Effect of Heroin Used in Iran on Male Fertility of Mice

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Abstract: The aim of this study, was to determining the effects of the heroin used in Iran that there has been no study on this subject on fertility indices in mice (Balb/c). These factors include sperm motility, sperm viability, daily sperm production, epididymal sperm reserve, serum testosterone concentration, body weight, testis weight and gonado-somatic index. For this study a total number of 177 mice (105 male and 72 female) were used. The male mice were divided into 5 groups (3 control and 2 experimental). From each group 3 male were chosen for fertility rate. Different experimental groups of heroin-dependant mice (50 mg kg⁻¹ IP for 3 days, twice daily), were divided into two groups. One of which received heroin with a dose of 5 mg kg⁻¹, IP and the other 5 mg mL⁻¹, IP twice daily for a period of 40 days. The Results showed that the heroin used in Iran could exert a significant effect on the sperm motility, sperm viability and serum testosterone concentration; Also significant changes in the body weight, testis weight and fertility were observed. But no significant changes in the daily sperm production, epididymal sperm reserve and gonado-somatic index were seen. The data suggests that the heroin used in Iran could affect some of the spermatogenesis functions.

Key words: Heroin, mice, sperm, fertility

INTRODUCTION

Drug abuse is a serious concern in male adolescents. Administration of opiates during prepubescent and adolescent periods produces long-term physiological and endocrine disturbances (Yilmaz et al., 1998). There was also effect of toxins on the adrenocorticotropic hormone cell in the pituitary gland (Dangxia et al., 2006). It has been shown that immature or prepubescent rats are more sensitive than adults, to the effects of morphine and the other toxins affect on male reproductive system (Cicero et al., 1989; Zhou, 2005). Some studies have been performed on the effect of certain drugs on the sperm count, sperm motility, sperm viability, metabolism and the morphology of sperms. It has also been demonstrated that morphine depresses basal LH release and blocks proestrous and sex steroid induced LH surges (Ieiri et al., 1980; Kalra and Simpkins, 1981). In addition cannabinoid compounds reliably inhibit ovulation in animals and are associated with depressed Luteinizing Hormone (LH) levels in both female and male animals. The decreased LH levels appear to be due to both hypothalamic and ovarian sites of action. Other studies have showed that nicotine caused a reduction in the weight of epididymis and vasa deferens and epididymal sperm count (Londonkar et al., 1998). The other findings indicated that exposure of non-smoker's spermatozoa to seminal plasma from smokers significantly reduced sperm motility and membrane functional integrity. Cigarette smoking has also been associated with detrimental effects on sperm concentration, motility and morphology (Stillman et al., 1986; Little and Vainio, 1994; Vine, 1994, 1996). The negative effects on sperm viability increased during short and long term incubation in smoker's seminal plasma (Zavos et al., 1998). Some studies have been conducted on the effect of opioids on the hormones of reproductive system. As shown in these studies, morphine has an inhibitory influence on gonadotropin releasing hormone (GnRH). Endogenous opioids also appear to affect testosterone secretion and testicular functions (Fabbri et al., 1989). Other studies suggest that morphine can change the body and testis weight in male rats (Yilmaz et al., 1999). So sperm quality and quantity is a critical factor to male fertility (Shewetta et al., 2005).

This study was undertaken to evaluate the effects of the administration of the heroin used in Iran on sperm motility, sperm viability, daily sperm production, epididymal sperm reserve, serum testosterone

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concentrations, body and testicular weight, gonadosomatic index and fertility in the male mice (Considering the fact that street heroin in other countries contains different components (Anonymous, 2001), also in Iran, after analyzing such differences were observed). This research is the first study on the effects street heroin on male fertility in Iran.

MATERIALS AND METHODS

In this study, a total number of 105 male and 72 female adult mice were obtained from experimental animal center of Razi institute in 2005. The drug was the heroin used in Iran and it was injected intra-peritoneally (IP). At first we determined the body weight of male mice. Then male mice were divided into 5 groups:

The first group: (n = 21) intact; Did not receive any drug.

The second group: (n = 21) Sham I; Received normal saline injections for a period of 40 days with the following pattern: In the first 3 days they were injected 3 times daily, with the dosage of 150 μL in the first day, 180 μL in the second day and 210 μL in the third day. The last dose was then injected twice daily for the rest of the 40 days period.

The third group: (n = 21) The third group for determining the effects of lemon juice Sham II; Received normal saline and lemon juice by injection for a period of 40 days with the following pattern. In the first 3 days they were injected 3 times daily: with the dosage of 150 μL normal saline plus 0.4 μL lemon juice in the first day; 180 μL normal saline plus 0.48 μL lemon juice in the second day and 210 μL normal saline plus 0.56 μL lemon juice in the third day. The last dose was then injected twice daily for the rest of the 40 days period.

The fourth group: (n = 21) Experiment I: Received normal saline, lemon juice (heroin solvent) and heroin by injection for a period of 40 days. In the first 3 days, they were injected 3 times daily with the 50 mg kg⁻¹ dosage for independency to heroin (Homayoun et al., 2002). Then they were treated with injection solutions of 1 mg heroin in 1 mL normal saline and 0.0026 mL lemon juice. The dosage of the mentioned solution was 150 μL in the first day, 180 μL in the second day and 210 μL in the third day which continued twice daily for 40 days. (Dosage of heroin for this group was 5 mg kg⁻¹).

The fifth group: (n = 21) Experiment II: Received normal saline, lemon juice and heroin by injection with the dosage of 5 mg mL⁻¹ for a period of 40 days. In this group the pattern of using the solution was similar to that of the 4th group.

From each group six mice were selected and after anesthetizing the animals, architectomy was performed. After putting the testicles into normal saline and using homogenizer, one drop of the solution was put on the Neobar slide. The sperms were counted by using light microscope and the number of sperms in each cubic milliliter was calculated (Robb et al., 1978).

Determining the Daily Sperm Production (DSP): For determining the number of sperms in each gram of the testicle, the number of the sperms was divided to the weight of the testicles and by dividing the result to 4.84 the rate of daily sperm production was calculated (Robb et al., 1978).

Determining the percentage of viability: For determining the percentage of viability of the sperms, the above solution was put on the slide and by adding eosine-nigrosine solution, the percentage of the live sperms, which didn’t stain, was calculated.

Determining the percentage of motility: For determining the percentage of sperm motility, ductus deferens was removed from the body and was incubated in normal saline solution in 37°C. Then the ductus deferens was sectioned to extract the sperms; and one drop of the solution containing sperms was put on the Neobar slide by slow shaking. The sperms were counted in 10 different visual fields. The number of sperms moving forward was determined and the percentage of the moving sperms was calculated.

Calculating the Epididymal Sperm Reserve (ESR): After opening the abdominal wall of the mice, one of the epididymides was removed and weighed, the other one was treated for determining the ESR; The epididymis was put in 2 cc normal saline for 20 min in incubator under 37°C. During this time, all the sperms come out of the epididymis and enter the solution. After the incubation, the sections of epididymis were removed and one drop of the solution was put on the Neobar slide. In order to calculating the ESR the number of sperms was counted and the result was divided to the weight of epididymis.

Determining the serum testosterone concentration: From each group, 6 mice were selected and their blood was poured into lab tubes and kept in -40°C for 24 h. Then the blood of the mice was centrifuged and transferred to the laboratory for determining the serum concentration of testosterone (RIA method).
Table 1: Sperm motility, sperm viability, epididymal sperm reserve, daily sperm production, serum testosterone concentration, body weight differences, testis weight, gonado-somatic index and fertility rate of control and experimental groups of males rats exposed to heroin injection

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Intact</th>
<th>Sham 1</th>
<th>Sham 2</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm motility (%)</td>
<td>90.59±1.98a</td>
<td>90.30±1.89a</td>
<td>90.50±0.85a</td>
<td>79.23±0.88a</td>
<td>57.70±11.25a</td>
</tr>
<tr>
<td>Sperm viability (%)</td>
<td>92.22±2.29a</td>
<td>92.40±2.37a</td>
<td>90.03±0.90a</td>
<td>90.90±3.57a</td>
<td>72.33±6.24a</td>
</tr>
<tr>
<td>Epididymal sperm reserve (%)</td>
<td>14.07±2.56b</td>
<td>14.82±2.56b</td>
<td>8.25±1.43b</td>
<td>5.00±2.56b</td>
<td>11.80±2.06b</td>
</tr>
<tr>
<td>Daily sperm production (%)</td>
<td>6.50±0.47a</td>
<td>5.00±0.53a</td>
<td>4.73±0.22a</td>
<td>4.86±0.13a</td>
<td>5.00±0.50a</td>
</tr>
<tr>
<td>Testosterone concentration (ng/mL)</td>
<td>473.35±38.48a</td>
<td>462.33±55.11a</td>
<td>347.67±14.69a</td>
<td>298.75±33.65a</td>
<td>22.77±4.98a</td>
</tr>
<tr>
<td>Testis weight (g)</td>
<td>0.23±0.0240a</td>
<td>0.26±0.0170a</td>
<td>0.25±0.0204a</td>
<td>0.16±0.0149a</td>
<td>0.18±0.0178a</td>
</tr>
<tr>
<td>Body weight differences (g)</td>
<td>16.80±1.657a</td>
<td>17.37±2.040a</td>
<td>19.07±2.101a</td>
<td>9.90±1.95a</td>
<td>9.93±0.717a</td>
</tr>
<tr>
<td>Gonado-somatic index (g)</td>
<td>0.006±0.0008a</td>
<td>0.006±0.0006a</td>
<td>0.006±0.0005a</td>
<td>0.006±0.0005a</td>
<td>0.005±0.0005a</td>
</tr>
<tr>
<td>Fertility rate (%)</td>
<td>99.35±0.409a</td>
<td>99.35±0.648a</td>
<td>97.43±2.567a</td>
<td>47.74±21.374a</td>
<td>37.50±8.102a</td>
</tr>
</tbody>
</table>

Means±SE, N = 6, unequal letter(s) in each data indicate significant difference at the level of p<0.05

Determining the changes in body weight, testis weight and Gonado Somatic Index (GSI): From each group six mice were selected. To determine the changes in body weight (at the beginning of the experiment), each of the male mice was weighed before being anesthetized. After washing the testicles, their weight was measured. The ratio of the weight of both testicles to the body weight was calculated and the percentage was determined and recorded as GSI.

Determining the fertility rate: From each group 3 male rats were chosen and each of them was kept with 3 female mice in a separate cage. After 10 days the female mice were taken out, anesthetized and the embryos in their uterus were counted. The ovaries were then removed and washed in normal saline (sodium chloride solution 0.9%). The number of corpus luteum was counted and the fertility rate was determined by dividing the number of embryos to the corpus luteum. In the cases that no embryos were found, the above experiment was repeated for three times.

Statistical methods: One-way analysis of variance plus complementary Post Hoc Tukey test.

RESULTS

The results showed no significant difference (p = 0.543) between the experimental and control groups in daily sperm production, epididymal sperm reserve and gonado-somatic index (Table 1). However in the motility of sperms and serum testosterone concentration, no significant difference (p = 0.543) between the experiment 1 and 2, but significant differences were observed between the experiment 2 and control groups (p<0.05). Body weight differences and fertility rate (Table 1) significant differences were observed between the experimental and control groups (p<0.05), but no significant difference (p = 0.543) between the experiment 1 and 2. Also the results showed that the percentage of the sperm viability (Table 1) in the test experimental 2 group had a significant reduction in comparison with experiment 1 and control groups (p<0.05). As for the testis weight, a significant difference was observed only between sham 2 and test experiment 1 groups (Table 1); but there were no significant reduction between the control groups.

DISCUSSION

The action of opioids on the reproductive function has received renewed attention in the recent years (Lakshman et al., 1988). However, no study has been conducted on the exact effects of the heroin used in Iran on the male reproductive system. Therefore, it was decided to examine the effect of the heroin used in Iran on the motility, viability, daily sperm production, epididymal sperm reserve, serum testosterone concentration, body and testis weight, gonado-somatic index and fertility rate. Previous findings indicated that there are significantly reduced in sperm motility and membrane functional integrity of smoker's spermatozoa in seminal plasma than non-smokers (Zavos et al., 1996). Irreversible damage to spermatozoa in smokers was confirmed in a second study, in which the architectural elements of the sperm tail axoneme in smokers were assessed at the ultra structural levels (Zoa et al., 1998), in contrast to an earlier published report of reduced sperm motility in methadone and heroin users (Hargreaves et al., 1983). This study shows the heroin used in Iran could also change the sperm motility. The negative effects on sperm viability increased during short and long-term incubation in smoker's seminal plasma (Zavos et al., 1996). It was suggested that heroin could decrease the viability of
sperms as well. Previous studies on male rats have shown that the sperm number from cauda epididymis and daily sperm production was significantly decreased with higher doses of lithium carbonate, whereas, lower doses did not show any untoward effect (Thakur et al., 2003). Also some researches have been conducted to study the effect of propyl parabene on the male reproductive system, suggesting that the cauda epididymal sperm reserves and concentrations decreased in a dose-dependent manner and the differences were significant. Nicotine caused a decrease in the epididymal sperm count (Londonec et al., 1998). In this study no significant differences was observed in the epididymal sperm reserve. Daily sperm production and its efficiency in the testis of all groups receiving propyl parabene significantly decreased (Oishi et al., 2002). In addition have corroborated the adverse effects of cannabinoid compound on sperm production (Abel, 1981). In this study no significant difference was observed in daily sperm production of control groups and the other groups which had received heroin. Another parameter assessed in this study was the effect of heroin on serum testosterone concentration. It was observed that heroin can decrease the concentration of testosterone in serum. Similar studies have been performed on the effect of some opioids on serum testosterone concentration, showing that endogenous opioids appear to affect testosterone secretion and testicular functions, by two mechanisms. These studies suggest that this effect might be through (1) the hypothalamo-hypophyseal-gonadal axis, or (2) putative opioid receptors in the testis (Margioris et al., 1989, Wittert et al., 1996). The functionality of these testicular opioid receptors has been questioned (Fabbri et al., 1988). Endogenous opioid peptides can be synthesized in the testis in different components of the male reproductive tract (Yilmaz et al., 1999). Also the previous study has shown that morphine exposure during sexual maturation causes significant disruption in the hypothalamo-hypophyseal-testicular axis in rat. Testosterone supplementation may be required to restore sexual dysfunction in male morphine addicts during rehabilitation (Yilmaz et al., 1999). Present study shows that the heroin used in Iran can also change the body weight and testicular weight, but there is no significant difference in the GSI of control and experimental groups. Other studies suggest that chronic morphine administration progressively reduces body weight and testicular weight (Yilmaz et al., 1999), because Inhibition of androgen production would be expected to be associated with its decreased metabolism. Weight of the testis remained unaltered following morphine exposure (Yilmaz et al., 1999). In this study, it was observed that in female mice despite more presence of corpus luteum, fertility percentage was obviously decreased. This reduction in the fertility of female mice might be due to the adverse effects of heroin on male sexual organs (Öberlander et al., 1994). Almost all of the adverse effects of cannabinoid exposure on reproductive organs can be attributed to these secondary effects (Abel, 1981).

CONCLUSION

Increasing infertility through decreasing the motility and viability of sperms and not lowering the sperm production. Results from this study suggest that the heroin used in Iran which is a combination of opioids and non-opioids, called street heroin, might be able to increase infertility in men.

It is suggested that the effect of street heroin on histological structure of male reproduction system should be studied in further investigation.

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REFERENCES


