Effects of Methamphetamine on Testes Histopathology and Spermatogenesis Indices of Adult Male Rats

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Abstract

**Background:** Methamphetamine (MAMP) as a recreational drug has devastating effects on the central nervous system (CNS). Several studies have shown that MAMP has inhibitory effects on oogenesis and spermatogenesis, and causes impaired fertility. This study designed to investigate the effect of mAMP administration on histological changes and spermatogenesis indices in the testis of adult male rats.

**Methods:** In this experimental study, 50 male Wistar rats were randomly divided into control (received no treatment, n = 10), vehicle (received saline for 7 and 14 days, n = 20), and experimental group [received MAMP, 5 ml/kg, intraperitoneal (IP) for 7 and 14 days, n = 20]. Testicular tissue samples were stained by hematoxylin and eosin (H&E) technique. For histological study, we counted the number of spermatogonia, spermatocytes and Leydig cells. Spermatogenesis indices which include: tubular differentiation index (TDI), spermiogenesis index (SI), repopulation index (RI) and the mean seminiferous tubules diameter (MSTD) were studied. Data were analyzed by one-way ANOVA, using SPSS software. P < 0.05 was considered statistically significant.

**Findings:** This study showed that MAMP caused a significant decrease in number of seminiferous tubules cells and spermatogenesis in treated group compared with the control group. Moreover, results showed a significant decrease in spermatogenesis indices including TDI, SI, RI, and MSTD in 14th day, compared to control group (P < 0.001).

**Conclusion:** The data showed the adverse effects of MAMP administration (for 7 and 14 days) on testes structure and spermatogenesis indices in rat testis tissue. The underlying mechanism(s) needs further investigation.

**Keywords:** Methamphetamine; Histology; Spermatogenesis; Testis; Rats

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Introduction

Methamphetamine (MAMP), a central nervous system (CNS) stimulant used as a recreational drug, is a very addictive drug. It is often abused worldwide at rates that are lower than only alcohol and marijuana. The estimates of the global prevalence of MAMP use is as high as 1.2% (24.7 million), which is particularly common in Asia, Oceania and North America. It is reported that 8.6% of 18 to 49 years old of USA population had lifetime illicit use of MAMP. There is a growing tendency to amphetamine abuse among young adults in most parts of the world, including Iran. Ziaaddini et al. reported that 6.6% of pre-university male students in Kerman City, Iran, were using ecstasy.

The main consumers of these drugs are the youth and adolescents who are at the age of reproduction. Despite the number of MAMP users, there is no universally effective pharmacological treatment for MAMP abuse. The mechanisms underlying MAMP-induced neurotoxicity have been identified and include oxidative stress, toxicity, apoptosis and damage of cellular macromolecules like lipids, proteins, and DNA. There are many reports regarding the effects of this drug on various organs of the body, such as the cardiovascular, pulmonary, renal and hepatic systems, and reproductive organs.

Previous studies showed that amphetamine and its derivatives [MAMP, 3,4-methylenedioxyamphetamine (MDMA) or ecstasy] consumption has significant results including sustained hyperthermia, a slight decrease in body and liver weight, increased plasma levels of aspartate transaminase (AST) and alanine transaminase (ALT) as well as liver damage as indicated by histological analyses. Mohammadi et al. reported that MAMP caused destructive effects on kidney tissue including agglomerular sclerosis and renal congestion as well as significant increase in serum creatinine levels in adult male mice.

Other researchers reported that illicit use of MAMP not only causes adverse effects on CNS, but also it could be associated with reproductive toxic effects including apoptosis in seminiferous tubules in male mice testis, decrease in sperm motility, decrease in plasma testosterone concentration, abnormal sperm morphology, and low sperm concentration in male rats. Other investigators reported that gonadal steroid hormones are important in modulating MAMP neurotoxicity. Despite the widespread use of MAMP by young adults, however few studies have reported MAMP effects on male reproductive structure; so, the aim of this study was to investigate the effects of MAMP administrations on histological and spermatogenesis indices of testes in adult male rats.

Methods

This experimental study was conducted according to the National Institute of Health (NIH) guidelines on ethical standards for investigation of animals, which were approved by the Animal Experimentation Ethic Committee of Kerman Neuroscience Research Center (EC/KNRC 96000534).

In this experimental study, 50 adult (10-weeks old) male Wistar rats, weighing 250-280 g were used. The rats were housed four in the cage in an air-conditioned animal house at 23 ± 2 °C, a relative humidity of 55 ± 5%, and on a 12 hour light/dark cycle. The animals had free access to standard pellet food and water.

MAMP was dissolved in normal saline (0.9% sodium chloride, 1 mg MAMP per 1 ml normal saline); stock solutions were prepared and administered via intraperitoneal (IP) injection at the daily dose of 5 mg/kg either for 7 or 14 days. Animals were randomly divided to 5 groups including control (received no treatment), vehicle (received saline) and MAMP (received MAMP).

At the end of each experiment, rats were sacrificed by cervical dislocation and testes were removed and fixed in 10% neutral buffered formalin for 48 h for histological examinations. Testes tissues were washed through graded concentrations of ethanol saturated. Then, the samples were dehydrated, cleared and embedded in paraffin wax. Sections were cut with a rotary microtome at 5 mm thicknesses, then stained with hematoxylin and eosin (H&E). The sections were studied under light microscope for spermatogenesis process. For histological study, we counted the number of spermatogonia, primary and secondary spermatocytes, Leydig cells and vascularity, and compared them with control group. For spermatogenesis indices, we measured tubular differentiation index (TDI), spermatogenesis index (SI), repopulation index...
(RI) as a measure of the length of total colonies per testis, and the mean seminiferous tubule diameter (MSTD).

For measuring TDI, SI and RI, 200 seminiferous tubule cross sections were scored. The TDI is the percentage of tubules that contained three or more differentiated spermatogenic cells more advanced than spermatogonia type (i.e., intermediate or type B spermatogonia, spermatocytes, or spermatids), and is a measure of survival and differentiation of the stem cells, type A spermatogonia. The RI is the ratio of active to inactive spermatogonia cells. For the SI, the ratio of seminiferous tubules containing sperm to the tubules without sperm was calculated.\(^\text{26,29}\)

MSTD of each testis was measured by Image Tools software version 2.\(^\text{30}\)

Data were expressed as mean ± structural equation model (SEM) of 10 rats per group and compared for statistical significance using analysis of variance (ANOVA) followed by Tukey post hoc test to assess the significance of changes between control and experimental groups. \(P < 0.05\) was considered statistically significant. All data were processed by SPSS software (version 20, IBM Corporation, Armonk, NY, USA).

### Results

Considering the examination of testicular tissue samples, results showed normal seminiferous tubules in control group, spermatogonia, spermatocytes and spermatids exhibited normal arrangement in different stages of spermatogenesis in vehicle groups. MAMP treatment (5 mg/kg either for 7 or 14 days) had no significant changes in the testis weight compared to control group. However, mAMP treatment for 14 days caused a significant decrease in testes histological parameters including spermatogonia, primary and secondary spermatocytes, compared to control group. \(P < 0.001\), but mAMP had no significant effect on Leydig cells (Table 1) (Figure 1).

![Figure 1. Photomicrograph testis sections in the mAMP and control groups. Normal spermatogenesis is seen in the control group (A) and vehicle group (B). Nearly all lineage of spermatogonia cells were damaged and their number were decreased in mAMP group either in 7 days (C) or 14 days (D). [Hematoxylin and eosin (H&E) staining. ×400].](image)

#### Table 1. Effects of 7 and 14 days methamphetamine (MAMP) treatment on testes weight and testes structure in male rats

| Groups          | Testes weight (g) (mean ± SD) | P* | Spermatogonia (n) (mean ± SD) | P* | 1st Spermatocytes (n) (mean ± SD) | P* | 2nd Spermatocytes (n) (mean ± SD) | P* | Leydig cells (n) (mean ± SD) | P* |
|-----------------|-------------------------------|----|-------------------------------|----|-----------------------------------|----|-------------------------------|----|-------------------------------|----|
| Control         | 2.70 ± 0.21                   | -  | 52.50 ± 0.65                  | -  | 52.70 ± 0.60                      | -  | 50.40 ± 0.65                  | -  | 10.40 ± 0.26                   | -  |
| Vehicle (7 days)| 2.68 ± 0.28                   | 0.96| 53.30 ± 1.14                  | 0.96| 52.90 ± 0.69                      | 0.98| 54.20 ± 0.80                  | 0.86| 10.03 ± 0.39                   | 0.94|
| Vehicle (14 days)| 2.80 ± 0.27                   | 0.84| 55.60 ± 0.97                  | 0.190| 54.60 ± 1.19                      | 0.380| 53.30 ± 0.70                  | 0.970| 10.00 ± 0.37                   | 0.980|
| MAMP (7 days)   | 2.77 ± 0.28                   | 0.780| 46.60 ± 0.76                  | 0.001| 48.10 ± 0.53                      | 0.001| 54.00 ± 0.56                  | 0.001| 10.30 ± 0.43                   | 0.950|
| MAMP (14 days)  | 2.68 ± 0.13                   | 0.780| 42.50 ± 0.69                  | 0.001| 43.70 ± 0.47                      | 0.001| 49.20 ± 0.49                  | 0.001| 10.04 ± 0.48                   | 0.840|

MAMP: Methamphetamine; SD: Standard deviation
*Compared with control group
The present results indicated that consecutive administration of MAMP for either 7 or 14 days caused significant histopathological changes in testes structure and spermatogenesis indices in adult male rats. In addition, MAMP caused structural abnormalities in testes of male rats, indicated as a significant decrease in the number of spermatogonia, primary and secondary spermatocytes, as well as a significant decrease in spermatogenesis indices including TDI, SI, RI and MSTD, compared with the control group. Our results are in agreement with some previous reports that demonstrated the adverse effects of MAMP on fertility indices in both male and female animals.\(^{16,22,31,32}\)

Sabour et al. reported that MAMP administration for 35 days caused significant changes in sperm morphology, sperm chromatin abnormalities, DNA integrity impairment and apoptotic activities.\(^{16}\) Nudmamud-Thanoi and Thanoi reported that MAMP induced highly significant decrease in percentage of normal. Sperm morphology, total sperm counts, and significant increase in apoptotic activities in the seminiferous tubules in the testes of male rats in a dose dependent manner.\(^{22}\)

Alavi et al. showed that repeated administration of MAMP (10 mg/kg/14 days) caused a significant effect on spermatogenesis process including a significant impairment in spermatogonia and primary spermatocytes as well as increase in apoptosis in seminiferous tubules of rat testes.\(^{31}\) Yamamoto et al. reported MAMP-induced apoptosis in seminiferous tubules in male mice testes following a single dose administration of MAMP (10 and 15 mg/kg).\(^{34}\) Fazelipour et al. reported that methylphenidate (Ritalin), one of the isomers of amphetamine, caused a significant decline in serum testosterone concentration and a decrease in the number of Leydig cells and fertility rate in male mice; however, it did not show any significant changes in the weight or morphometric parameters of testes.\(^{33}\) Barenys et al. showed that rats exposure to ecstasy (MDMA) during developmental periods caused a significant higher incidence of DNA damage in sperm and interstitial edema in testes;\(^{34}\) however, Kwack et al. showed that the adverse effects of ecstasy on some organs, including reproductive system, is dose dependent and no observed adverse-effect level of MDMA is estimated to be 1.25 mg/kg bw/d.\(^{35}\) Since the reproductive effects of amphetamine and its derivatives could be mediated by different factors such as drug dosage, exposure duration, and gender, it is suggested that further experiments are needed to elucidate the effects of short and long-term administration of MAMP on reproductive function in both animals and humans.\(^{4,35,36}\)

Wang et al. showed the adverse impact of MAMP on female ovarian tissue and their fertility ability, including morphological-apparent, an activated apoptosis pathway in the ovarian tissue, mitochondrial damage, a decreased number of primordial and growing follicles estradiol and progesterone from granulosa cells in adult mice.\(^{32}\) Taghavi et al. reported that acute administration of MAMP (5, 10, and 15 mg/kg) caused a significant reduction in number of sperms, but it did not affect the motility and morphology of sperms.\(^{37}\) MAMP exposure during pregnancy caused significant impairments in neurodevelopmental parameters such as pinna unfolding and alteration of behavioral development such as disturbed surface righting test, impairment of plane and forelimb grip tests in rat offsprings.\(^{36}\)

The underlying mechanism(s) is not

### Table 2. Effects of 7 and 14 days methamphetamine (MAMP) treatment on spermatogenesis indices in male rats

| Groups          | TDI (%) (mean ± SD) | SI (%) (mean ± SD) | RI (%) (mean ± SD) | MSTD (µm) (mean ± SD) |
|-----------------|---------------------|--------------------|--------------------|-----------------------|
| Control         | 93.50 ± 0.70        | 82.50 ± 0.92       | 85.00 ± 0.54       | 301.10 ± 1.37         |
| Vehicle (7 days)| 92.70 ± 0.60        | 83.40 ± 0.86       | 84.50 ± 0.83       | 299.40 ± 1.13         |
| Vehicle (14 days)| 89.70 ± 0.42      | 81.60 ± 0.96       | 82.60 ± 0.93       | 297.50 ± 1.44         |
| MAMP (7 days)   | 84.00 ± 1.29        | 75.90 ± 0.75       | 75.20 ± 1.05       | 264.30 ± 2.25         |
| MAMP (14 days)  | 64.20 ± 1.10        | 46.80 ± 0.84       | 44.90 ± 1.44       | 256.30 ± 1.86         |

MAMP: Methamphetamine; TDI: Tubular differentiation index; SI: Spermatogenesis index; RI: Repopulation index; MSTD: Mean seminiferous tubule diameter; SD: Standard deviation

*Compared with control group
determined yet, and needs further investigation. Most studies on the effects of illicit drug abuse including amphetamines and ecstasy on male fertility are studied through animal models. Other investigators reported that ecstasy decreased Gonadotropin-releasing hormone (GnRH) and serum testosterone levels through the alteration in hypothalamic-pituitary-testicular axis. 30,39

In summary, our results showed the adverse impact of MAMP on testes structures, indicated as a significant decrease in the number of spermatocytes, spermatogonia and spermatogenesis indices (TDI, SI, RI, and MSTD) in adult male rats. The underlying mechanism(s) is not determined yet and needs further investigation.

Since MAMP use is common during adolescent period, and MAMP abuse could be associated with significant public health problems, so prevention strategies on adverse effects of drug abuse including MAMP and its derivatives, marijuana, opioids, and alcohol, should be integrated into education programs for high school students.40

**Conclusion**

Use of MAMP in adult male rats can cause changes in the structure of the testicles, which also affects their fertility.

**Conflict of Interests**

The Authors have no conflict of interest.

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اهداف: با مقدمه، مت-آمفتامین به عنوان یک ماده مخدر، تأثیرات زایده بر سیستم اعصاب می‌گذارد. مطالعات گذشته نشان داده است که این ماده مخدر اثر مهاری بر ساخت اسپرم و تخمک‌گذاری دارد. پژوهش حاضر با هدف انجام تجربیات مت-آمفتامین بر تقسیم و تغییرات بافتی بیضه و معیارهای اسپرم‌سازی در بافت بیضه موش، با روش‌های تجربی در کرمان ایران بر انجام می‌گردد.

روش‌ها: در این مطالعه تجربی، 50 نر موش بالغ و نژاد ویستار در گروه‌های دوodynamic در وزن 7.8±0.4 کیلوگرم محروم شدند. گروه‌هایی مشابه دو ترکیب 0% و 5% و ترکیب 10% و 50% از مت-آمفتامین در روز نوزدهم توزیع گردیدند. سپس از گذشت 7 روز به بعد، گروه‌ها به مقدار ماده مخدر مذکور در روزهای دوم و ششم دریافت گردیدند. به هر گروه، 50 نمونه از بافت بیضه گرفته شد. مطالعات تجربی که شامل ضریب تمایز لوله‌ای (TDI) و ضریب بازسازی (SI) اسپرم‌سازی (Spermiogenesis index) در گروه‌های دوodynamic تجزیه و تحلیل گردید.

نتیجه‌گیری: مصرف مت-آمفتامین به مدت 14 روز، باعث کاهش اسپرم‌سازی و تغییرات بافتی بیضه می‌شود که ممکن است از طریق کاهش تقسیم سلولی و اثرات سطحی در بافت بیضه باشد. این اثرات بیانگر اثرات جانبی این دارو و باعث کاهش اسپرم‌سازی و ناباروری انسان، به مطالعات پیشین نیز دارد.

واژگان کلیدی: مت-آمفتامین، بافت-بیضه‌سازی، بیضه، موش، موش‌سازی

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