solution) and group 2 in the sperm count variable. However, no significant difference was observed for group 4. There was a significant difference between the mean sperm viability (%) in groups 2, 3, 4, 5, and 6 with control group. Mean sperm viability (%) between group 6 and groups 2 and 4 showed a significant difference. Also this difference was observed between groups 5 with groups 2 and 3 in sperm viability (%) variable. In this study, there existed a significant difference between the mean normal sperm morphology (%) of groups 2, 3, 4, 5, and 6 with the control group (P <0.05). The mean normal sperm morphology (%) between group 6 and groups 2 and 4 showed a significant difference, as well as between group 5 and groups 2 and 3.

In this study, there was a significant difference between the mean sperm motility with class a (%) (Rapid progressive sperm) in groups 2, 3, 4, 5, and 6 with the control group (P <0.05). Mean sperm

motility with class a (%) between group 6 and group 2 showed a significant difference. Also, between group 5 and groups 2 there was a significant difference between groups 5 and 6 from the viewpoint of rapid progressive sperm (effect of opium solution in same noise exposure) However, there was no significant difference between group 3 and 4 in this variable. There was a significant difference between the mean sperm motility with class b (%) (Slow progressive sperm) in groups 2, 4, 5, and 6 with the control group. There was a significant difference between groups 2, 3, 4, 5, and 6 compared to the control group in terms of total motility (%). A significant difference was also observed between the groups receiving opium solution regarding total motility, which highlights the role of opium dosage in this decreasing trend. The average percentage of immotile sperm (%) between groups that received opium (3 and 4) and the group with combined exposure (5 and 6) was significantly different from the control group, while there was no significant difference observed between the control group and the group 2 in the case of immotile sperm (%). There was a significant difference between groups 2 and 3 with group 5 and 2 and 4 with groups 6 in relation to immotile sperm (%). There was a significant difference between groups 3 and 4, but there was no significant difference between group 5 and group 6 when considering immotile sperm (%) (Table 2).

Table 2. Mean and standard deviation of sperm motility by classified classes in the study groups

Motility	Groups					
	1 (mean±SD)	2 (mean±SD)	3 (mean±SD)	4 (mean±SD)	5 (mean±SD)	6 (mean±SD)
Motility class a (%)	47±3	30.33±2.88a	20.33±1.52a,b	16.33±3.51a,b	10.33±2.30a,b,c	2.66±2.51a,b,c, d, e
Motility class b (%)	28.66±1.52	19.66±.057a	22.66±3.51	18.33±2.88a	19.66±3.51a	19±3.60a
Motility class c (%)	15±3	29.66±4.04a	19±3.60b	17.66±3.21b	17.33±2.08b	21±1b
Motility class d (%)	9.33±1.52	9.66±1.52	36.66±2.88a,b	47.66±2.51a, b, c	52.66±3.51a, b, c	57.33±2.51a, b, c, d
Motile sperm (%) (a+b+c)	90.66±1.52	79.66±1.52a	63.33±2.88a,b	52.33±2.51a,b, c	47.33±3.51a, b, c	42.66±2.51a, b, c, d
Progressive sperm(%)(a+b)	57.66±2.88	66±3a	43±2a,b	34.66±1.52a,b,c	30±1.73a,b, c	21.66±2.30a,b,c, d, e

a P <0.05, vs. control group, b P <0.05, vs. noise group, c P <0.05, vs. opium (50 mg/case) group, d P <0.05, vs. opium (100 mg/case) group, e P <0.05, vs. opium(50 mg/case) group+ noise exposure.(according to ANOVA test (Tukey))

Discussion

This study was conducted to investigate the effect of exposure to noise stress on sperm parameters among opium-addicted rats. In this study, noise stress significantly reduced sperm count (Table 1), which is consistent with the results of Bisong et al. [11].

The normal sperm morphology (%) in the noise-exposed group was also significantly decreased (Table 1); the finding is in line with the results of Pramanik et al. This effect has been reported due to decreased elliptical protein due to decreased testosterone levels due to noise exposure [46]. The results also highlighted that noise-induced stress reduces sperm viability (%) (Comparison of control and noise-exposed groups). In this regard, the study of Vosoughi et al. also reported such findings about sperm viability in their study [35].

Similar to results from the study conducted by Shahramian et al. (48), both doses of oral opium solution in groups 3 and 4 reduced the sperm count, sperm viability (%), and normal sperm morphology (%) (Table 1). In contrast, Assaei and colleagues found no significant difference between the addicted and non-addicted groups regarding sperm count [49]. The study of Safarinejad et al. in 2013 showed that sperm count per ejaculation, sperm concentration, sperm motility, and normal sperm morphology (%) in opiate addicts was significantly lower than that of normal people [26]. Yasin's study showed that the use of opium increases the percentage of sperms with abnormal morphology, and this increase is related to the increase in the destruction of seminiferous tubules by opium [50]. The reason for the decrease in sperm count due to opium addiction can be found in the study of Asadikaram et al., which states the programmed death rate (apoptosis) in testicular cells in addicted rats was significantly higher than the control group [42].

Increasing the dosage of opium solution from 50 mg to 100 mg reduced the sperm count, viability, and normal sperm morphology (%) significantly (Table 1). This implies that by increasing the dose of opium, its degradation effects on sperm parameters are also increasing. Oral solution of

opium in the rat appears to play a role as an oxidant. It disturbs the body's oxidant-antioxidant defense system as Safarinejad's study showed that the activity of catalase and superoxide dismutase enzymes (as antioxidant enzymes) in addicted people was significantly lower than in normal subjects [26].

Total motility and progressive sperm motility (%) in group 2 (exposed to noise) were significantly lower than the control group (Table 2), which is consistent with the results from Vosoughi et al. [35]. In this study, both doses of opium solution significantly reduced the total motility (%) and progressive motility (%). The increase in the opium dose has also accelerated the decline in total motility (%) and progressive motility (%) (Table 2). Nonetheless, no difference was observed in the levels of sperm motility with class a (%) between two doses of opium in groups 3 and 4. Assaei et al. concluded that total motility in the addict group was significantly lower than that of non-addicts, and non-moving sperm (immotile) in the addict group was significantly higher than the non-addicted group [48]. The difference between the present study's results and the Assaei et al. study can be related to the time of opium consumption.

Exposure to noise and receiving an oral solution of opium in both doses of opium significantly reduced the sperm count, viability, and normal sperm morphology (%) (Table 1). Also, there was a significant difference between the results of groups 5 and 6 in the three mentioned variables (sperm count, viability, and normal sperm morphology (%). This means that with the same noise exposure level, due to increasing the dose of opium solution, the studied variables decreased.

Given that the prevalence of noise has been reported to be excessively high in many workplaces, it is suggested that:

a) Hormonal studies such as the determination of testosterone levels should be put on the agenda during periodic examinations of addicts located in noisy places because, according to studies, both noise and opium reduce testosterone levels, reducing testosterone levels can be a prognosis for fertility disorders such as reducing the sperm count and sperm motility.
b) More studies are required to shed light on the possible adverse health effects of concomitant exposures such as opium and noise on the expression of effective genes in the spermatogenesis process.
c) The use of natural antioxidant substances to overcome the diminishing effects of noise and opium on sperm quality parameters is suggested. For example, in the study by Nadri et al., Cinnamon extract reduced the effects of noise on sperm parameters (51).
d) The study time was chosen equal to one cycle of spermatogenesis, and it is better to follow up the effect of opium solution in longer times and even in the next generations, as in the study of Pirami et al. (52).
e) Another limitation of the study is the noise level, and it is suggested to use different noise levels in some studies to explain the subject.

Conclusion

Regarding the combination of noise and opium, it can be deduced that the noise exposure and the use of opium reinforced the adverse effects, particularly on reducing sperm count, sperm viability, and normal sperm morphology (%). One of the major exclusive approaches in this animal study was that the researchers investigated the effect of taking pure opium on sperm parameters. However, in studies conducted on human populations, an abundance of exposure to different opiates is involved, and there is considerable uncertainty about using a particular type of narcotics such as opium.

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